



International Journal of Environment Agriculture and Biotechnology

(IJEAB)

An open access Peer-Reviewed International Journal



DOI: 10.22161/ijeab.105

Vol.- 10 | Issue - 5 | Sep-Oct 2025

editor.ijeab@gmail.com | editor@ijeab.com | <https://www.ijeab.com/>

International Journal of Environment, Agriculture and Biotechnology

(ISSN: 2456-1878)

DOI: 10.22161/ijeab

Vol-10, Issue-5

September - October, 2025

Editor in Chief

Dr. Pietro Paolo Falciglia

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Publisher

Infogain Publication

Email: editor.ijeab@gmail.com ; editor@ijeab.com

Web: www.ijeab.com

FOREWORD

I am honoured to introduce this latest issue to the International Journal of Environment, Agriculture and Biotechnology (IJEAB). Our journal is dedicated to disseminating high-quality research and innovative findings that contribute to advancing knowledge in these critical fields.

In this issue, we present a collection of papers that exemplify the diversity and depth of contemporary environmental, agriculture, and biotechnology research. The articles include various topics, from sustainable agricultural practices and environmental conservation strategies to cutting-edge biotechnological innovations. Each contribution has undergone a rigorous peer-review process, ensuring the publication of only the most significant and original research.

Our commitment at IJEAB is to provide a robust platform for researchers, academicians, and practitioners to share their work and engage with a global audience. By fostering an interdisciplinary approach, we aim to bridge the gaps between different areas of study and promote holistic understanding and solutions to the challenges we face in these domains.

We are grateful to our dedicated authors, whose hard work and intellectual rigour are the backbone of our journal. We also extend our appreciation to our reviewers and editorial board members, whose expertise and diligence ensure the high standards of our publication. Finally, we thank our readers for their continued support and engagement.

We hope you find the articles insightful and inspiring as you explore this issue. We encourage you to contribute your research to future issues and join us in our mission to advance knowledge and drive positive change in the environment, agriculture, and biotechnology fields.

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Author(s): K.M.D.W.P. Nishantha, M.H.N.Y. Malwenna, W.M.C. Weerakoon, A.R. Attanayake, S.W.G.A.N. Kumari, D.M.I.C.B. Dissanayake, M.A.G.C.N. Premaratne

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 DOI: [10.22161/ijeab.105.25](https://doi.org/10.22161/ijeab.105.25)

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Utilizing Locally Isolated Parasitoid, *Encarsia guadeloupae*, (Hymenoptera: Aphelinidae), for Biological Control of Coconut Whitefly, (*Aleurodicus rugioperculatus*), in Sri Lanka.

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Received: 28 Jul 2025; Received in revised form: 27 Aug 2025; Accepted: 30 Aug 2025; Available online: 06 Sep 2025

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Abstract— Coconut Whitefly (*Aleurodicus rugioperculatus*) infestation possess a growing threat to various coconut growing regions in Sri Lanka since 2019. The National Plant Protection Service in collaborated with Horticultural Crops Research and Development Institute conducted a series of studies to investigate the potential use of locally isolated parasitoid, *Encarsia guadeloupae* for the control of coconut whitefly. The parasitoid *E. guadeloupae* identified as a promising biocontrol agent of coconut whitefly due to its efficacy in damaging the nymphal stages of whitefly. The study initiated with a comprehensive field survey across seven districts to assess the severity of whitefly damage followed by a laboratory analysis of whitefly parasitoids for identification and rearing. Mass production of *A. rugioperculatus* and *E. guadeloupae* was carried out using various host-plant species under the laboratory conditions. Field release of parasitoids were conducted in selected locations, using two distinct methods. Results indicated successful taxonomic identification of *A. rugioperculatus* and *E. guadeloupae*, with coconut identified as the preferred host for whitefly rearing. The field release of parasitoids indicates a significant increase in parasitism level and a corresponding decrease in whitefly populations in coconut plantations. In conclusion, the study establishes the feasibility of mass rearing and field release of *E. guadeloupae* as an environmentally friendly and effective strategy for biological control of coconut whitefly infestations.



Keywords— *Aleurodicus rugioperculatus*, *Encarsia guadeloupae*, Biological control, Coconut whitefly, Parasitoid wasp, Mass rearing

I. INTRODUCTION

Aleurodicus rugioperculatus (Hemiptera: Sternorrhyncha: Aleyrodidae) commonly known as Coconut whitefly is a serious pest causing economic losses to ornamentals and tropical crops in many countries [2]. Recently, the infestation of coconut whitefly has been observed in various coconut growing regions in Sri Lanka including Colombo, Kaluthara, Gampaha, Kegalle, Batticaloa, Kandy and Jaffna.

Whitefly feeding induces stress to the host plants by extracting water and nutrients while also secreting honeydew, which favors the growth of sooty mold on the leaf surface resulting a massive threat to coconut cultivation. Management of coconut Whitefly possess significant challenges because of their wide host range. Biological control of pest becomes an ecologically sound and effective solution for whitefly infestation. Whiteflies have a number

of naturally occurring parasites with them *Encarsia guadeloupae* (Hymenoptera: Aphelinidea) has been identified as the most commonly found natural enemy of *A. rugioperculatus* [8]. *E. guadeloupae* is an obligate endoparasitoid against *A. rugioperculatus* nymphs [11]. The adult stage of this parasite damages the nymphal stage of the whitefly. Therefore, *E. guadeloupae* can be utilized as a biocontrol agent for managing coconut whitefly populations. This approach is environmentally friendly and can serve as an alternative to chemical pesticides.

The National Plant Protection Service of the Department of Agriculture as the mandated institute responsible for implementing the Plant Protection Act 1999, No 35, in collaboration with Horticultural Crops Research and Development Institute, conducted a comprehensive survey to assess the severity of whitefly damage in these areas. Through precise laboratory analysis of collected samples, whitefly parasitoids were identified and preserved for further rearing and identification.

II. METHODOLOGY

2.1. Field survey

Field survey was conducted across Kaluthara, Colombo, Gampaha, Kegalle, Kandy, Batticaloa and Kurunegala districts, where severe whitefly pandemic was recorded for the first time in Sri Lanka. Whitefly infested fields were selected and coconut leaf samples infested with whiteflies were collected from each location. Leaflet parts measuring 4cm × 2cm were examined under the dissecting microscope (20x). The number of observed whitefly adults, nymphs, parasitized pupae and parasitoid emerged pupal cases were recorded to determine the population density of whitefly and the natural parasitism of *E. guadelouoae*. These leaflet parts were stored in well ventilated 200 ml plastic cups for three weeks until the emergence of possible whitefly and parasitoid adults. Emerged whiteflies and parasitoids were collected in vials containing 70% alcohol for taxonomic identification.

2.2. Identification of whitefly and parasitoids

Taxonomic identification was conducted based on morphological characters following the identification key developed by Martin (2004) and Hernandaz *et al.* (2003). Pupal cases of adult whiteflies and adult parasitoids were slide mounted using the protocols described by Nelson *et al* (2001) and Gill (1990). Specimens were observed using both a dissecting microscope (20x) and a compound light microscope (100x and 400x).

2.3. Mass production of *A. rugioperculatus* and *E. guadeloupae*

The identified prominent parasitoid species, *Encarsia guadeloupae*, isolated from the field samples, was reared in the laboratory for mass production. Six host plant species viz Poinsettia (*Euphorbia pulcherrima*), Canna (*Canna indica*), Banana (*Musa spp*), Fan palm (*Livistona chinensis*), Ground nut (*Arachis hypogaea*), and Coconut (*Cocos nucifera*) were tested under laboratory conditions to select the most suitable host-plant for mass rearing.

Each host plant was placed in a single rearing cage (1.5 × 2 × 1 ft). Raring cages were placed under the room temperature. One hundred whitefly adults were introduced at a time, in 2 times with 1-day interval. The number of egg masses laid/leaf and the number of days taken to develop eggs, nymphs, pupa and adults were observed.

After selecting the most suitable host plant, mass rearing was commenced. Mass raring process consists of the following steps.

1. Maintenance of host plants for rearing *A. rugioperculatus*
2. Establishment of pure culture of *A. rugioperculatus*
3. Establishment of parasitoid (*E. guadeloupae*) culture

2.3.1. Maintenance of host plants for raring *A. rugioperculatus*

Coconut seedlings (2 ft height) were selected from plant nurseries as the most suitable host plants for raring *A. rugioperculatus*. The collected plants were maintained in mini-protected plant houses under the controlled environmental conditions.

2.3.2. Establishment of pure culture of *A. rugioperculatus*

Coconut seedlings were transferred into insect raring cages. The adult whiteflies aspirated from field samples were released into raring cage. One hundred adult whiteflies at a time were introduced up to 3 days into the cage in order to establish a pure culture of *A. rugioperculatus*. Adults were kept undisturbed for oviposition and allowed to develop a new life cycle on the host plants. New coconut plants were introduced in four-week interval to be attacked by the newly emerging whitefly adults.

2.3.3. Establishment of parasitoid (*E. guadeloupae*) culture

Coconut plants with egg masses were selected from the pure culture of *A. rugioperculatus* and transferred into parasite raring cages. The plants were allowed to develop up to 2nd and 3rd nymphal stages for introducing parasites. Adults of

E. guadeloupae emerged from coconut leaf samples collected in the field, were aspirated and introduced into the cage. The adult introduction occurred in four stages, with a four-day interval, releasing ten adults at each stage. After 20-23 days from the introduction of adult parasitoids, the host plants were transferred into collection cages.

2.4. Field release of parasitoids

Field release was conducted in late *maha* season in 2022/2023. Thirty-two locations were selected in Kaluthara, Colombo, Gampaha, Kegalle, Kandy and Batticaloa districts where severe whitefly pandemic was recorded.

Two methods were used to field release of parasitoids:

2.4.1. Introduction of adult parasitoids to whitefly infested plants

Adult parasitoids reared in the laboratory were aspirated from the rearing cages and placed in 250 mL plastic containers. To provide an artificial food source, a 10% sugar solution was supplied to these containers. These cups were hung near the canopy area of coconut trees and the lid of the cups were opened to release adult parasitoids.

2.4.2 Introduction of parasitoid cards to infested fields

Parasitized pupae of whiteflies were carefully separated from the leaf using no 10-paint brush. Fifty pupae were then mounted on a cardboard-card using special gum tape, all under observing from a dissecting microscope. These parasitoid containing cards could be stored in the refrigerator for one to two weeks until field release. Two parasitoid cards were introduced to each plant, totaling 20 cards per acre. These cards were placed to the canopy area of the coconut tree by hanging on leaflets.

This introduction process was repeated five times at two-week intervals in each location. After each round of releasing parasitoids into the field, coconut leaf samples

were collected from each location. Number of existing whiteflies puparial cases, both with and without emergence hole were recorded to determine the percentage of parasitism.

III. RESULT AND DISCUSSION

3.1. Taxonomic identification

3.1.1. Identification of *Aleurodicus rugioperculatus*

Adult whiteflies are about three times (2.5 mm) larger than commonly found whiteflies. They can be distinguished by their larger size and the existence of a two irregular light brown bands across the wings. The eyes are dark reddish brown in color. Antenna consists of seven segments. Females are larger than males. Males have a pincer like structure at the end of the abdomen (Fig.1.a).

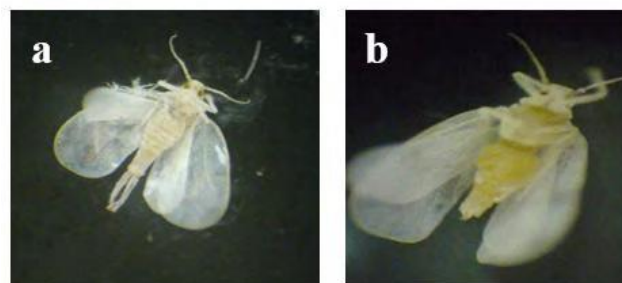


Fig 1: Adult whiteflies. a. Male; b. Female

Whitefly identification is mostly based on the characters of the puparial case. Distinguishable features of the puparial case were rugose nature of the operculum (Fig.2.a), triangular nature of the lingula (Fig.2.b), occurrence of the reticulated margin on dorsum (Fig.2.c), compound pores with dagger like process (Fig.2.d) and presence of smaller compound pores in VII and VIII segments (Fig.2.e).

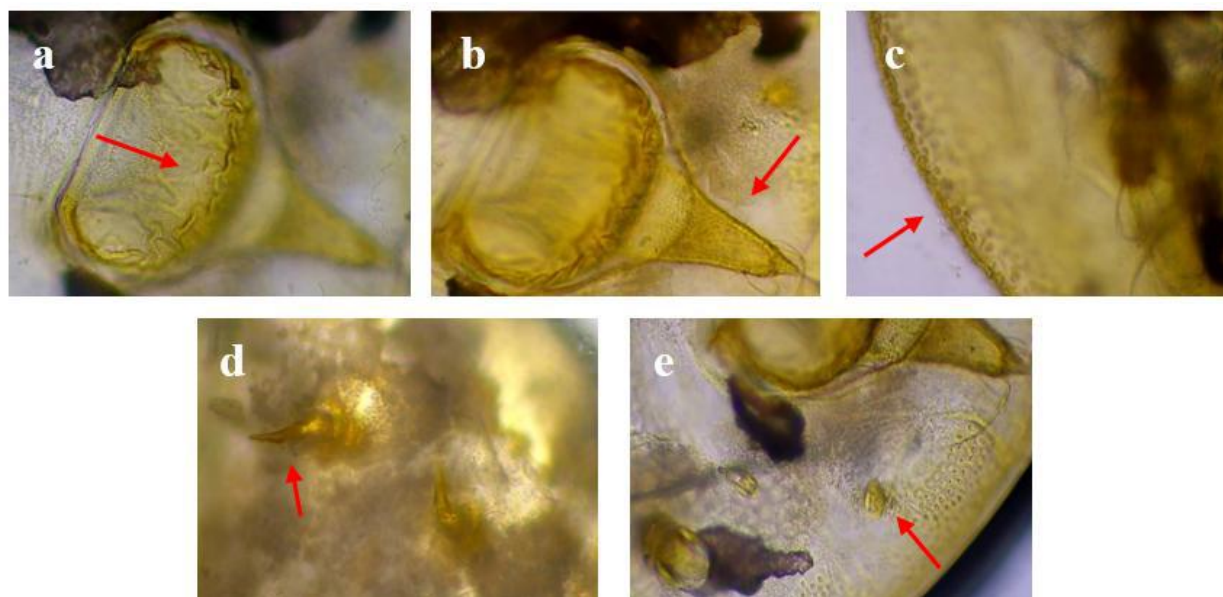


Fig 2: Characters of puparial case. a. rugose operculum; b. triangular lingua; c. reticulated margin on dorsum; d. compound pores with dagger like process; e. presence of smaller compound pores



Fig. 3: Adult *E. guadeloupae*

3.1.2. Identification of *Encarsia guadeloupae*

An average of 98% of emerged parasitoids from the samples showed similar characteristics, and they were identified as *Encarsia guadeloupae* based on the specific features of the species. Adults were dark brown with yellow scutellum. Antenna pale with radical and scape brown. Legs pale except hind coxae and hind femur. Antennal formula 1-1-4-

2. Tarsal formula 5-4-5. In the mesosoma 9-11 pairs of setae on the mesocutum and 2 pairs of setae on the scutellum. More than 2 setae on each side of gastral tergites II and III. Fore wings with 3 setae on basal cell, 2 setae on sub marginal vein and 6-7 long setae on the anterior margin of the marginal vein (Fig. 4).

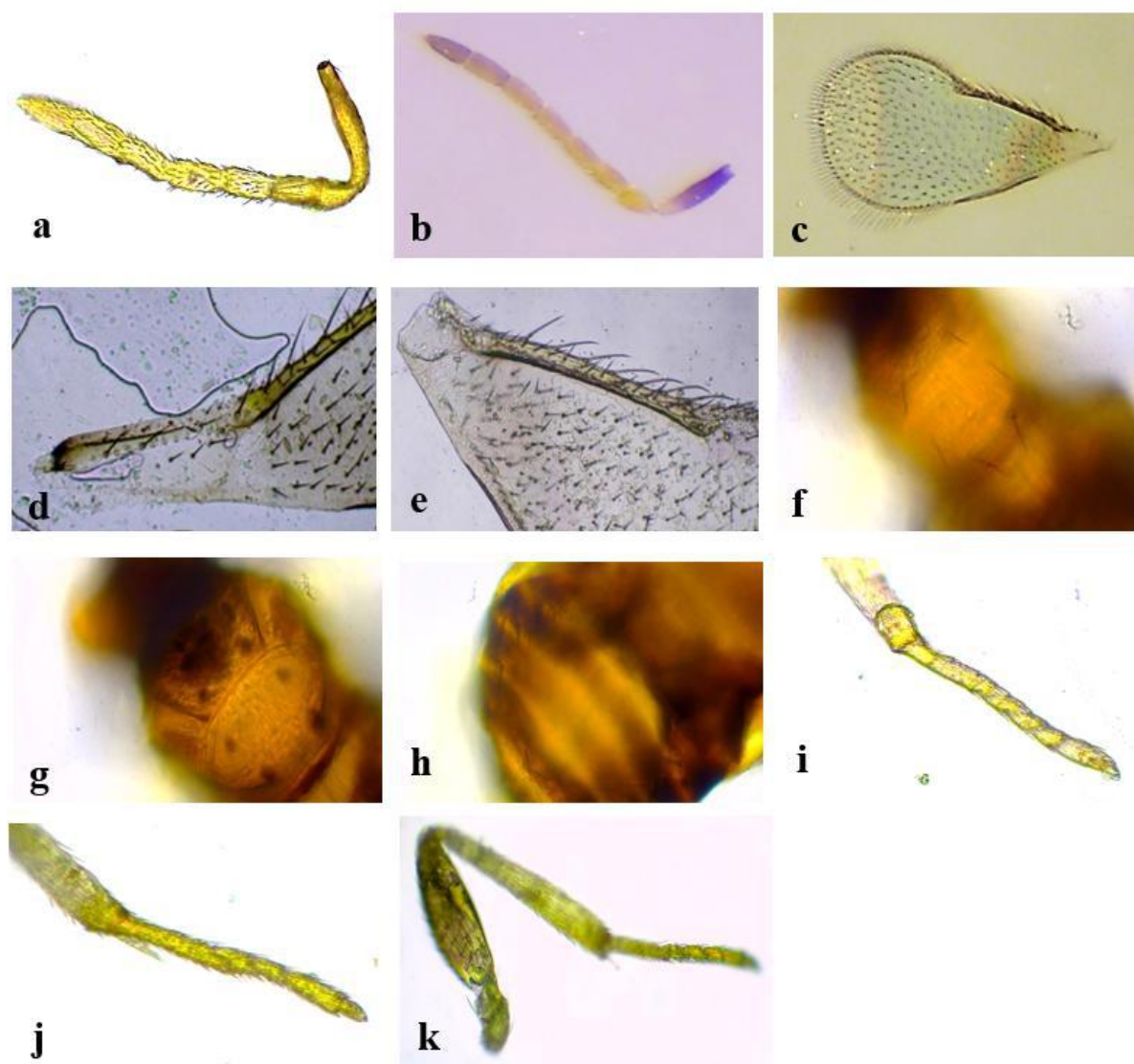


Fig 4: Body parts of *E. guadeloupae* a-b antenna; c-e fore wing; f-h thorax; i. fore leg; j. mid leg; k. hind leg

3.2. Mass production of *A. rugioperculatus* and *E. guadeloupae*

Six host plants were selected for rearing coconut whitefly based on the earlier reports. All the six host plants tested were found to be favorable for the oviposition of whitefly.

Highest mean egg spirals were observed in coconut (18), while the lowest were observed in groundnut (2). Oviposition preference of whitefly is influenced by the leaf hairiness. They more prefer rough leaf surfaces. Coconut having rough leaf surface was more favored by whiteflies for their oviposition.

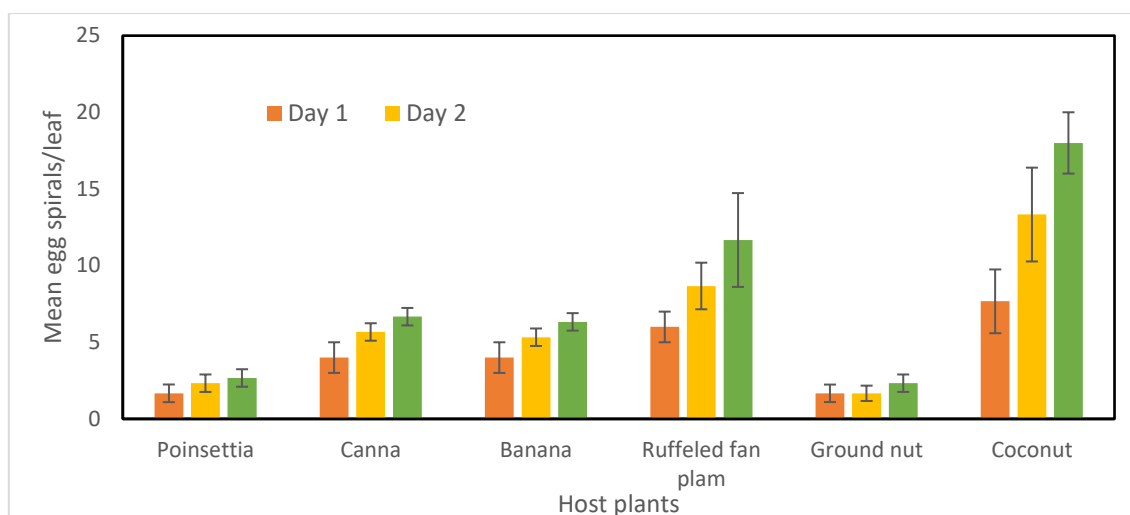


Fig 5: Mean egg spirals per leaf in tested host plants

When consider the number of days taken to develop the stages of the life cycle among six host plants, there was no significant difference of mean number of days taken to develop 2nd instar larvae and pupae. All the other development stages were significant (Table 1). All the treatments except groundnut were capable to develop all the stages of whitefly. In groundnut whiteflies were unable to initiate and develop their lifecycle. Shortest life cycle of whitefly was observed in coconut (29.67 ± 0.57) while the longest was in banana (37.67 ± 0.57).

Coconut has been selected as the optimal host plant to rare whitefly providing an environment conducive to both whitefly oviposition and the successful parasitism of *E. guadeloupae*. This preference is attributed to the favorable conditions that support the entire life cycle of whitefly. Additionally, the relatively short life cycle of the whitefly makes coconut an efficient choice for mass rearing, enabling a rapid and efficient production process.

Table 1: Mean number of days taken to develop eggs, larvae, pupae and adults per plant

Host plant	Number of days taken to develop						Total life cycle
	Eggs	1st Instar larvae	2nd Instar larvae	3rd Instar larvae	Pupae	Adult	
Poinsettia	1.33 ± 0.57 ab	3.67 ± 1.15 b	5.33 ± 0.57 a	6.67 ± 0.57 ab	6.33 ± 0.57 a	7 ± 0 ab	30.33 ± 0.57 bc
Canna	2 ± 0 a	5.5 ± 0.70 ab	6 ± 0 a	7.5 ± 0.70 ab	7 ± 0 a	8 ± 0 a	36 ± 0.57 a
Banana	1 ± 0 b	6.67 ± 0.57 a	7 ± 0 a	8 ± 0 a	7 ± 0 a	8 ± 0 a	37.67 ± 0.57 a
Fan palm	1 ± 0 b	3.67 ± 0.57 b	6.67 ± 0.57 a	7 ± 1 ab	7.33 ± 0.57 a	6 ± 0 b	31.67 ± 0.57 b
Ground nut	2 ± 0 a	0	0	0	0	0	0
Coconut	1 ± 0 b	3.33 ± 0.57 b	6 ± 1 a	5.67 ± 0.57 b	6.67 ± 1.15 a	7 ± 1 ab	29.67 ± 0.57 c

3.3. Field survey

Field survey revealed that all the locations were severely infested with coconut whitefly. Natural parasitism level was

significantly lower in each location. This may be due to the usage of synthetic insecticides and the changes of the climatic conditions.

Table 2: Average percentage of whitefly (*A. rugioperculatus*) and parasitoid (*E. guadeloupae*) population in Coconut cultivations in selected locations

District	Location	% of whitefly	% of parasitism
Gampaha	Ganemulla	90.51	9.48
	Panadura	89.47	10.52
Kaluthara	Horana	85.07	3.05
	Nabada -1	76.45	4.22
	Nabada -2	81.06	1.1
	Gamagoda	75.97	4.68
	Galpatha	77.25	2.79
	Wadduwa	75.97	3.72
	Wadduwa -2	82.46	0.97
	Kolonnawa	91.53	8.46
	Rathmalana	89.63	10.36
Colombo	Katunayaka	88.59	11.4
	Gammanpila	88.61	11.38
	Wavita (Ganegoda)	90.98	9.01
	Polgasowita	89.02	10.97
	Katana	88.09	11.9
	Aluthpola temple	83.83	10.2
	Galthude	88.61	11.38
	Aluthpola -Amandoluwa	91.48	8.51
	Aluthpola -Kontharaduwa	92.5	7.5
	Aluthpola -Nilpanagoda	88.89	11.11
	Aluthpola – 87 Kosgolla	94.59	5.4
	Aluthpola – Miriswellalanda	93.1	6.89
	Aluthpola – Mahawatta	91.89	8.1
	Aluthpola – Delgodalla	90.9	9.09
	Aluthpola – 112	93.87	6.12
Kegalle	Marukwathura	91.13	8.86
	Tholangamuwa	91.03	8.96
	Nangalla	90.9	9.09
	Ibulgoda temple	93.89	6.1
	Gamagedara	73.06	5.64
	Devalegama -1	88.37	4.1
	Devalegama -2	80.9	7.51

	Devalegama -3	80.64	7.95
	Devalegama -4	80.45	3.85
	Devalegama -5	77.57	10.86
	Devalegama -6	85.3	6.54
	Devalegama -7	86	3.15
	Devalegama -8	91.42	2.54
	Devalegama -9	87.2	3.53
	Devalegama -10	75.71	8.79
	Devalegama -11	80.83	4.6
	Devalegama -12	77.28	8.04
	Ibulgasdeniya	87.45	3.99
	Ibuldeniya	85.57	6.53
	Halabada -1	92.51	6.3
	Halabada -2	88.08	4.19
	Halabada -3	86.52	4.5
Kandy	Kundasale	91.56	8.43
	FRI (Gannoruwa)	88.74	11.25
	SCPPC(Gannoruwa)	92.85	7.14
Batticaloa	Coconut Seedling Nursery	93.79	6.2
	Vipulananelapuram	92.04	7.95
	Thanamunei	89.28	10.71
	Meerakermey	88.15	11.84
	Erawur	90.9	9.09
	Pasikuda	97.15	2.84

The Pearson correlation coefficient of 0.225 and a p value of 0.089 indicates a positive correlation between the percentage of whitefly and the percentage of parasitism, but the correlation is not statistically significant (Fig. 6). The positive correlation suggests as the percentage of whitefly increases; there is a tendency for the percentage of natural

parasitism to also increase. The behavioral response of *E. guadeloupae* involves more attraction to the whitefly infesting host plants [7]. Therefore, natural parasitism level of *E. guadeloupae* increase as the whitefly infestation increase.

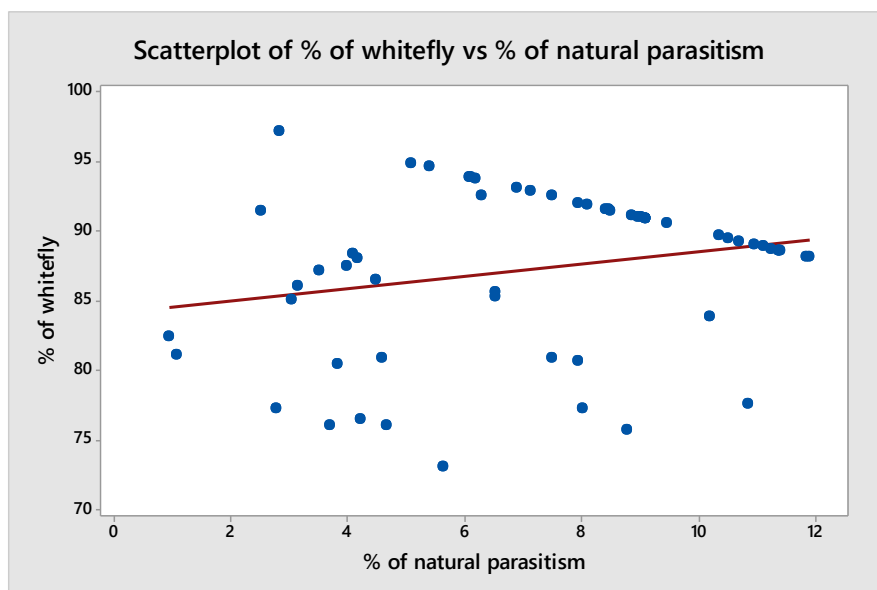


Fig 6: Relationship between Whitefly (*A. rugioperculatus*) and Parasitoid (*E. guadeloupae*) populations in Coconut Cultivations in selected locations at the initial stage

3.4. Field release of parasitoids

There is a significant difference between the percentage of parasitism before field release and the after the field release of parasitoids. Parasitoid population in coconut cultivations

were gradually increase after the field release of parasitoids. It indicates that, *E. guadeloupae* has successfully established in whitefly infested coconut plantations with the time.

Table 3: Percentage of parasitoid (*E. guadeloupae*) population in coconut cultivations at selected locations before and after the field release of parasitoids

Location	% of parasitism (Before the 1 st release)	% of parasitism (After the final release)
Ganemulla	9.48	87.35
Panadura	10.52	83.82
Kolonnawa	8.46	83.82
Rathmalana	10.36	86.2
Katunayaka	11.4	72.41
Gammanpila	11.38	82.25
Wavita (Ganegoda)	9.01	71.18
Polgasowita	10.97	71.66
Katana	11.9	67.08
Aluthpola temple	10.2	83.83
Galthude	11.38	40.21
Aluthpola -Amandoluwa	8.51	70.64
Aluthpola -Kontharaduwa	7.5	56.66
Aluthpola -Nilpanagoda	11.11	72.47
Aluthpola - 87 Kosgolla	5.4	49.5
Aluthpola - Miriswellalanda	6.89	50.61

Aluthpola - Mahawatta	8.1	61.53
Aluthpola - Delgodalla	9.09	53.68
Aluthpola - 112	6.12	50.53
Marukwathura	8.86	82.08
Tholangamuwa	8.96	71.42
Nangalla	9.09	73.91
Kundasale	8.43	61.34
FRI (Gannoruwa)	11.25	78.56
SCPPC(Gannoruwa)	7.14	68.14
Coconut Seedling Nursery	6.2	71.65
Vipulananelapuram	7.95	76.92
Thanamunei	10.71	73.33
Meerakermey	11.84	69.83
Erawur	9.09	67.39
Pasikuda	2.84	48.33
Bopitiya	5.1	60.26

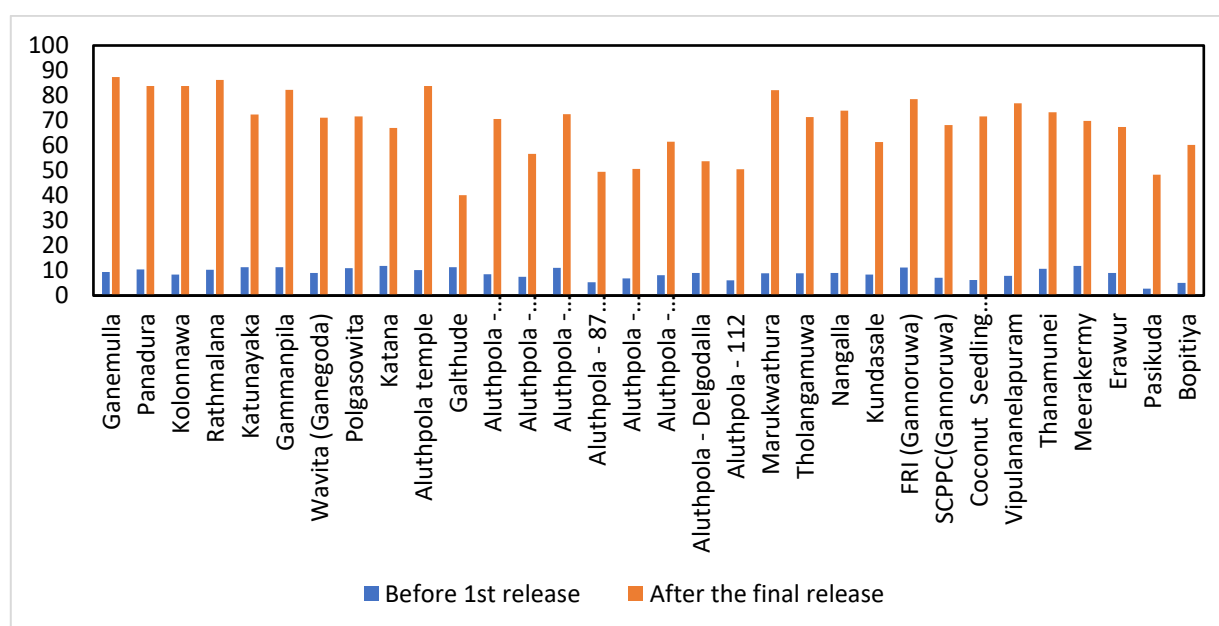


Fig 7: Percentage of parasitoid (*E. guadeloupae*) population in coconut cultivations at selected locations before and after field release of parasitoids

Table 4: Average percentage of whitefly (*A. rugioperculatus*) and parasitoid (*E. guadeloupae*) population in coconut cultivations at selected locations after the field release of parasitoids

Location	% of parasitism after the field release of parasitoids	% of whitefly after the field release of parasitoids
Ganemulla	87.35	12.65
Panadura	83.82	16.18

Kolonnawa	83.82	16.18
Rathmalana	86.2	13.8
Katunayaka	72.41	27.59
Gammanpila	82.25	17.75
Wavita (Ganegoda)	71.18	28.82
Polgasowita	71.66	28.34
Katana	67.08	32.92
Aluthpola temple	83.83	16.17
Galthude	40.21	59.79
Aluthpola -Amandoluwa	70.64	29.36
Aluthpola -Kontharaduwa	56.66	43.34
Aluthpola -Nilpanagoda	72.47	27.53
Aluthpola - 87 Kosgolla	49.5	50.5
Aluthpola - Miriswellalanda	50.61	49.39
Aluthpola - Mahawatta	61.53	38.47
Aluthpola - Delgodalla	53.68	46.32
Aluthpola - 112	50.53	49.47
Marukwathura	82.08	17.92
Tholangamuwa	71.42	28.58
Nangalla	73.91	26.09
Kundasale	61.34	38.66
FRI (Gannoruwa)	78.56	21.44
SCPPC(Gannoruwa)	68.14	31.86
Coconut Seedling Nursery	71.65	28.35
Vipulananelapuram	76.92	23.08
Thanamunei	73.33	26.67
Meerakermey	69.83	30.17
Erawur	67.39	32.61
Pasikuda	48.33	51.67
Bopitiya	60.26	39.74

Observed a perfect negative correlation (-1.000) between the percentage of parasitism and the percentage of whitefly after the field release of parasitoids (Fig. 8). The correlation is highly significant. It revealed that when there is an increase in parasitism, there is a corresponding tendency for a decrease in the percentage of whitefly. It is due to the fact

that, the adult stage of *E. guadeloupae* damages the larval stage of the whitefly. Here, the parasite sucks the essence of the whitefly, which is mainly in the second larval stage and lays its eggs on it. As a result, the parasite population grows, and the whitefly population decreases over time.

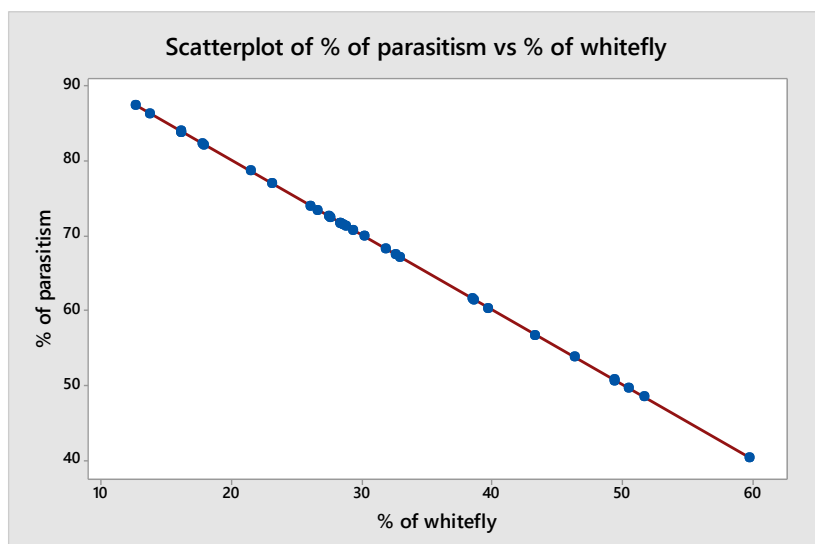


Fig 8: Relationship between whitefly (*A. rugioperculatus*) and parasitoid (*E. guadeloupae*) populations in coconut cultivations after field release of parasitoids

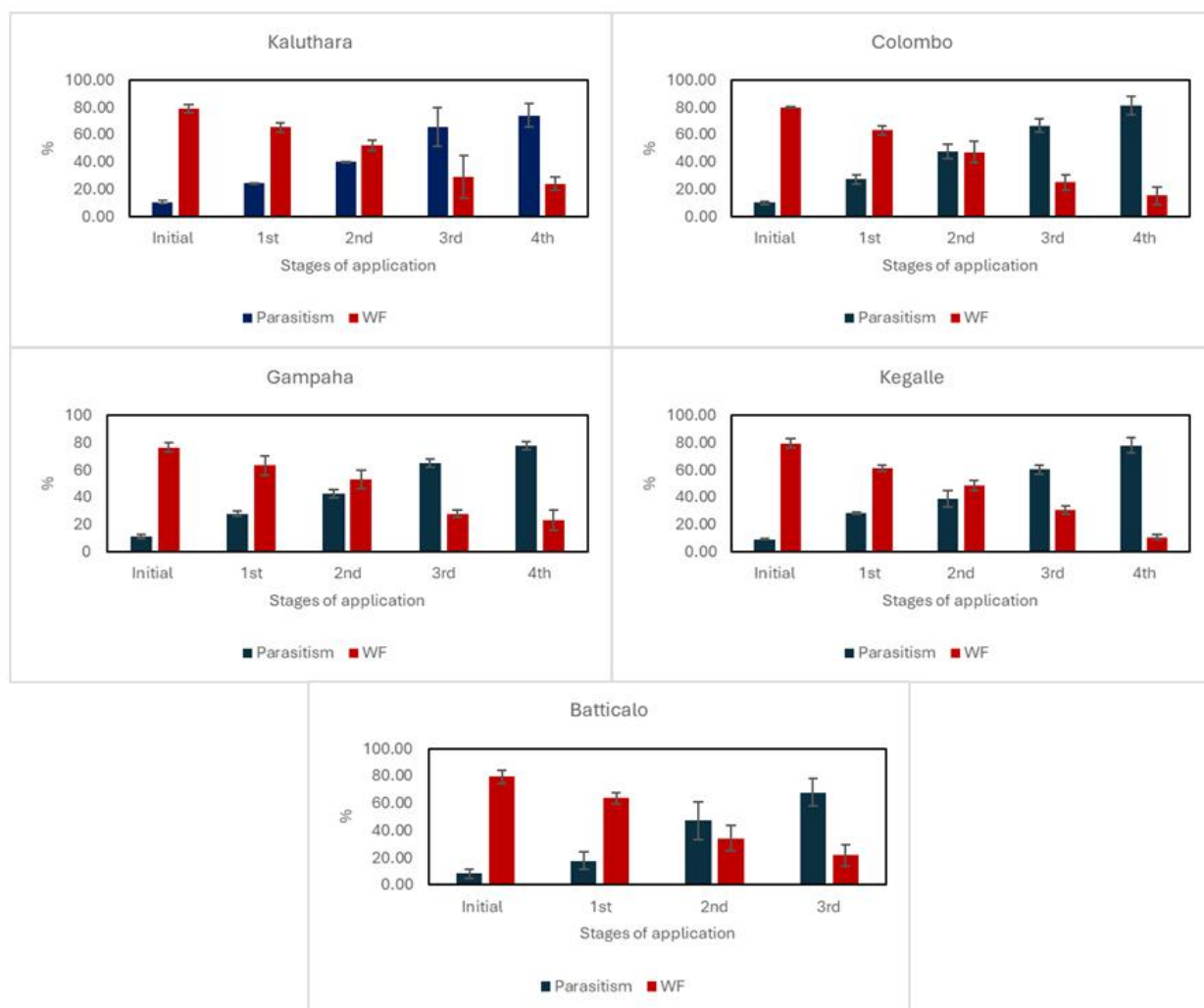


Fig 9: Average percentage of whitefly (*A. rugioperculatus*) and parasitoid (*E. guadeloupae*) population in Coconut cultivations in selected districts at different stages of release of parasitoids

The following graphs depict the percentage of population density of whitefly and its parasitoid throughout different stages of field release of parasitoids (Fig.9). It is noteworthy that a consistent trend is observed in each district, where the whitefly population undergoes a gradual reduction concurrent with an increase in parasitoid population.

In Kaluthara district, the initial whitefly and parasitoid populations were recorded at 79% and 10%, respectively.

After the introduction of parasitoids, whitefly population exhibited a decline to 24% by the end of the 4th release stage, while the parasitoid population increased to 74%. This trend of whitefly population reduction with an increase in parasitoid population can be observed in all other districts. Ultimately, whitefly populations decreased to 24%, 15%, 23%, 11%, 22% in Kaluthara, Colombo, Gampaha, Kegalle, and Batticaloa districts, respectively.

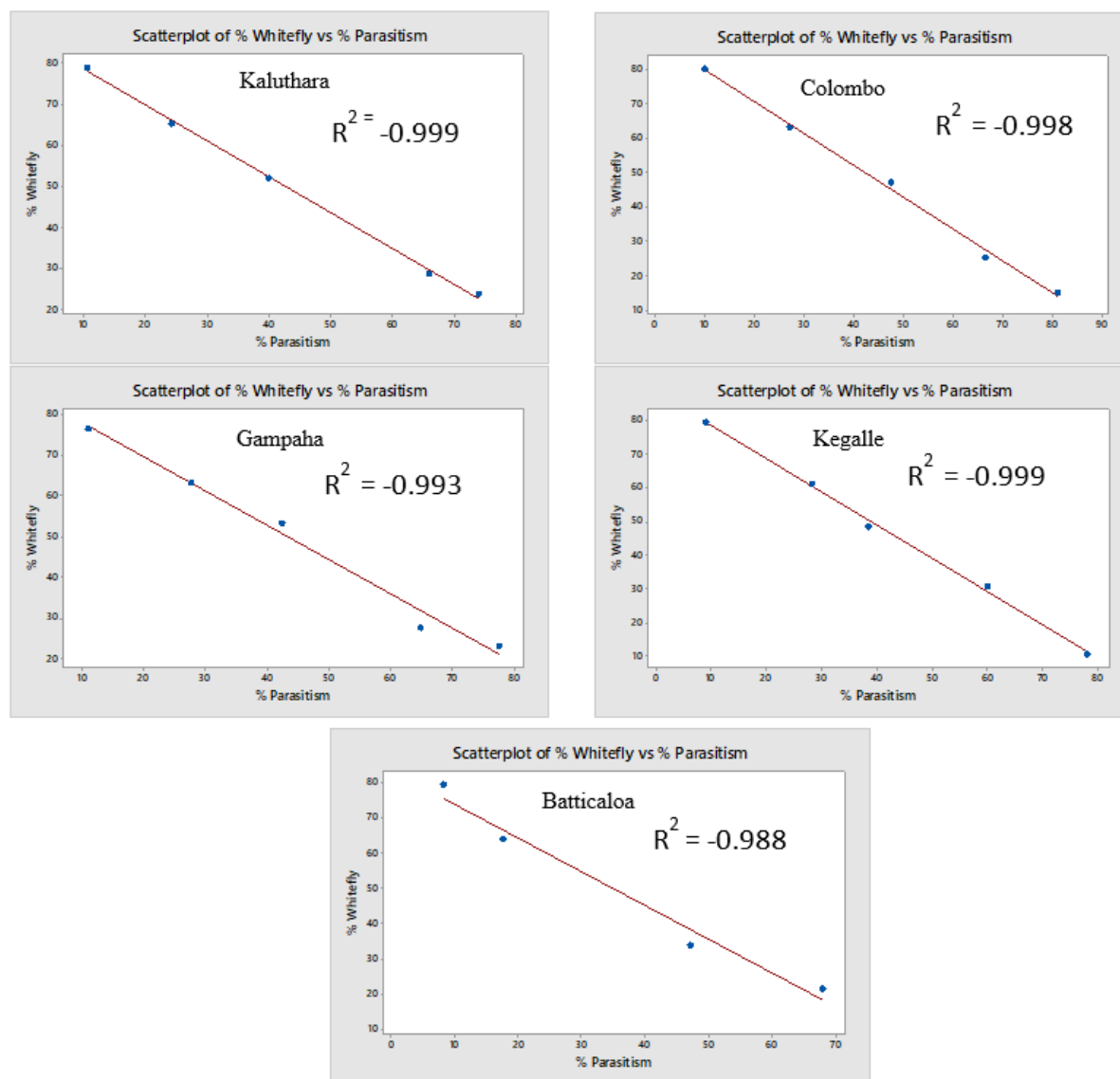


Fig 10: Correlation between population of whitefly and parasitoids in selected locations at different stages of release of parasitoids

There is a perfect negative correlation between the percentage of parasitism and the percentage of whitefly after the field release of parasitoids in each location revealed that when there is an increase in parasitism (Fig. 10), there is a corresponding tendency for a decrease in the percentage of whitefly. These findings indicate possibilities

of successful control of the *A. rugioperculatus* populations through the application of *E. guadeloupae*.

IV. CONCLUSION

The parasitic wasp, *E. guadeloupae*, identified from the natural environment, can be successfully mass reared in the laboratory and release into invaded areas to effectively

control coconut whitefly infestations. Both adult parasitoids of *E. guadeloupae* and parasitized pupae-cards can be used for field release to control the whitefly populations. This control strategy involves releasing the parasitoids five times at two-week intervals. Therefore, this parasitoid has proven to be effective in controlling existing whitefly damage in coconut cultivations in Sri Lanka.

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Estimation of Economic Heterosis for Grain Yield and its Attributing Traits in Macaroni Wheat (*Triticum durum* Desf.)

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Received: 27 Jul 2025; Received in revised form: 29 Aug 2025; Accepted: 01 Sep 2025; Available online: 07 Sep 2025

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Abstract— The present investigation was undertaken in order to estimate the heterosis for grain yield and its attributing traits in Macaroni wheat (*Triticum durum* Desf.). The crosses were attempted by using line × tester mating design among eight lines and four testers during rabi 2023-24. The resultant 32 hybrids together with 12 parents and 1 standard check (GW 1339) were tested using randomized block design with three replications at Wheat Research Station, Junagadh Agricultural University, Junagadh during Rabi 2024-25. A total of six and five hybrids exhibited significant desirable heterobeltiosis and standard heterosis, respectively for grain yield per plant. The heterobeltiosis for grain yield per plant ranged from -43.43 % to 83.99 %, while standard heterosis ranged from -58.69 % to 42.36 %. The highest heterosis over better parent in desirable direction was recorded by cross MACS 3949 × GDW 1255 (83.99%), UAS 475 × GDW 1255 (69.02%) and HD 4758 × HI 8737 (61.26%). The highest significant heterosis towards positive direction over standard check were recorded by five crosses viz., MACS 3949 × GDW 1255 (42.36%), HD 4758 × WHD 965 (28.66%), HD 4758 × HI 8737 (27.40%), UAS 475 × GDW 1255 (19.36%) and MACS 3949 × WHD 965 (9.45%). Hybrids with desirable traits for yield components showed increased grain yield, which is likely due to the combined effect of those improved traits.

Keywords— Heterosis, *Triticum durum* Desf.



I. INTRODUCTION

Wheat is a most extensively grown food crop in the world. Wheat is prized for its high nutritious content. Approximately 32% of all cereal growing land worldwide is planted with wheat, which is cultivated throughout a variety of latitudes. India's major wheat-growing states

include Uttar Pradesh, Madhya Pradesh, Punjab, Haryana, Rajasthan, Maharashtra and Gujarat.

In India, farmers cultivated 31.83 million hectares of land, yielding a total of 113.29 million tonnes, which breaks down to an average productivity of 3559 kilograms per hectare. While Gujarat accounted for 1.24 million

hectares of land, 3.77 million tonnes of production and 3027 kg of productivity per hectare [2]. In India there are six mega wheat-growing environments *i.e.* North-Western Plains Zone (NWPZ), North-Eastern Plains Zone (NEPZ), Central Zone (CZ), Peninsular Zone (PZ), Northern Hills Zone (NHZ) and Southern Hills Zone (SHZ) [1].

The durum wheat is higher in protein, β -carotene and vital micronutrients like iron and zinc, so it offers greater nutrition [14]. Durum wheat contains high level of folate. which is much important during pregnancy time. A single cup of uncooked enriched durum wheat semolina is a great source of folate, providing you with 306 micrograms of this important nutrient. Durum wheat pasta, unlike regular wheat pasta, doesn't spike your blood sugar as much. Durum wheat pasta has a lower glycemic index (47), which means it's digested more slowly and won't cause your blood sugar to spike as quickly as common wheat pasta (68). Additionally, durum wheat contains about twice as much lutein, a beneficial antioxidant that's great for eye health. These are great for your health, particularly for your eyes.

The choice of parents to be incorporated in hybridization programme is a crucial step for breeders, particularly if the aim is improvement of complex quantitative characters, such as grain yield and its components. The use of parents of known superior genetic worth ensures much better success. We need to thoroughly analyze the genes of both current plant varieties and new promising ones so that we can use them to develop better crops or release them directly as new cultivars. Nature and magnitude of heterosis is one of the important aspects for selection of right parents for crosses and also help in identification of superior cross combinations that produce desirable transgressive segregants in advanced generations.

II. MATERIALS AND METHODS

The field experiment was conducted at Wheat Research Station, Junagadh Agricultural University, Junagadh during *Rabi*, 2023-24 and 2024-25. This region has a typical sub-tropical climate. The soil of the experimental site was medium black, alluvial in origin and poor in organic matter. The experimental material of present study was comprised of 32 elite hybrids developed by crossing eight lines and four testers in line $\times$ teste mating design along with one standard check (GW 1339). DDW 48, GW 1348, HD 4758, HI 8841, MACS 3949, MPO 1357, RAJ 3307 and UAS 475 used as lines and GDW 1255, HI 8737, WHD 965 and NIDW 1158 used as testers. The genotypes were obtained from the Junagadh Agricultural University's Wheat Research Station in Junagadh.

The crossing programme was carried out during *Rabi*, 2023-24 at Wheat Research Station, Junagadh

Agricultural University, Junagadh. At the same time, the male and female parents were selfed to get pure seeds of parents for the experiment. The experimental material consisting of 45 entries, including 12 parents, 32 crosses and one standard check (GW 1339) were tested in randomized block design with three replications during *Rabi*, 2024-25. A single row plot of 2.5 m was allotted randomly to each entry. The space between plants was maintained at 10 cm and 22.5 cm from row to row.

Five competitive plants per genotype in each replication in each environment were selected randomly for recording observations on plant height, number of effective tillers per plant, length of main spike, number of spikelets per main spike, number of grains per main spike, 100-grain weight, grain yield per plant, biological yield per plant and harvest index (except days to anthesis, grain filling period and days to maturity) and their average values were used in the statistical analysis.

III. RESULTS AND DISCUSSION

Analysis of variance

The genotypes in our experiment showed significant differences across all traits, confirming that we have enough genetic variation to study. The genotype variance was further subdivided into parent, hybrid, and parent vs. hybrid variance. For every character under study, it was also determined that the differences between the hybrids and parents were highly significant. Mean squares due to parents vs hybrids were found highly significant for all the characters except for plant height and number of spikelets per main spike were found significant and 100-grain weight was found non-significant. These differences in parents and hybrids were found significant due to recombination of genes derived from diverse parents leading to generation of an array of variability for different traits. This suggested the existence of overall heterosis and the chance of significant differences between the parents and crosses with respect to these characters. By seeing possibility of heterosis among these selected genotypes further analysis was needed. Similar observations were also reported Dedaniya *et al.* (2018) [3], Joshi and Kumar (2020) [8], Kumar *et al.* (2021) [9] and Dudhat *et al.* (2022) [4] in wheat.

Heterosis

The percentage increase or reduction in F1 over the better parent (heterobeltiosis) and over the standard check (standard heterosis) for twelve characters was used to measure heterosis. Standard heterosis is more useful than the measure of heterosis over superior parents. character-wise results on heterosis over better parent (heterobeltiosis)

and over standard check, GW 1339 (standard heterosis) were presented in Table 2 to Table 5 and described as under.

Table 1 Analysis of variance (mean squares) for grain yield and its attributing traits in durum wheat

Source of variation	d. f.	Days to anthesis	Grain filling period	Days to maturity	Plant height	Number of effective tillers per plant	Length of main spike	Number of spikelets per main spike	Number of grains per main spike	100-grain weight	Grain yield per plant	Biological yield per plant	Harvest index
Replications	2	5.14**	0.29	6.19**	1.49	0.06	0.14	1.43	5.91	0.45*	0.03	0.68	2.11
Genotypes	43	36.21*	41.39**	17.06*	51.89**	7.05**	1.26*	6.94**	36.42*	0.55*	42.99**	670.89*	306.74**
Parents (P)	11	55.12*	60.64**	36.03*	95.78**	1.23**	1.01*	3.13**	13.75*	0.80*	14.86**	219.48*	333.06**
Hybrids (H)	31	26.20*	33.31**	10.62*	35.92**	8.49**	1.28*	8.33**	42.19*	0.48*	53.03**	781.87*	301.50**
P vs. H	1	138.33**	80.18**	7.88**	64.57*	26.14*	3.16*	5.89*	106.92**	0.01	41.09**	2195.73**	179.72**
Error	86	0.61	0.36	0.55	15.18	0.18	0.23	0.91	4.72	0.14	1.25	7.92	9.65

*, ** Significant at 5% and 1% levels, respectively

Table 2 Per cent heterobeltiosis (HB) and standard heterosis (SH) for days to anthesis, grain filling period and days to maturity in durum wheat

Sr. No.	Crosses	Days to anthesis		Grain filling period		Days to maturity	
		HB (%)	SH (%)	HB (%)	SH (%)	HB (%)	SH (%)
1	DDW 48 × GDW 1255	0.49	10.81**	-18.63**	-15.31**	-2.70**	1.77**
2	DDW 48 × HI 8737	-4.52**	14.05**	-10.78**	-7.14**	1.68**	6.71**
3	DDW 48 × WHD 965	4.64**	9.73**	-13.73**	-10.20**	-1.69**	2.83**
4	DDW 48 × NIDW 1158	9.79**	15.14**	-27.93**	-18.37**	-1.01	3.53**
5	GW 1348 × GDW 1255	-11.76**	-2.70**	28.57**	19.39**	4.95**	4.95**
6	GW 1348 × HI 8737	-5.43**	12.97**	-9.89**	-16.33**	-2.02**	2.83**
7	GW 1348 × WHD 965	-1.60	-0.54	11.34**	10.20**	2.82**	3.18**
8	GW 1348 × NIDW 1158	7.57**	7.57**	-14.41**	-3.06*	0.00	3.89**
9	HD 4758 × GDW 1255	3.92**	14.59**	-23.97**	-6.12**	-1.62**	7.42**
10	HD 4758 × HI 8737	-0.90	18.38**	-34.71**	-19.39**	-3.56**	5.30**
11	HD 4758 × WHD 965	4.26**	5.95**	-16.53**	3.06*	-3.88**	4.95**
12	HD 4758 × NIDW 1158	12.23**	14.05**	-33.88**	-18.37**	-5.83**	2.83**
13	HI 8841 × GDW 1255	4.88**	16.22**	-7.50**	-24.49**	1.40*	2.12**
14	HI 8841 × HI 8737	-4.07**	14.59**	-2.50	-20.41**	-2.36**	2.47**
15	HI 8841 × WHD 965	0.98	11.89**	-9.28**	-10.20**	3.51**	4.24**
16	HI 8841 × NIDW 1158	0.49	11.35**	-23.42**	-13.27**	-1.02	2.83**
17	MACS 3949 × GDW 1255	-0.48	12.97**	-8.79**	-15.31**	-2.99**	3.18**

18	MACS 3949 × HI 8737	-4.98**	13.51**	-13.19**	-19.39**	-3.99**	2.12**
19	MACS 3949 × WHD 965	-5.24**	7.57**	-6.19**	-7.14**	-3.65**	2.47**
20	MACS 3949 × NIDW 1158	-10.00**	2.16**	-16.22**	-5.10**	-6.31**	-0.35
21	MPO 1357 × GDW 1255	-3.70**	12.43**	2.25	-7.14**	-1.97**	5.65**
22	MPO 1357 × HI 8737	-6.33**	11.89**	10.11**	0.00	0.00	7.77**
23	MPO 1357 × WHD 965	0.46	17.30**	-27.84**	-28.57**	-5.90**	1.41*
24	MPO 1357 × NIDW 1158	-3.24**	12.97**	-26.13**	-16.33**	-4.59**	2.83**
25	RAJ 3307 × GDW 1255	-3.92**	5.95**	-1.09	-7.14**	1.41*	1.41*
26	RAJ 3307 × HI 8737	-8.14**	9.73**	0.00	-6.12**	-0.67	4.24**
27	RAJ 3307 × WHD 965	11.11**	13.51**	-15.46**	-16.33**	2.82**	3.18**
28	RAJ 3307 × NIDW 1158	5.82**	8.11**	-16.22**	-5.10**	-0.34	3.53**
29	UAS 475 × GDW 1255	0.00	10.27**	-9.52**	-3.06*	0.34	5.65**
30	UAS 475 × HI 8737	-5.88**	12.43**	-9.52**	-3.06*	1.68**	7.07**
31	UAS 475 × WHD 965	6.22**	10.81**	-6.67**	0.00	1.68**	7.07**
32	UAS 475 × NIDW 1158	2.59**	7.03**	-8.11**	4.08**	0.67	6.01**
S.Em ±		0.64	0.64	0.48	0.48	0.60	0.60
Range of heterosis		-11.76	-2.70	-34.71	-28.57	-6.31	-0.35
		to 12.23	to 18.38	to 28.57	to 19.39	to 4.95	to 7.77
No. of crosses with significant and desirable heterosis (negative)		13	1	25	26	15	0

*, ** Significant at 5% and 1% levels, respectively

Table 3 Per cent heterobeltiosis (HB) and standard heterosis (SH) for plant height, number of effective tillers per plant and length of main spike in durum

Sr. No.	Crosses	Plant height		Number of effective tillers per plant		Length of main spike	
		HB (%)	SH (%)	HB (%)	SH (%)	HB (%)	SH (%)
1	DDW 48 × GDW 1255	4.79	-4.68	-8.26*	-5.66	5.30	-13.23**
2	DDW 48 × HI 8737	-7.47*	-3.00	34.38**	21.70**	-6.19	-9.12*
3	DDW 48 × WHD 965	-1.22	-4.47	-7.34	-4.72	-0.60	-14.60**
4	DDW 48 × NIDW 1158	4.70	-3.22	10.31*	0.94	1.49	-6.55
5	GW 1348 × GDW 1255	15.67**	5.21	-18.02**	-14.15**	12.49*	-12.03**
6	GW 1348 × HI 8737	-4.60	0.01	-24.32**	-20.75**	-7.25	-10.15*
7	GW 1348 × WHD 965	-0.23	-3.52	18.02**	23.58**	11.86*	-3.90
8	GW 1348 × NIDW 1158	7.80*	-0.36	-12.61**	-8.49*	-5.12	-12.63**
9	HD 4758 × GDW 1255	14.17**	3.85	-25.69**	-23.58**	0.51	-14.95**
10	HD 4758 × HI 8737	-9.31**	-4.93	72.22**	46.23**	-10.34*	-13.15**
11	HD 4758 × WHD 965	4.54	1.10	16.51**	19.81**	3.29	-11.26*
12	HD 4758 × NIDW 1158	6.12	-1.91	46.39**	33.96**	-12.65*	-19.57**

13	HI 8841 × GDW 1255	-10.01**	-2.85	-48.62**	-47.17**	-4.70	-6.21
14	HI 8841 × HI 8737	-0.33	7.59*	35.63**	11.32*	14.27**	12.46**
15	HI 8841 × WHD 965	-8.18*	-0.88	4.59	7.55	-8.31	-9.76*
16	HI 8841 × NIDW 1158	-13.28**	-6.39	-2.06	-10.38*	1.22	-0.39
17	MACS 3949 × GDW 1255	6.32	3.16	44.95**	49.06**	26.05**	12.72**
18	MACS 3949 × HI 8737	-11.85**	-7.59*	28.41**	6.60	-9.02*	-11.86*
19	MACS 3949 × WHD 965	-0.36	-3.32	22.94**	26.42**	12.45*	0.56
20	MACS 3949 × NIDW 1158	-0.57	-3.53	12.37*	2.83	3.35	-4.84
21	MPO 1357 × GDW 1255	-2.53	5.39	-26.61**	-24.53**	10.30	-12.89**
22	MPO 1357 × HI 8737	-12.90**	-5.83	2.11	-8.49*	-9.55*	-12.38**
23	MPO 1357 × WHD 965	-6.57*	1.01	12.84**	16.04**	7.88	-7.32
24	MPO 1357 × NIDW 1158	-8.61*	-1.19	-14.43**	-21.70**	-12.47*	-19.40**
25	RAJ 3307 × GDW 1255	5.99	3.03	21.10**	24.53**	-6.09	-15.46**
26	RAJ 3307 × HI 8737	-16.46**	-12.43**	22.47**	2.83	0.71	-2.44
27	RAJ 3307 × WHD 965	2.83	-0.05	3.67	6.60	2.95	-7.32
28	RAJ 3307 × NIDW 1158	-5.13	-7.79*	-36.08**	-41.51**	-6.88	-14.26**
29	UAS 475 × GDW 1255	6.86	-2.80	44.95**	49.06**	8.26	-13.58**
30	UAS 475 × HI 8737	-5.51	-0.95	11.46*	0.94	-12.38**	-15.12**
31	UAS 475 × WHD 965	-5.42	-8.54*	13.76**	16.98**	9.77*	-5.70
32	UAS 475 × NIDW 1158	9.12*	0.87	14.43**	4.72	-24.84**	-30.79**
SE ±		3.14	3.14	0.34	0.34	0.39	0.39
Range of heterosis		-16.46 to 15.67	-12.43 To 7.59	48.62 to 72.22	-47.17 to 49.06	-28.84 to 26.05	-30.79 to 12.72
No. of crosses with significant and desirable heterosis (positive)		10	4	18	12	5	2

*, ** Significant at 5% and 1% levels, respectively

Table 4 Per cent heterobeltiosis (HB) and standard heterosis (SH) for number of spikelets per main spike, number of grains per main spike and 100-grain weight in durum wheat

Sr. No.	Crosses	Number of spikelets per main spike		Number of grains per main spike		100-grain weight	
		HB (%)	SH (%)	HB (%)	SH (%)	HB (%)	SH (%)
1	DDW 48 × GDW 1255	-9.29*	-10.87*	-4.11	-10.78*	3.49	-17.42**
2	DDW 48 × HI 8737	3.10	1.30	13.33**	0.52	-2.20	-19.48**
3	DDW 48 × WHD 965	-14.16**	-15.65**	-1.32	-9.04*	-7.47	-11.62*
4	DDW 48 × NIDW 1158	-10.18*	-11.74*	-8.83*	-12.00**	-12.53**	-7.92
5	GW 1348 × GDW 1255	-8.80	-14.35**	-7.48	-13.91**	-13.07*	-21.54**
6	GW 1348 × HI 8737	-1.46	-11.74*	-0.78	-12.00**	-14.48*	-22.81**
7	GW 1348 × WHD 965	9.35*	1.74	1.51	-6.43	9.44	4.54

8	GW 1348 × NIDW 1158	-16.89**	-18.70**	-15.32**	-18.26**	-22.13**	-18.03**
9	HD 4758 × GDW 1255	0.93	-5.22	1.31	-5.74	10.39	-11.92*
10	HD 4758 × HI 8737	15.53**	3.48	15.88**	2.78	15.14*	-5.20
11	HD 4758 × WHD 965	4.67	-2.61	5.47	-2.78	-7.73	-11.86*
12	HD 4758 × NIDW 1158	-15.56**	-17.39**	-14.05**	-17.04**	-22.24**	-18.15**
13	HI 8841 × GDW 1255	-12.50*	-17.83**	-4.67	-11.30**	4.40	-16.70**
14	HI 8841 × HI 8737	49.03**	33.48**	50.00**	33.04**	10.21	-9.26*
15	HI 8841 × WHD 965	8.88	1.30	9.81*	1.22	-3.42	-7.74
16	HI 8841 × NIDW 1158	2.67	0.43	3.60	0.00	-23.10**	-19.06**
17	MACS 3949 × GDW 1255	12.96**	6.09	11.21*	3.48	10.28	-1.39
18	MACS 3949 × HI 8737	-3.40	-13.48**	0.39	-10.96**	-2.37	-12.70*
19	MACS 3949 × WHD 965	-1.40	-8.26	-0.57	-8.35*	-3.04	-7.38
20	MACS 3949 × NIDW 1158	0.44	-1.74	1.44	-2.09	-22.93**	-18.87**
21	MPO 1357 × GDW 1255	-15.74**	-20.87**	-11.96**	-18.09**	-11.55*	-12.89*
22	MPO 1357 × HI 8737	-7.51	-14.35**	-5.58	-14.61**	-11.43*	-12.76*
23	MPO 1357 × WHD 965	0.47	-6.52	1.32	-6.61	-8.85	-10.22*
24	MPO 1357 × NIDW 1158	-23.56**	-25.22**	-17.30**	-20.17**	-35.40**	-32.00**
25	RAJ 3307 × GDW 1255	-6.02	-11.74*	-3.74	-10.43*	-2.11	-18.57**
26	RAJ 3307 × HI 8737	-3.88	-13.91**	-2.55	-13.57**	-4.80	-20.81**
27	RAJ 3307 × WHD 965	-3.27	-10.00*	-2.64	-10.26*	-8.23	-12.34*
28	RAJ 3307 × NIDW 1158	-12.44**	-14.35**	-10.63*	-13.74**	-24.31**	-20.33**
29	UAS 475 × GDW 1255	-1.85	-7.83	-0.93	-7.83*	20.77**	-3.63
30	UAS 475 × HI 8737	-2.91	-13.04**	0.00	-11.30**	-7.27	-23.65**
31	UAS 475 × WHD 965	3.74	-3.48	2.26	-5.74	-7.16	-11.31*
32	UAS 475 × NIDW 1158	-15.56**	-17.39**	-13.69**	-16.70**	-19.43**	-15.18**
SE ±		0.77	0.77	1.76	1.76	0.30	0.30
Range of heterosis		-23.56 to 49.03	-25.22 To 33.48	-17.30 to 50.00	-20.17 to 33.04	-35.40 to 20.77	-32.00 to 4.54
No. of crosses with significant and desirable heterosis (positive)		4	1	5	1	2	0

*, ** Significant at 5% and 1% levels, respectively

Table 5 Per cent heterobeltiosis (HB) and standard heterosis (SH) for biological yield per plant and harvest index in durum wheat

Sr. No.	Crosses	Grain yield per plant		Biological yield per plant		Harvest index	
		HB (%)	SH (%)	HB (%)	SH (%)	HB (%)	SH (%)
1	DDW 48 × GDW 1255	-25.36**	-36.50**	-36.12**	-40.21**	-23.34**	6.56

2	DDW 48 × HI 8737	8.01	-8.11	41.79**	32.73**	-43.15**	-30.65**
3	DDW 48 × WHD 965	-14.23**	-9.09*	3.04	-3.55	-49.29**	-5.34
4	DDW 48 × NIDW 1158	-28.23**	-34.52**	3.17	-3.43	-51.47**	-32.34**
5	GW 1348 × GDW 1255	0.91	-23.92**	29.40**	-37.93**	-28.11**	22.47**
6	GW 1348 × HI 8737	-25.59**	-41.22**	105.68**	33.19**	-51.40**	-17.21*
7	GW 1348 × WHD 965	-3.78	1.99	66.64**	-5.49	-42.12**	8.04
8	GW 1348 × NIDW 1158	-19.00**	-26.10**	-34.71**	-57.34**	1.53	72.96**
9	HD 4758 × GDW 1255	-12.23	-41.52**	-34.03**	-50.33**	-15.19**	17.89*
10	HD 4758 × HI 8737	61.26**	27.40**	76.57**	32.94**	-20.92**	-3.53
11	HD 4758 × WHD 965	21.38**	28.66**	41.49**	6.53	-35.21**	20.93**
12	HD 4758 × NIDW 1158	-0.90	-9.58*	-16.69**	-37.27**	3.34	44.09**
13	HI 8841 × GDW 1255	-42.31**	-58.69**	-33.57**	-52.93**	-36.72**	-12.04
14	HI 8841 × HI 8737	29.58**	2.37	34.13**	-4.97	-11.16	8.37
15	HI 8841 × WHD 965	1.98	8.09	0.12	-29.06**	-18.04**	52.97**
16	HI 8841 × NIDW 1158	2.78	-6.22	-10.05*	-36.27**	5.41	46.97**
17	MACS 3949 × GDW 1255	83.99**	42.36**	128.33**	9.52**	-24.16**	30.46**
18	MACS 3949 × HI 8737	-18.05**	-35.26**	75.93**	13.92**	-51.40**	-16.41*
19	MACS 3949 × WHD 965	3.26	9.45*	70.99**	-3.03	-39.58**	12.77
20	MACS 3949 × NIDW 1158	2.72	-6.28	-5.49	-38.24**	-11.84**	51.64**
21	MPO 1357 × GDW 1255	-36.32**	-41.83**	19.66**	-4.30	-47.60**	-27.15**
22	MPO 1357 × HI 8737	-9.99*	-17.77**	-37.50**	-50.01**	34.89**	64.54**
23	MPO 1357 × WHD 965	-12.48**	-7.24	-16.17**	-32.95**	-25.97**	38.18**
24	MPO 1357 × NIDW 1158	-43.43**	-48.32**	-31.10**	-44.89**	-32.76**	-6.25
25	RAJ 3307 × GDW 1255	24.64**	-16.95**	16.53**	-16.66**	-28.04**	0.03
26	RAJ 3307 × HI 8737	-9.62	-28.60**	-32.63**	-51.82**	21.57**	48.30**
27	RAJ 3307 × WHD 965	-19.63**	-14.81**	-11.55*	-36.74**	-27.86**	34.65**
28	RAJ 3307 × NIDW 1158	-36.18**	-41.77**	-49.59**	-63.95**	15.85**	61.54**
29	UAS 475 × GDW 1255	69.02**	19.36**	80.46**	15.37**	-25.59**	3.44
30	UAS 475 × HI 8737	-2.90	-23.29**	28.80**	-16.60**	-24.31**	-7.67
31	UAS 475 × WHD 965	-6.34	-0.73	4.26	-33.35**	8.12*	101.80**
32	UAS 475 × NIDW 1158	-9.12	-17.09**	41.99**	-7.22*	-35.94**	-10.68
SE ±		0.90	0.90	0.90	2.29	2.50	2.50
Range of heterosis		-43.43 to 83.99	-58.69 to 42.36	-49.59 to 128.33	-63.95 to 33.19	-51.47 to 34.89	-32.34 to 101.80
No. of crosses with significant and desirable heterosis (positive)		6	5	15	6	4	15

*, ** Significant at 5% and 1% levels, respectively



For wheat days to anthesis, the heterotic effect in a negative direction was preferable. Heterobeltiosis ranged from -11.76 per cent (GW 1348 × GDW 1255) to 12.23 per cent (HD 4758 × NIDW 1158) for days to anthesis. Thus, the earliest hybrids were GW 1348 × GDW 1255 (-11.76 %) followed by MACS 3949 × NIDW 1158 (-10.00 %) and RAJ 3307 × HI 8737 (-8.14 %). Out of 32 hybrids, 13 hybrids manifested significant and desirable (negative) estimate of heterobeltiosis. The range of standard heterosis was varied from -2.70 per cent (GW 1348 × GDW 1255) to 18.38 per cent (HD 4758 × HI 8737). The results were in accordance with the finding of Singh *et al.* (2012) [13] and Singh *et al.* (2013) [11].

For grain filling period ranged from -34.71 per cent (HD 4758 × HI 8737) to 28.57 per cent (GW 1348 × GDW 1255) and top cross combinations viz., HD 4758 × HI 8737 (-34.71%), HD 4758 × NIDW 1158 (-33.88%) and DDW 48 × NIDW 1158 (-26.13%), which showed significant and negative heterotic effect for grain filling period. Out of 32 hybrids, 25 hybrids manifested significant and desirable (negative) estimate of heterobeltiosis. Heterosis over standard check ranged from -28.57 per cent (MPO 1357 × WHD 965) to 19.39 per cent (GW 1348 × GDW 1255). The most preferable standard heterosis over standard check was shown by the cross MPO 1357 × WHD 965 (-28.57%), which was followed by HI 8841 × GDW 1255 (-24.49%) and HI 8841 × HI 8737 (-20.41%). Out of 32 hybrids, 26 hybrids showed significant and desirable (negative) heterosis over standard check. The results were in accordance with the finding of Dedaniya *et al.* (2018) [3] and Dudhat *et al.* (2022) [4].

Negative heterosis for days to maturity is believed to be beneficial for wheat crop earliness. The range of heterobeltiosis varied from -6.31 per cent (MACS 3949 × NIDW 1158) to 4.95 per cent (GW 1348 × GDW 1255). The earliest hybrid was MACS 3949 × NIDW 1158 (-6.31%) followed by MPO 1357 × WHD 965 (-5.90%) and HD 4758 × NIDW 1158 (-5.83%). Fifteen of the thirty-two hybrids had negative heterosis over the superior parent. The range of standard heterosis varied from -0.35 per cent (MACS 3949 × NIDW 1158) to 7.77 per cent (MPO 1357 × HI 8737). Out of 32 hybrids, none of the hybrid exhibited significant and negative heterosis over standard check (Table 4). The similar findings were observed Reddy *et al.* (2023) [11] and Puri *et al.* (2025) [10].

The heterotic effect in negative direction is desirable for plant height in wheat. Heterobeltiosis ranged

from -16.46 per cent (RAJ 3307 × HI 8737) to 15.67 per cent (GW 1348 × GDW 1255) for plant height. Highest desirable heterobeltiosis was recorded by the cross RAJ 3307 × HI 8737 (-16.46%) followed by HI 8841 × NIDW 1158 (-13.28%) and MPO 1357 × HI 8737 (-12.90%). Out of 32 hybrids, 10 hybrids shown significant and desirable (negative) heterosis over better parent for this trait. Heterosis over standard check ranged from -12.43 per cent (RAJ 3307 × HI 8737) to 7.59 per cent (HI 8841 × HI 8737). The cross RAJ 3307 × HI 8737 (-12.43%) exhibited the highest desirable standard heterosis over standard check followed by UAS 475 × WHD 965 (-8.54%), RAJ 3307 × NIDW 1158 (-7.79%) and MACS 3949 × HI 8737 (7.59%). Out of 32 hybrids, four hybrids showed significant and desirable (negative) heterosis over standard check. The results were in confirmation with the findings of Fouad *et al.* (2023) [6] and Reddy *et al.* (2023) [11].

The minimum and maximum values for heterobeltiosis recorded were -48.62 per cent (HI 8841 × GDW 1255) and 72.22 per cent (HD 4758 × HI 8737) for number of effective tillers per plant. The highest significant positive heterosis over better parent was recorded by the hybrid HD 4758 × HI 8737 (72.22 %) followed by HD 4758 × NIDW 1158 (46.39 %) and UAS 475 × GDW 1255 (44.95%). Out of 32 hybrids, 18 hybrids showed significant and positive heterosis over better parent. The magnitude of standard heterosis ranged from -47.17 per cent (HI 8841 × GDW 1255) to 49.06 per cent (MACS 3949 × GDW 1255 and UAS 475 × GDW 1255) for number of tillers per plant and total 12 crosses were showing positive and significant effect. Top three crosses were MACS 3949 × GDW 1255 (49.06%), UAS 475 × GDW 1255 (49.06%) and HD 4758 × HI 8737 (46.23%). The results were in similarity with the findings of Dudhat *et al.* (2022) [4] and Puri *et al.* (2025) [10].

The range of heterosis over better parent was recorded from -24.84 per cent (UAS 475 × NIDW 1158) to 26.05 per cent (MACS 3949 × GDW 1255) for length of main spike. The highest desirable heterosis was recorded by the hybrid MACS 3949 × GDW 1255 (26.05%) followed by HI 8841 × HI 8737 (14.27%) and MACS 3949 × WHD 965 (12.45%). Out of 32 hybrids, 5 hybrids showed significant and positive heterosis over better parent for length of main spike. Heterosis over standard check ranged from -30.79 per cent (UAS 475 × NIDW 1158) to 12.72 per cent (MACS 3949 × GDW 1255). The highest desirable heterosis were recorded in two hybrids MACS 3949 × GDW 1255 (12.72%) and HI 8841 × HI 8737 (12.46%). The results

were in accordance with the findings of Kumar *et al.* (202) [9], Dudhat *et al.* (2022) [3] and Fouad *et al.* (2023) [6].

For Number of spikelets per main spike heterobeltiosis ranged from -23.56 per cent (MPO 1357 $\times$ NIDW 1158) to 49.03 per cent (HI 8841 $\times$ HI 8737) for number of spikelets per main spike. Four hybrids *viz.*, HI 8841 $\times$ HI 8737 (49.03%), HD 4758 $\times$ HI 8737 (15.53%), MACS 3949 $\times$ GDW 1255 (12.96%) and GW 1348 $\times$ WHD 965 (9.35) exhibited significant and positive heterotic effect over better parent. The range of standard heterosis was -25.22 per cent (MPO 1357 $\times$ NIDW 1158) to 33.48 per cent (HI 8841 $\times$ HI 8737). Out of 32 hybrids, only HI 8841 $\times$ HI 8737 (33.48%) exhibited significant and positive heterosis over standard check. The results were in accordance with the findings of Joshi and Kumar (2020) [8], Dudhat *et al.* (2022) [4] and Fouad *et al.* (2023) [6].

Number of grains per main spike was one of the most important traits contributing to the grain yield and hence, their positive values are beneficial in wheat. The range of heterosis over better parent varied from -17.30 per cent (MPO 1357 $\times$ NIDW 1158) to 50.00 per cent (HI 8841 $\times$ HI 8737). The highest heterosis over better parent in desirable direction was recorded by crosses HI 8841 $\times$ HI 8737 (50.00%) followed by HD 4758 $\times$ HI 8737 (15.88%) and DDW 48 $\times$ HI 8737 (13.33%). The range of standard heterosis for number of grains per main spike varied from -20.17 per cent (MPO 1357 $\times$ NIDW 1158) to 33.04 per cent (HI 8841 $\times$ HI 8737). Out of 32 hybrids, only HI 8841 $\times$ HI 8737 (33.04%) exhibited significant and positive heterosis over standard check. These results were in agreement with the earlier studies carried out by Reddy *et al.* (2023) [11] and Puri *et al.* (2025) [10].

The heterobeltiosis for 100-grain weight ranged from -35.40 per cent (MPO 1357 $\times$ NIDW 1158) to 20.77 per cent (UAS 475 $\times$ GDW 1255). The highest heterosis over better parent in desirable direction was recorded by two crosses *viz.*, UAS 475 $\times$ GDW 1255 (20.77%) and HD 4758 $\times$ HI 8737 (15.14) for 100-grain weight. The range of standard heterosis for 100-grain weight varied from -32.00 per cent (MPO 1357 $\times$ NIDW 1158) to 4.54 per cent (GW 1348 $\times$ WHD 965). Out of 32 hybrids, none of the hybrid exhibited significant and positive heterosis over standard check for 100-grain weight. The results were in accordance with the findings of Kumar *et al.* (2021) [9] and Dudhat *et al.* (2022) [4].

The estimates of heterosis over better parent varied from -43.43 per cent (DDW 48 $\times$ NIDW 1158) to 83.99 per cent (MACS 3949 $\times$ GDW 1255) for grain yield per plant. The significantly highest heterosis over better parent in desirable direction was recorded by cross MACS 3949 $\times$ GDW 1255 (83.99%) followed by UAS 475 $\times$ GDW 1255

(69.02%) and HD 4758 $\times$ HI 8737 (61.26%). Out of 32 hybrids, 6 hybrids expressed significant positive heterosis over better parent for grain yield per plant. The economic heterosis for grain yield per plant ranged from -58.69 per cent (MPO 1357 $\times$ NIDW 1158) to 42.36 per cent (MACS 3949 $\times$ GDW 1255). The highest significant heterosis towards positive direction over standard check were recorded by five crosses *viz.*, MACS 3949 $\times$ GDW 1255 (42.36%) followed by HD 4758 $\times$ WHD 965 (28.66%), HD 4758 $\times$ HI 8737 (27.40%), UAS 475 $\times$ GDW 1255 (19.36%) and MACS 3949 $\times$ WHD 965 (9.45%). The results were in accordance with the findings of Joshi and Kumar (2020) [8], Kumar *et al.* (2021) [9], Dudhat *et al.* (2022) [4], Fouad *et al.* (2023) [6], Reddy *et al.* (2023) [11], Fareed *et al.* (2024) [5] and Puri *et al.* (2025) [10].

For biological yield per plant heterosis over better parent ranged from -49.59 per cent (RAJ 3307 $\times$ NIDW 1158) to 128.33 per cent (MACS 3949 $\times$ GDW 1255). The highest significant heterobeltiosis was recorded by the cross MACS 3949 $\times$ GDW 1255 (128.33%) followed by GW 1348 $\times$ HI 8737 (105.68%) and UAS 475 $\times$ GDW 1255 (80.46%). Out of 32 hybrids, 15 hybrids expressed significant and positive heterosis over better parent for biological yield per plant. The range of heterosis over standard check observed from -63.95 per cent (RAJ 3307 $\times$ NIDW 1158) to 33.19 per cent (GW 1348 $\times$ HI 8737). The cross GW 1348 $\times$ HI 8737 (33.19%) exhibited the highest significant heterosis over standard check followed by HD 4758 $\times$ HI 8737 (32.94%) and DDW 48 $\times$ HI 8737 (32.73%). For biological yield per plant six of the 32 hybrids showed significant and favourable heterosis over the standard check. The results were in accordance with the findings of Reddy *et al.* (2023) [11] and Puri *et al.* (2025) [10].

The estimates of heterobeltiosis for harvest index varied from -51.47 per cent (DDW 48 $\times$ NIDW 1158) to 34.89 per cent (MPO 1357 $\times$ HI 8737). The highest significant and desirable heterosis over better parent was recorded by the cross MPO 1357 $\times$ HI 8737 (34.89%) followed by RAJ 3307 $\times$ HI 8737 (21.57%), RAJ 3307 $\times$ NIDW 1158 (15.85%) and UAS 475 $\times$ WHD 965 (8.12%). Out of 32 hybrids, four hybrids demonstrated significant and positive heterosis over better parent for harvest index. The range of heterosis over standard check observed from -32.34 per cent (DDW 48 $\times$ NIDW 1158) to 101.80 per cent (UAS 475 $\times$ WHD 965). The cross UAS 475 $\times$ WHD 965 (101.80%) exhibited the highest significant heterosis over standard check followed by GW 1348 $\times$ NIDW 1158 (72.96%) and MPO 1357 $\times$ HI 8737 (64.54%). 15 of the hybrid plants showed a better performance than the standard check. The results were in accordance with the findings of Kumar *et al.* (2021) [9] and Dudhat *et al.* (2022) [4].

Comparative studies of standard heterotic crosses along with *per se* performance for grain yield corresponding to other attributes are presented in Table 4. It was revealed that high, significant and positive heterosis for grain yield per plant in these crosses were not accompanied by single unique trait. These crosses also exhibited significant and desirable heterosis for component traits.

For grain yield, the five best-performing crosses were MACS 3949 × GDW 1255, HD 4758 × WHD 965, HD 4758 × HI 8737, UAS 475 × GDW 1255, and MACS 3949 × WHD 965, all of which significantly outperformed both their parent lines and the standard varieties. These crosses also showed significant and desirable heterobeltiosis and standard heterosis for grain yield and attributing traits *viz.*, days to anthesis, grain filling period, days to maturity, plant height, number of effective tillers per plant length of main spike, number of spikelets per main spike, number of grains per main spike, 100-grain weight, grain yield per plant, biological yield per plant and harvest index.

IV. CONCLUSION

High heterotic hybrids had also shown high mean performance, so it revealed that the selection of hybrids either on the basis of *per se* performance or on the basis of magnitude of heterotic effects would be equally reliable. On the basis of *per se* performance, heterotic response involved in the inheritance of grain yield and its attributing traits, the three crosses *viz.*, MACS 3949 × GDW 1255, HD 4758 × WHD 965 and HD 4758 × HI 8737 appeared to be the most superior cross combinations. These hybrids recorded 42.36, 28.66 and 27.40 per cent higher yield over standard check (GW 1339), respectively. Therefore, these crosses could be exploited for heterosis breeding programme to boost the grain yield in durum wheat.

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Estimation Combining Ability for Grain Yield and its attributing Traits in Macaroni Wheat (*Triticum durum* Desf.)

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Received: 29 Jul 2025; Received in revised form: 30 Aug 2025; Accepted: 02 Sep 2025; Available online: 08 Sep 2025

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Abstract— The goal of the current study to estimate the combining ability and gene effects for grain yield and its attributing traits in Macaroni wheat (*Triticum durum* Desf.). During Rabi 2023-24, eight lines and four testers were used in an attempt to make crossovers utilizing a line × tester mating scheme. In Rabi 2024–2025, the 32 hybrids that were produced, along with 12 parents and one standard check (GW 1339), were examined at the Wheat Research Station, Junagadh Agricultural University, Junagadh, using a randomized block design with three replications. The analysis of variance for combining ability revealed that the mean square due to lines and testers were significant for all the characters except mean square due to testers for plant height and 100-grain weight. Likewise, mean squares due to line × tester interaction was also found significant for all characters under investigation except plant height. For every individual in the study, the influence of specific gene combinations (SCA) on their traits was more significant than the general influence of their parents (GCA). This was further supported by the ratio of $\sigma^2_{gca}/\sigma^2_{sca}$ was less than unity for all characters confirmed the preponderance of non-additive gene action for all the traits. The estimates of gca effect indicated that among the lines, MACS 3949, GW 1348, MPO 1357 and HD 4758 were found to be good general combiners for grain yield per plant, while GDW 1255 and NIDW 1158 identified as good general combiner for this trait among testers. Parent UAS 475 was found to be good general combiners for early maturity due to negative and significant gca effects for days to anthesis and days to maturity. For grain yield per plant out of 32 crosses 11 were evaluated for their sca effects exhibited significant and favourable sca effects. Among them, the highest sca effects was manifested by the cross GW 1348 × GDW 1255 followed by HD 4758 × WHD 965 and HI 8841 × HI 8737.

Keywords— *Triticum durum* Desf., combining ability, gene action



I. INTRODUCTION

Wheat is a most extensively grown food crop in the world. Wheat is prized for its high nutritious content. Approximately 32% of all cereal growing land worldwide is planted with wheat, which is cultivated throughout a variety of latitudes. India's major wheat-growing states include Uttar Pradesh, Madhya Pradesh, Punjab, Haryana, Rajasthan, Maharashtra and Gujarat.

In India, farmers cultivated 31.83 million hectares, yielding 113.29 million tons with an average productivity of 3559 kg per hectare. While Gujarat accounted for 1.24 million hectares of land, 3.77 million tonnes of production and 3027 kg of productivity per hectare (Anon., 2024). In India there are six mega wheat-growing environments i.e. North-Western Plains Zone (NWPZ), North-Eastern Plains Zone (NEPZ), Central Zone (CZ), Peninsular Zone (PZ), Northern Hills Zone (NHZ) and Southern Hills Zone (SHZ) (Anon., 2007).

The durum wheat is higher in protein, β -carotene and vital micronutrients like iron and zinc, so it offers greater nutrition (ZukGolaszewska *et al.*, 2016). Durum wheat contains high level of folate, which is much important during pregnancy time. The glycemic index of durum wheat pasta is substantially lower than that of regular wheat pasta. Regular pasta causes a quicker spike in blood sugar (GI 68) compared to pasta made from durum wheat, which leads to a more gradual rise (GI 47). Durum wheat is a potentially for maintaining the health of our eyes because it contains roughly twice as much lutein than bread wheat.

The choice of parents to be incorporated in hybridization programme is a crucial step for breeders, particularly if the aim is improvement of complex quantitative characters, such as grain yield and its components. The use of parents of known superior genetic worth ensures much better success. Geneticists need to thoroughly analyze the genes of current plant varieties and new promising lines to identify the best ones for future breeding programs or direct release as new crop types after testing. Nature and magnitude of heterosis is one of the important aspects for selection of right parents for crosses and also help in identification of superior cross combinations that produce desirable transgressive segregants in advanced generations.

II. MATERIALS AND METHODS

The field experiment was conducted at Wheat Research Station, Junagadh Agricultural University, Junagadh during *Rabi*, 2023-24 and 2024-25. This region has a typical sub-tropical climate. The soil of the experimental site was medium black, alluvial in origin and

poor in organic matter. The experimental material of present study was comprised of 32 elite hybrids developed by crossing eight lines and four testers in line $\times$ tester mating design along with one standard check (GW 1339). DDW 48, GW 1348, HD 4758, HI 8841, MACS 3949, MPO 1357, RAJ 3307 and UAS 475 used as lines and GDW 1255, HI 8737, WHD 965 and NIDW 1158 used as testers. The genotypes were collected from Wheat Research Station, Junagadh Agricultural University, Junagadh.

The crossing programme was carried out during *Rabi*, 2023-24 at Wheat Research Station, Junagadh Agricultural University, Junagadh. At the same time, the male and female parents were selfed to get pure seeds of parents for the experiment. The experimental material consisting of 45 entries, including 12 parents, 32 crosses and one standard check (GW 1339) were tested in randomized block design with three replications during *Rabi*, 2024-25. A single row plot of 2.5 m was allotted randomly to each entry. The row-to-row and plant-to-plant distance was kept 22.5 cm and 10 cm, respectively.

Five competitive plants per genotype in each replication in each environment were selected randomly for recording observations on plant height (cm), number of effective tillers per plant, length of main spike (cm), number of spikelets per main spike, number of grains per main spike, 100-grain weight (g), grain yield per plant(g), biological yield per plant (g) and harvest index (%) (except days to anthesis, grain filling period and days to maturity) and their average values were used in the statistical analysis.

III. RESULTS AND DISCUSSION

3.1 Analysis of variance for combining ability and gene action

The analysis of variance for combining ability for all the twelve traits is presented in Table 1. The analysis of variance for combining ability revealed that the mean square due to lines and testers were significant for all the characters except mean square due to testers for plant height and 100-grain weight. Likewise, mean squares due to line $\times$ tester interaction was also found significant for all characters under investigation except plant height.

The SCA variations were greater than the GCA variances for every character in the study, according to the magnitude of the GCA and SCA variants. This suggested that non-additive gene action plays a significant role in the inheritance of these traits. This was further supported by the ratio of $\sigma^2_{gca}/\sigma^2_{sca}$ was less than unity for all characters confirmed the preponderance of non-additive gene action for all the traits. The predominance of non-additive gene action for grain yield and its attributing traits was also reported by Riaz *et*

al. (2021), Mousa et al. (2022), Rauf et al. (2023) and Talpur et al. (2024).

3.2 Estimation of general combining ability effects

The estimation of general combining ability effects revealed that for days to anthesis DDW 48, MACS 3949 and UAS 475 among the lines, whereas HI 8737 among the testers were found good general combiners. For grain filling period lines viz., HD 4758, MPO 1357 and RAJ 3307, whereas among testers, GDW 1255 considered as good combiner. Lines viz., RAJ 3307 and UAS 475 were good general combiners for days to maturity, whereas GDW 1255 as tester parent. The lines GW 1348 and HI 8841 registered good general combiners, while none of the testers was good combiner for plant height. The lines viz., GW 1348, HD 4758, MACS 3949 and MPO 1357 excretes good general combining effects for number of effective tillers per plant. Lines HD 4758 and MPO 1357 whereas in tester GDW 1255 exhibited good general combiners for length of

main spike. The lines, HD 4758 and MACS 3949, while NIDW 1158 among the testers were considered as good general combiners for number of spikelets per main spike. The estimation of general combining ability effect indicated that good combiners for number of grains per main spike were HD 4758 and MACS 3949, among the lines, whereas NIDW 1158 among the parents. For a 100-grain weight, lines GW 1348 and MACS 3949 as well as no tester parents demonstrated good overall combining effects. For grain yield per plant, among the lines, GW 1348, HD 4758, MACS 3949 and MPO 1357 were identified as good general combiners, GDW 1255 and NIDW 1158 were identified as good general combiners among the testers. For biological yield per plant, good general combining effect was registered in three lines GW 1348, HD 4758 and MACS 3949; GDW 1255 and NIDW 1158 testers. For harvest index two lines MPO 1357 and RAJ 3307 and testers, WHD 965 and NIDW 1158 were considered as good general combiners. (Table 2)

Table 1 Analysis of variance for combining ability and variance components for grain yield and its attributing traits in durum wheat

Source	d.f.	Days to anthesis	Grain filling period	Days to maturity	Plant height	Number of effective tillers per plant	Length of main spike
Replications	2	2.697**	0.375	3.885**	7.163	0.342	0.207
Lines	7	32.994**	28.166**	5.375**	53.598*	15.321**	1.170**
Testers	3	13.819**	21.305**	13.736**	30.685	8.587**	1.219**
Lines × Testers	21	25.708**	36.742**	11.926**	30.774	6.205**	1.334**
Error	62	0.536	0.331	0.595	18.304	0.167	0.229
Variance Components							
σ^2_l		2.704	2.319	0.398	2.941	1.262	0.078
σ^2_t		0.553	0.873	0.547	0.515	0.350	0.041
$\sigma^2_{sca} (\sigma^2_{lt})$		8.390**	12.136**	3.777**	4.156	2.012**	0.368**
σ^2_{gea}		1.270	1.355	0.497	1.324*	0.654**	0.053
$\sigma^2_{gea}/\sigma^2_{sca}$		0.151	0.111	0.131	0.318	0.325	0.145

*, ** Significant at 5% and 1% against error, respectively

+, ++ Significant at 5% and 1% levels, respectively against line × tester interaction

The estimation of genetic variance contributed by lines (σ^2_l) and testers (σ^2_t)

Table 1 Cont...

Source	d.f.	Number of spikelets per main spike	Number of grains per main spike	100-grain weight	Grain yield per plant	Biological yield per plant	Harvest index
Replications	2	1.750	5.790	0.919**	0.914	0.881	4.861
Lines	7	13.745**	70.709**+	0.898**	101.290**+	2125.200**++	359.699**
Testers	3	11.274**	81.472**	0.189	42.623**	875.204**	277.965**

Lines x Testers	21	6.105**	27.076**	0.395**	38.443**	320.772**	285.474**
Error	62	1.082	5.094	0.137	1.226	10.576	9.958
Variance Components							
σ^2_l		1.055	5.467*	0.063	8.338*	176.218**	29.145
σ^2_t		0.424	3.182	0.002	1.724	36.026	11.166
$\sigma^2_{sca} (\sigma^2_{lt})$		1.674**	7.327**	0.086**	12.405**	103.398**	91.838**
σ^2_{gca}		0.634**	3.944**	0.022*	3.929**	82.757**	17.159
$\sigma^2_{gca}/\sigma^2_{sca}$		0.379	0.538	0.262	0.316	0.800	0.186

*, ** Significant at 5% and 1% against error, respectively

+, ++ Significant at 5% and 1% levels, respectively against line × tester interaction

The estimation of genetic variance contributed by lines (σ^2_l) and testers (σ^2_t)

Table 2 General combining ability effects of parents for grain yield and its attributing traits in durum wheat

Sr. No.	Parents	Days to anthesis	Grain filling period	Days maturity to	Plant height	Number of effective tillers per plant	Length of main spike
Lines							
1	DDW 48	-0.604**	0.750**	0.146	1.740	-1.910**	-0.161
2	GW 1348	-0.188	0.250	0.063	3.157*	1.423**	0.174
3	HD 4758	2.646**	-2.250**	0.296	1.378	0.723**	0.354*
4	HI 8841	0.729**	0.583**	1.313**	-3.798**	-0.277*	-0.071
5	MACS 3949	-2.438**	2.333**	-0.104	-0.077	0.506**	-0.027
6	MPO 1357	0.979**	-1.333**	-0.354	-0.688	0.856**	0.357*
7	RAJ 3307	0.813**	-1.417**	-0.604**	-0.880	-0.027	-0.019
8	UAS 475	-1.938**	1.083**	-0.854**	-0.833	-1.294**	-0.606**
	SE (g _i)	0.211	0.166	0.222	1.235	0.118	0.138
Testers							
1	GDW 1255	0.021	-1.083**	-1.063**	-1.165	0.548**	0.286**
2	HI 8737	-1.063**	1.208**	0.146	1.513*	-0.827**	-0.140
3	WHD 965	0.354**	-0.167	0.188	-0.428	0.298**	-0.215*
4	NIDW 1158	0.688**	0.042	0.729**	0.080	-0.019	0.070
	SE (g _i)	0.149	0.117	0.157	0.873	0.083	0.097
*, ** Significant at 5% and 1% against error, respectively							

Table 2 Cont.....

Sr. No.	Parents	Number of spikelets per main spike	Number of grains per main spike	100-grain weight	Grain yield per plant	Biological yield per plant	Harvest index
Lines							
1	DDW48	-0.602*	-1.021	-0.159	-4.525**	-15.090**	-2.841**

2	GW1348	-0.069	-0.171	0.269*	2.615**	11.645**	-4.968**
3	HD4758	2.265**	5.313**	-0.010	1.633**	24.621**	-8.737**
4	HI8841	-0.852**	-1.854**	-0.192	-2.093**	-3.998**	1.237
5	MACS3949	0.665*	1.346*	0.404**	3.781**	6.522**	0.317
6	MPO1357	0.165	0.013	0.203	1.905**	-4.223**	8.703**
7	RAJ3307	-0.569	-1.554*	-0.098	-0.849*	-8.297**	4.486**
8	UAS475	-1.00**	-2.071**	-0.418**	-2.466**	-11.180**	1.804
	SE (g _i)	0.300	0.651	0.107	0.319	0.938	0.9110
Testers							
1	GDW1255	0.206	0.621	0.105	0.785**	8.748**	-4.850**
2	HI8737	-0.860**	-2.296**	-0.094	-1.975**	-3.328**	0.406
3	WHD965	-0.127	-0.413	-0.049	0.365	-4.595**	2.993**
4	NIDW1158	0.781**	2.087**	0.038	0.824**	-0.826	1.451*
	SE (g _i)	0.212	0.460	0.075	0.226	0.663	0.644
** Significant at 5% and 1% against error, respectively							

Table 3 Specific combining ability effects for days to anthesis, grain filling period, days to maturity and plant height in durum wheat

Sr. No.	Hybrids	Days to anthesis	Grain filling period	Days to maturity	Plant height
1	DDW 48 × GDW 1255	0.646	-1.750**	-1.104*	-2.796
2	DDW 48 × HI 8737	-6.604**	7.292**	0.683	2.263
3	DDW 48 × WHD 965	2.646**	0.333	2.979**	3.140
4	DDW 48 × NIDW 1158	3.313**	-5.875**	-2.563**	-2.608
5	GW 1348 × GDW 1255	1.563**	-1.250	0.313	1.921
6	GW 1348 × HI 8737	2.313**	-0.875*	1.438*	0.986
7	GW 1348 × WHD 965	-3.104**	0.500	-2.604**	1.080
8	GW 1348 × NIDW 1158	-0.771	1.625**	0.854	-3.987
9	HD 4758 × GDW 1255	-0.604	3.917**	3.313**	-1.117
10	HD 4758 × HI 8737	-0.188	-1.375**	-1.563**	-1.441
11	HD 4758 × WHD 965	1.729**	-1.000**	0.729	-3.367
12	HD 4758 × NIDW 1158	-0.938*	-1.542**	-2.479**	5.925*
13	HI 8841 × GDW 1255	0.979*	-2.917**	-1.938**	0.466
14	HI 8841 × HI 8737	1.063*	1.125**	2.188**	-0.835
15	HI 8841 × WHD 965	-1.688**	0.500	-1.188**	-4.051
16	HI 8841 × NIDW 1158	-0.354	1.292**	0.938*	4.421
17	MACS 3949 × GDW 1255	1.813**	-1.667**	0.146	-0.815
18	MACS 3949 × HI 8737	-3.438**	2.708**	-0.729	-2.743
19	MACS 3949 × WHD 965	-0.854*	1.750**	0.896*	2.805

20	MACS 3949 × NIDW 1158	2.479**	-2.792**	-0.313	0.753
21	MPO 1357 × GDW 1255	-2.938**	3.000**	0.063	0.696
22	MPO 1357 × HI 8737	4.146**	-6.292**	-2.146**	1.408
23	MPO 1357 × WHD 965	0.396	-0.917**	-0.521	2.522
24	MPO 1357 × NIDW 1158	-1.604**	4.208**	2.604**	-4.626
25	RAJ 3307 × GDW 1255	1.896**	-0.583	1.313**	0.968
26	RAJ 3307 × HI 8737	-1.688**	2.125**	0.438	0.530
27	RAJ 3307 × WHD 965	0.896*	-1.500**	-0.604	1.257
28	RAJ 3307 × NIDW 1158	-1.104*	-0.042	-1.146*	-2.754
29	UAS 475 × GDW 1255	-3.354**	1.250**	-2.104**	0.677
30	UAS 475 × HI 8737	4.396**	-4.708**	-0.313	-0.167
31	UAS 475 × WHD 965	-0.021	0.333	0.013	-3.386
32	UAS 475 × NIDW 1158	-1.021*	3.125**	2.104**	2.876
SE (S _{ij})±		0.422	0.332	2.470	2.470
Range of sca effects		-6.604 to 4.396	-6.292 to 7.292	-2.604 to 3.313	-4.626 to 5.925
No. of crosses with significant and desirable sca effects		11	14	10	0

*, ** Significant at 5% and 1% against error, respectively

Table 4 Specific combining ability effects for number of effective tillers per plant, length of main spike, number of spikelets per main spike and number of grains per main spike in durum wheat

Sr. No.	Hybrids	Number of effective tillers per plant	Length of main spike	Number of spikelets per main spike	Number of grains per main spike
1	DDW 48 × GDW 1255	0.652**	-0.412	-0.023	-0.754
2	DDW 48 × HI 8737	1.427**	0.107	0.510	0.962
3	DDW 48 × WHD 965	-0.365	-0.045	1.177	2.213
4	DDW 48 × NIDW 1158	-1.715**	0.350	-1.665**	-2.421
5	GW 1348 × GDW 1255	1.185**	1.273**	2.044**	3.863**
6	GW 1348 × HI 8737	-2.640**	-0.295	-1.023	-1.488
7	GW 1348 × WHD 965	-0.298	-0.420	-0.356	-0.438
8	GW 1348 × NIDW 1158	1.752**	-0.558*	-0.665	-1.938
9	HD 4758 × GDW 1255	-0.048	-0.607*	-1.023	-2.754*
10	HD 4758 × HI 8737	-1.673**	-0.261	-1.956**	-4.637**
11	HD 4758 × WHD 965	1.935**	-0.420	-0.356	-0.854
12	HD 4758 × NIDW 1158	-0.215	1.288**	3.335**	8.246**
13	HI 8841 × GDW 1255	-0.115	-0.396	-0.173	0.012

14	HI 8841 × HI 8737	0.194	-0.010	0.760	1.529
15	HI 8841 × WHD 965	-0.131	0.839**	0.094	0.046
16	HI 8841 × NIDW 1158	0.052	-0.433	-0.681	-1.588
17	MACS 3949 × GDW 1255	-1.698**	-0.653*	-2.023**	-2.454
18	MACS 3949 × HI 8737	1.677**	0.606*	1.710**	1.463
19	MACS 3949 × WHD 965	0.285	0.108	0.310	0.979
20	MACS 3949 × NIDW 1158	-0.265	-0.061	0.002	0.013
21	MPO 1357 × GDW 1255	0.152	0.143	-0.390	0.854
22	MPO 1357 × HI 8737	0.794**	-0.045	0.944	2.729*
23	MPO 1357 × WHD 965	-0.998**	0.030	-0.323	-0.554
24	MPO 1357 × NIDW 1158	0.052	-0.128	-0.231	-1.321
25	RAJ 3307 × GDW 1255	-0.765**	-0.034	-0.190	-0.688
26	RAJ 3307 × HI 8737	-0.056	-0.081	0.190	-0.171
27	RAJ 3307 × WHD 965	1.819**	-0.546	-0.723	-1.587
28	RAJ 3307 × NIDW 1158	-0.998**	0.662*	1.102	2.446
29	UAS 475 × GDW 1255	0.635**	0.686*	1.777*	3.629**
30	UAS 475 × HI 8737	0.277	-0.021	-0.756	-0.387
31	UAS 475 × WHD 965	-2.248**	0.454	0.177	0.196
32	UAS 475 × NIDW 1158	1.335**	-1.118**	1.198	-3.438**
SE (S_{ij})±		0.236	0.276	0.600	1.303
Range of sca effects		-2.640 to 1.935	-1.118 to 1.288	-2.023 to 3.335	-4.637 to 8.246
No. of crosses with significant and desirable sca effects		10	6	4	4

*, ** Significant at 5% and 1% against error, respectively

Table 5 Specific combining ability effects for 100-grain weight, grain yield per plant, biological yield per plant and harvest index in durum wheat

Sr. No.	Hybrids	100-grain weight	Grain yield per plant	Biological yield per plant	Harvest index
1	DDW 48 × GDW 1255	-0.134	-0.147	-5.781**	4.194*
2	DDW 48 × HI 8737	-0.162	4.810**	7.609**	3.755*
3	DDW 48 × WHD 965	0.323	-0.604	1.722	-0.215
4	DDW 48 × NIDW 1158	-0.027	-4.059**	-3.550	-7.73**
5	GW 1348 × GDW 1255	0.321	6.483**	-3.822*	13.558**
6	GW 1348 × HI 8737	-0.113	-5.457**	0.280	-9.141**
7	GW 1348 × WHD 965	-0.472*	-3.454**	-5.583**	-3.495
8	GW 1348 × NIDW 1158	0.264	2.428*	9.125**	-0.923
9	HD 4758 × GDW 1255	-0.397	-1.348*	-3.408	-1.174
10	HD 4758 × HI 8737	-0.381	-4.368**	8.935**	-2.359

11	HD 4758 × WHD 965	0.554*	5.272**	10.058**	-0.806
12	HD 4758 × NIDW 1158	0.234	0.444	-15.584**	4.339*
13	HI 8841 × GDW 1255	0.158	-2.362**	14.360**	-6.837**
14	HI 8841 × HI 8737	0.354	3.451**	-10.454**	12.414**
15	HI 8841 × WHD 965	-0.134	-0.779	-10.230**	4.914**
16	HI 8841 × NIDW 1158	-0.378	-0.310	6.324**	-10.491**
17	MACS 3949 × GDW 1255	-0.378	-3.666**	-6.243**	-2.567
18	MACS 3949 × HI 8737	0.712**	1.027	4.713*	-3.769*
19	MACS 3949 × WHD 965	-0.237	3.344**	12.920**	-2.453
20	MACS 3949 × NIDW 1158	0.097	-0.705	-11.389**	8.789**
21	MPO 1357 × GDW 1255	0.057	1.446*	4.806*	-5.466**
22	MPO 1357 × HI 8737	0.099	1.293*	-0.385	-3.032
23	MPO 1357 × WHD 965	-0.063	-2.370**	-1.305	-6.685**
24	MPO 1357 × NIDW 1158	-0.093	-0.369	-3.117	15.183**
25	RAJ 3307 × GDW 1255	0.328	-3.476**	8.647**	-14.906**
26	RAJ 3307 × HI 8737	-0.029	0.754	-10.380**	11.715**
27	RAJ 3307 × WHD 965	-0.081	1.297*	2.463	0.388
28	RAJ 3307 × NIDW 1158	-0.218	1.425*	-0.729	2.803
29	UAS 475 × GDW 1255	0.045	3.070**	-8.557**	13.199**
30	UAS 475 × HI 8737	-0.479*	-1.510*	-0.318	-9.583**
31	UAS 475 × WHD 965	0.119	-2.706**	-10.045**	8.353**
32	UAS 475 × NIDW 1158	0.315	1.145	18.920**	-11.968**
SE (S _{ij})±		0.214	0.639	1.877	1.822
Range of sca effects		-0.479 to 0.712	-5.457 to 6.483	-15.584 to 18.920	-14.906 to 15.183
No. of crosses with significant and desirable sca effects		2	11	11	11

*, ** Significant at 5% and 1% against error, respectively

The lines GW 1348, HD 4758, MACS 3949, and MPO 1357 were effective in producing high grain yield per plant, and they passed this trait on to their offspring for number of effective tillers per plant, length of main spike, number of spikelets per main spike, number of grains per main spike, 100-grain weight and biological yield per plant. The testers GDW1255 and NIDW 1158 were good general combiners for grain yield per plant were also found good combiners for length of main spike, number of spikelets per main spike, number of grains per main spike and biological yield per plant. Therefore, the parents that performed well in general combining for grain yield were also performing well in general combining for one or more component characteristics. Hence, these parents may be exploited well in the future breeding programme for grain yield improvement in durum wheat. These finding were in

accordance with Joshi and Kumar (2020), Kumar *et al.* (2021), Dudhat *et al.* (2022), Fouad *et al.* (2023), Reddy *et al.* (2023) and Fareed *et al.* (2024).

3.3 Estimation of specific combining ability effects

Here are the estimates of the specific combining ability (sca) effects of hybrids on yield and its attributing traits:

Out of 32 hybrids, 11 hybrids revealed significant negative sca effects for days to anthesis. The highest significant and negative sca effect was observed in cross DDW 48 × HI 8737 (-6.604) followed by MACS 3949 × HI 8737 (-3.438), UAS 475 × GDW 1255 (-3.354) and GW 1348 × WHD 965 (-3.104) (Table 3). For grain filling period (days), out of 32 hybrids, 14 hybrids exhibited significant negative sca effects. The highest significant and negative sca effects observed in cross MPO 1357 × HI 8737 (-6.292) followed by DDW 48 × NIDW 1158 (-5.875) and UAS 475

× HI 8737 (-4.708) (Table 3). 10 crosses exhibited significant and negative sca effects for early maturity. The highest significant and negative sca effect was observed in cross GW 1348 × WHD 965 (-2.604) followed by DDW 48 × NIDW 1158 (-2.563), HD 4758 × NIDW 1158 (-2.479), MPO 1357 × HI 8737 (-2.146) and UAS 475 × GDW 1255 (-2.104) indicating that they may be promising hybrids for exploiting earliness in durum wheat (Table 3). The ranged of sca effects for plant height in hybrids varied from -4.626 (MPO 1357 × NIDW 1158) to 5.925 (HD 4758 × NIDW 1158). None of the hybrids exhibited significant negative sca effects for dwarf plant height (Table 3). Out of 32 crosses, 10 crosses exhibited significant and positive sca effects for number of effective tillers per plant. The highest significant and positive sca effects was observed in cross HD 4758 × WHD 965 (1.935) followed by RAJ 3307 × WHD 965 (1.819), GW 1348 × NIDW 1158 (1.752) and DDW 48 × HI 8737 (1.427) indicating that they may be promising hybrids for number of effective tillers per plant (Table 4). The sca effect for length of main spike in hybrids, out of 32 crosses, six crosses exhibited significant and positive sca effects for length of main spike. The highest significant and positive sca effects were observed in cross HD 4758 ×

NIDW 1158 (1.288) followed by GW 1348 × GDW 1255 (1.273) and HI 8841 × WHD 965 (0.839) indicating that they may be promising hybrids for number of effective tillers per plant (Table 4). Four crosses exhibited significant and positive sca effects for number of spikelets per main spike. The highest significant and positive sca effect was observed in cross HD 4758 × NIDW 1158 (3.335) followed by GW 1348 × GDW 1255 (2.044), UAS 475 × GDW 1255 (1.777) and MACS 3949 × HI 8737 (1.710) (Table 4). Out of 32 crosses, four crosses exhibited significant and positive sca effects for number of grains per main spike. The highest significant and positive sca effects was observed in cross HD 4758 × NIDW 1158 (8.246) followed by GW 1348 × GDW 1255 (3.863), UAS 475 × GDW 1255 (3.629) and MPO 1357 × HI 8737 (2.729) indicating that they may be promising hybrids for number of grains per main spike (Table 4). The cross MACS 3949 × HI 8737 (0.712) and HD 4758 × WHD 965 (0.554) were identified as good specific combinations as they exhibit significant and positive sca effects for 100-seed weight. 11 crosses were identified as good specific combiners as they exhibit significant and positive sca effects for grain yield per plant. The most superior cross combiners were GW 1348 × GDW 1255 (6.483), HD 4758 × WHD 965 (5.272) and DDW 48 × HI 8737 for grain yield per plant (Table 5). Ten hybrids were identified as good specific combiners for biological yield per plant. The most superior cross combinations were UAS 475 × NIDW 1158 (18.920),

MACS 3949 × WHD 965 (12.920) and HI 8841 × GDW 1255 (14.360) (Table 5). The spectrum of variability for sca effects in hybrids for harvest index was varied from -14.906 (RAJ 3307 × GDW 1255) to 15.183 (MPO 1357 × NIDW 1158). Out of 32 crosses, 11 crosses exhibited significant and positive sca effects for harvest index. The highest significant and positive sca effect was observed in cross MPO 1357 × NIDW 1158 (15.183) followed by GW 1348 × GDW 1255 (13.558) and HI 8841 × HI 8737 (12.414) indicating that they may be promising hybrids for number of spikelets per main spike (Table 5).

It is general observation that good cross combinations obtained between good × good and poor ones between poor × poor general combiners. But in the present study, superior cross combinations viz., GW 1348 × GDW 1255 (good × good), HD 4758 × WHD 965 (good × average) and DDW 48 × HI 8737 (poor × poor) general combiners for the characters under study indicates that good cross combinations are not always obtained by crossing between good general combiners.

With respect to specific combining ability effects, following conclusion could be drawn from the present study. No cross combination exhibited consistently high specific combining ability effects for all the characters studied. Crosses having high sca effects for grain yield also depicted high sca effects for yield attributing characters. The crosses exhibiting high sca effects did not always involve parents with high gca effects suggesting that interallelic interaction was important for characters. These findings are in agreement with the findings of Motawea (2017), Joshi and Kumar (2020) and Kumar *et al.* (2021).

IV. CONCLUSION

Majority of the parents exhibited good gca effect for different traits also had acceptable *per se* performance, which suggested that the *per se* performance can be considered as a reliable criterion for selecting parents for hybridization. In case of line × tester, three cross combinations viz., GW 1348 × GDW 1255, HD 4758 × WHD 965 and DDW 48 × HI 8737 were found to be better specific combiners for grain yield per plant which were in combination of good × good, good × average and poor × poor combiners, respectively. Crosses with high sca effects for grain yield per plant also depicted high sca effects for important grain yield attributes viz., length of main spike, number of spikelets per main spike, number of grains per main spike and 100-grain weight. The combining ability analysis revealed predominance of non-additive gene action for the inheritance of grain yield and its attributes. At present heterosis breeding is not feasible in wheat at commercial level, above three crosses could be exploited to isolate

transgressive segregants in segregating generations to develop high yielding pureline in durum wheat.

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Evaluation of Berseem in Relay Cropping with Mustard as a Viable Climate Resilience Technology for Income Enhancement in semi-arid tropics Areas

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Received: 27 Jul 2025; Received in revised form: 29 Aug 2025; Accepted: 01 Sep 2025; Available online: 08 Sep 2025

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Abstract— *Trifolium alexandrinum*, commonly known as Berseem is the main legume fodder cultivated in the south-east Asia because of its more vegetative growth, multi-cut nature, better forage output after harvesting, prolonged time of forage provision, and prominent fodder yield with outstanding delicious and excessive beneficial values of 20-21% crude protein and 62% total edible food. The livestock is contributing up to 40 % of total annual income of small and marginal farmers of district. However, there was significant scarcity of green fodder during lean period. To increase the green fodder availability with increase in net farm income present study was undertaken to evaluate the berseem in relay cropping with mustard crop at farmers field in NICRA project at KVK Morena. The results revealed that the crop of berseem grown as relay cropping with mustard is shown higher net return and B:C ratio compared as sole crop of mustard or berseem. As per our findings, the mustard crop establishment and relay cropping with legume crop berseem with mustard have overall beneficial and wide scope for its adoption in similar agro-ecological circumstances under present changing climate condition.



Keywords— Relay cropping, Berseem, Mustard, Farm Income and Climate

I. INTRODUCTION

Trifolium alexandrinum, commonly known as Berseem is the main legume fodder cultivated in the south-east Asia because of its more vegetative growth, multi-cut nature, better forage output after harvesting, prolonged time of forage provision, and prominent fodder yield with outstanding delicious and excessive beneficial values of 20-21% crude protein and 62% total edible food (Yadav et al., 2015). This leguminous crop holds significant agricultural importance, particularly as a winter crop in Madhya Pradesh. Berseem Clover was introduced into northern India in the early 19th century and has found its place in various regions globally, including the United States, Europe, China, and Australia. Berseem clover typically grows between 30 to 100 cm height, featuring erect or ascending stems. It is used as green forage during the season

as well as hay or pallets during off season (Nigam et al., 2010). Moreover, it serves as an effective fodder crop, suppressing weeds and enriching the soil with nitrogen, providing up to 280 lbs/acre to subsequent crops (). India occupies the largest berseem growing area (2 m ha) followed by Egypt (1.1 m ha) and Pakistan (0.71 m ha) (Muhammad et al., 2014).

The inclusion of berseem into cropping systems presents opportunities for enhancing yield and income, particularly in mustard-based cropping systems. Recent studies showed that berseem can also be used for phytoremediation of heavy metals viz., Cd, Pb, Cu and Zn, due to its multi-cut nature, short life cycle and production of considerable biomass (Ali et al., 2012). The potential effect of cover crops on soil water conservation is especially significant because of the documented impact of soil water

on crop yield, especially for dry land cropping systems (Acharya, et al. 2019). The fodder and seed yield from improved varieties is significantly higher as compared to those as obtained by small holder farmers (Govt. of the Punjab, 2014).

In oilseed crops, Indian mustard (*Brassica juncea*) is second most important oilseed crop grown with fellow/pearl millet/cluster bean/green gram/black gram-based cropping systems. These cropping systems are grown in limited irrigated areas with intensive tillage, improper crop establishing and imbalanced use of chemical fertilizers (Singh, 2023). Mustard-based cropping systems have been experiencing a decline in productivity, economic profitability and soil quality in India's arid and semi-arid climate. Crop residues burning and imbalanced utilization of chemical fertilizers resulted in deteriorating soil quality and resources; are some of the factors contributing to the declining performance of mustard crop (Fustec et al., 2010). In gird region of Madhya Pradesh, fellow- mustard or pearl millet – mustard cropping system is mostly in practice. Among the oilseeds in India, Brassica ranks second in production (2015-16) after soybean (GOI, 2017).

Animals are the most important part of farming system in Morena district of Madhya Pradesh and it contributes approximately forty percent (40%) in annual income of small and marginal farmers. Livestock are indispensable for production of milk & meat and for work provisions (Anwar et al., 2012). Small & marginal farmers may have limited opportunities to cultivate green fodder, particularly during the lean season, where owning livestock is an alternate income generator (Sahu and Jha, 2022).

In this context, the current investigation aimed to investigate the impact of relay cropping of legume crop berseem with mustard-based cropping systems on the productivity of crops (mustard and berseem), yield attributes and economic parameters.

II. MATERIALS AND METHOD

Field experiments were conducted in two successive years i.e., 2023-24 and 2024-25 at on-farm locations viz., Kolua and Khargpura villages of Ambah Block of Morena district of Madhya Pradesh under the National Innovation on Climate Resilience Technology (NICRA) project. The study location has a semi-arid climate, severely cold from Dec to Jan (0°C minimum temp.), and hot from May to June (48°C maximum temp.).

The weekly mean of the minimum and maximum relative humidity ranged between 23.3-88.0% and 38.1-90.4%, respectively. The mean annual rainfall was 650 to 750 mm, mainly received in July and Aug months. At the time of the investigation, the minimum and maximum temperature were 2°C and 47°C, correspondingly. The total annual rainfall received was 942.5 and 848 mm during the year 2023-24 and 2024-25, respectively. The soils of selected fields were sandy loam with the texture of an old alluvial plain.

The spacing between row-to-row for mustard were kept 30 cm and 4.0 kg seed /ha was used for sowing. At 35-40 days after sowing (DAS) of mustard, berseem (Var.- Bundel Berseem -3) were sown uniformly by applying broadcasting @ 20.0 kg⁻¹ ha just prior to the first irrigation given to mustard. The seed treatment of berseem was done with captan (2.0 g/ kg seed) followed by *Rhizobium trifoli* and *Pseudomonas fluorescens*. A recommended agronomical package of practices was adhered to raise the experimental crops. The total plot area 4000 m² was divided into two equal parts. Each intervention had ten locations, with and without relayed berseem was established. Five locations were selected and every location was treated as a replication for statistical analysis in Randomized Block design.

As per recommendations for mustard crop, the full recommended dose of P, K, S and Zn was 50, 30, 40 and 5 kg ha⁻¹ applied at time of sowing, while N was applied in two splits of 50 kg ha⁻¹ at time of sowing and the remaining 50 kg⁻¹ ha at time of flower initiation (about 40 DAS). The berseem was grown in residual nutrition of mustard. The mustard crop was harvested in the first week of March of each year. After the harvesting of mustard, three irrigations were given to the berseem crop after harvesting of mustard, at head formation and seed filling stages. Spinosad @ 150 ml ha⁻¹ was applied to control pod borer (*Helicoverpa armigera*), at the economic threshold level in berseem. The berseem crop was harvested during 3rd to 4th week of may. The crop yield was recorded for individuals grown in the systems.

Seed and straw yield, gross and net returns and benefit cost ratio were calculated. The economics of the crops between the sowing and harvest of crops were examined. The system productivity of different crops was calculated with the yields of non-mustard crops converted into mustard equivalent yield (MEY) for berseem, as advised by Singh et al. (2020).

$$\text{MEY (t ha)} = \frac{\text{Berseem seed yield (q ha}^{-1}\text{)} \times \text{Prevailed Market price of Berseem (q}^{\wedge}(-1))}{\text{The minimum support price of mustard (q}^{\wedge}(-1))}$$

III. RESULTS AND DISCUSSION

The yield of mustard crop was increased under relay cropping system in comparison to sole crop while the berseem yield showed slight deterioration under relay cropping (Table1). However, total output as mustard equivalent yield of relay cropping is higher than both sole crops under demonstrated fields (Table 2). The mustard equivalent yield was comparably higher than sole crop of both crops grown individually during both the years at all locations of farmer's field. The yield of any crop plant depends upon the source sink relationship and is the cumulative function of various growth parameters and yield attributing components of sink viz. growth and dry matter content etc (Kumar et al. 2024). Under this situation, two crops of different root-shoot growing nature are grown at a certain proportion. Thereby increases the cropping intensity, total productivity and profitability under efficient utilization of soil, water, nutrients and sunlight in time and space (Panday et al. 2021)

Almost all observed yield attributes *i.e.*, plant height at harvest (cm), numbers of siliquae or pod per plant and numbers of seeds per siliquae or pod were also comparable in both crops as sole and relay crop (Table1). According to Singh et al. (2024), leguminous crop berseem is known to ameliorate soil quality over the years, contributing to system productivity. Likewise, Singh et al. (2020) reported that the relay berseem with mustard significantly enhanced the soil quality and system productivity.

The cost of cultivation for relay cropping was higher than sole crops while the net return Rs.84490/ha was also reported higher than sole crops of mustard and berseem Rs. 64882/ha and Rs. 4732/ha respectively. The higher net returns with berseem relay cropping with mustard probably due to improved soil fertility resulted in a higher yield of crops and additional net income (Singh et al. 2024).

Table1: Yield and its attributes of mustard and berseem crop as affected under different production systems (Mean of two successive years *i.e.*, 2023-24 & 2024-25)

Treatment	Yield (q/ha)	Straw yield (q/ha)	Plant height at harvest (cm)	Numbers of siliquae or pod/plant	Numbers of seeds/ siliquae/ pod
Berseem (Sole crop)	04.50	6.25	072.50	25	6.3
Mustard (Sole crop)	19.33	25.63	187.30	230	5.5
Mustard + Berseem (relay cop)	20.65 (03.94)	24.54 (5.32)	184.00 (074.09)	232 (22.4)	4.7 (4.5)

Note : Value of berseem in parenthesis

Table 2: Yield and economics of mustard & berseem as sole crop and with relay cropping (Mean of two successive years *i.e.*, 2023-24 & 2024-25)

Treatment	MEY (q/ha)	Cost of cultivation (Rs/ha)	Gross return (Rs/ha)	Net return (Rs/ha)	B:C Ratio
Berseem (Sole crop)	7.09	33500	38232	4732	1.14
Mustard (Sole crop)	19.33	39500	104382	64882	2.64
Mustard + Berseem (relay cop)	26.85	60500	144990	84490	2.39
SEm +_	0.087	NA	NA	NA	NA
CD@5%	0.253	NA	NA	NA	NA

IV. CONCLUSION

The results revealed that the crop of berseem grown as relay cropping with mustard is shown higher net return and B:C ratio compared as sole crop of mustard or berseem. As per our findings, the mustard crop establishment and relay cropping with legume crop berseem with mustard have overall beneficial and wide scope for its adoption in similar agro-ecological circumstances under present changing climate condition.

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Knowledge level of farmers on Recommended Package of Practices of Assam Lemon (*Citrus limon* L. Burm) Cultivation

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Received: 07 Aug 2025; Received in revised form: 02 Sep 2025; Accepted: 05 Sep 2025; Available online: 13 Sep 2025

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Abstract— Assam lemon is a unique citrus variety of Assam valued for its aroma, thin peel, juiciness, and near-seedless character. Despite its economic potential, productivity has not reached the desired level due to partial or incomplete adoption of scientific recommendations. The present study was conducted in Tinsukia district of Assam with a sample of 120 Assam Lemon growers. Data were collected and analysis was carried out using frequency and percentage. The results indicated that farmers exhibited high awareness of basic practices such as soil suitability, planting season, recommended varieties, irrigation, and orchard maintenance. However, considerable gaps existed in technical areas including scientific propagation techniques, pit preparation, plant spacing, canopy training, balanced fertilizer application, and use of micronutrients. Pest and disease management was largely confined to traditional methods, with limited adoption of chemical and preventive measures. In contrast, knowledge of harvesting practices was relatively better, as it is more experience-based and directly linked to market quality. The findings highlight that while indigenous knowledge has helped sustain Assam lemon cultivation, inadequate adoption of recommended package of practices remains a major constraint.



Keywords— Assam lemon, Knowledge, Package of Practices, Frequency and Percentage

I. INTRODUCTION

Citrus is regarded as one of the most significant fruit crops cultivated worldwide. In 2022, the global citrus cultivation area was estimated at about 9.8 million hectares, producing nearly 158.8 million metric tonnes (FAOSTAT, 2023). The average productivity across the world stands at around 16.2 tonnes per hectare, though this figure fluctuates among countries due to differences in climate, farming practices, and input use (FAO, 2023). In India, citrus fruits occupy an important place in the horticultural sector. The annual productivity of citrus in the country during 2022–23 was reported to be 10.30 MT/ha

(MoAFW, 2023). In the year 2023–24, India exported fruits and vegetables worth USD 1.814 billion, with citrus fruits such as oranges among the major contributors (APEDA, 2024). The principal citrus-growing states in the country include Andhra Pradesh, Maharashtra, Madhya Pradesh, Rajasthan, Karnataka, Assam, and Arunachal Pradesh.

Assam is home to several indigenous citrus species, of which the Assam Lemon (*Citrus limon* L. Burm), locally known as ‘Kaji Nemu,’ holds special importance (Gogoi, 2023). This variety is recognized for its characteristic elongated shape, thin rind, high juice content, and unique fragrance. Its near seedless nature

further distinguishes it from other lemon cultivars in India. Assam lemon has also entered the global market: in 2021 about 1200 kg of the fruit was exported from Chirang district to the UK, followed by 600 kg from Baksa in 2022 (G Plus, 2021). More recently, in 2023, approximately 5000 pieces were exported to London from Tinsukia district (The Sentinel, 2024). In 2015–16 the area, production, and productivity were 13,173 hectares, 108,492 MT, and 8236 kg/ha respectively (Statistical Handbook of Assam, 2016) which increased significantly to 18,036 hectares, 178,872 MT, and 9841 kg/ha during 2023–24 (Directorate of Horticulture and Food Processing, 2023–24). This steady growth demonstrates the rising economic significance of Assam lemon in the state's horticultural landscape.

The Government of Assam declared Assam lemon as the state fruit (*Jatiya Phal*) on 13th February 2024, while it had already received the Geographical Indication (GI) tag in 2019 under the “Geographical Indication of Goods (Registration and Protection) Act, 1999.” The GI recognition enhanced its brand value, market potential, and economic prospects for the region. Assam's subtropical humid climate also provides ideal conditions for its cultivation, ensuring good productivity per unit area.

With the demand for Assam lemon steadily increasing, it has become a vital source of livelihood and income for rural farmers. However, despite this economic importance, the productivity potential is not fully realized, largely because of gaps in farmers' knowledge and adoption of the recommended package of practices (POP). While traditional experience enables farmers to carry out basic practices such as soil selection, planting season, and irrigation, technical aspects like scientific propagation, canopy management, nutrient application, and integrated pest management remain poorly adopted. Therefore, assessing the knowledge level of growers on recommended

practices is essential for identifying existing gaps and guiding interventions that can enhance yield, quality, and profitability of Assam lemon cultivation. Keeping in view all these factors, the following objective was undertaken to conduct the study

Objective: To study the knowledge level of farmers on recommended package of practices on Assam Lemon Cultivation.

II. MATERIALS AND METHOD

Tinsukia district of Assam consisted of three sub-division namely Tinsukia, Margherita and Doomdooma. The Tinsukia subdivision was selected purposively as it has the highest numbers of Assam Lemon growers. There are eleven ADO circles in Tinsukia district, out of which two ADO circles namely Kakapather and Dholla. Four villages from each ADO circle were selected and from each village 15 numbers of Assam lemon growers were selected. Thus, a total of 120 Assam lemon growers from the district of Tinsukia, Assam were selected with a multi staged sampling procedure from eight sampled villages. Data was collected with the help of a one-to-one interview method. For collecting data, a structured scheduled was used. Data analysis was done using statistical tool such as frequency and percentage.

In order to test the reliability of the items Kuder Richardson (KR-20) reliability test was conducted. The result of the test indicate that the items are highly reliable.

Test name	No. of items	Result
Kuder Richardson (KR-20) reliability test	30	0.799

III. RESULT

Table 1. Distribution of respondents according to their level of knowledge on recommended package of practice. n=120

Sl. No.	Category	f	(%)	Mean	SD
1	Low (<16.48)	22	18.34	21.20	4.72
2	Medium (16.68-25.92)	62	51.66		
3	High (>25.92)	36	30.00		
	Total	120	100		

Findings from Table 1 reveal that the majority of respondents (51.66%), fell under the medium knowledge level category. Additionally, 30.00 per cent of the respondents had high and 18.34 per cent of the respondents

had low level of knowledge on recommended package on Assam Lemon cultivation practices. The result shows that majority of respondents had only an moderate level of knowledge concerning the recommended package of

practices for Assam lemon cultivation. This points to an existing gap in the flow of knowledge and information among the growers. Limited awareness of improved scientific methods, modern equipment, and updated cultivation practices can negatively impact overall productivity. Hence, the results underline the importance of

strengthening farmers' awareness and providing well-structured training programs on recommended practices, which could substantially enhance their knowledge base and ultimately boost the production and productivity of Assam lemon.

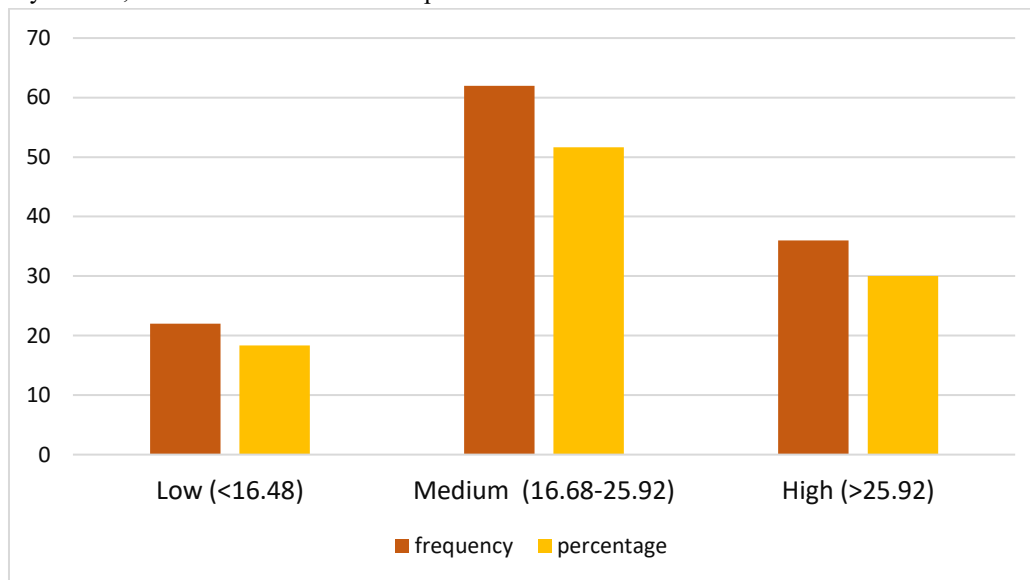


Fig 1. Distribution of respondents according to their knowledge level on recommended package of practices

Table 2. Distribution respondents according to their knowledge on recommended package of practices on Assam lemon cultivation. n=120

Sl no	statement	frequency	Percentage (%)
A	SOIL AND SITE SELECTION		
1	Do you know that loamy soil (3m depth) is ideal for Assam lemon?	110	91.67
2	Do you know that waterlogged condition is not suitable for Assam lemon cultivation?	120	100
B	PROPAGATION METHODS		
1	Do you use stem cutting, leaf-bud cutting, and air layering for propagation?	102	85.00
2	Do you prepare stem cuttings (18-20 cm) with proper node cuts?	19	15.83
3	Do you plant stem cuttings at 20 cm × 15 cm spacing?	20	16.67
4	Do you treat cuttings with IBA (2500 ppm) or other root stimulants like seradax and rootex?	75	62.50
5	Do you propagate between March and August for best results?	65	54.17
6	Do you plant leaf-bud cuttings at 10 cm × 5 cm spacing?	6	5.00
7	Do you treat cuttings with IBA (2500 ppm) or other root stimulants like seradax and rootex?	9	7.5
8	Do you propagate between March and August for best results?	3	2.5

C	PLANTING TIME, CULTIVAR AND SPACING						
1	Do you plant Assam lemon between May and August?				120	100	
2	Do you use cultivar CRS-AL-1/CRS-AL-2/ CRS AL-3/ CRS-AL-4 (seedless variety)				120	100	
3	Do you prepare 0.5m × 0.5m pits with a soil-manure mix?				11	9.17	
4	Do you maintain 3m × 3m spacing between plants?				10	8.33	
D	PRUNING AND TRAINING						
1	Do you remove side branches up to 50-60 cm for a strong trunk?				25	20.83	
2	Do you prune diseased, injured, and crossing branches regularly?				99	82.50	
3	Do you cut ground-touching branches close to the laterals?				45	37.50	
E	MANURE AND FERTILIZER APPLICATION						
1	Do you use two split fertilizer applications (Feb/Mar & Oct/Nov).?				21	17.50	
	Year	FYM	N	P ₂ O ₅			K ₂ O
	1 st	5 kg	150g	100g			145g
	2 nd	10 kg	300g	200g			290g
	3 rd	15 kg	450g	300g			435g
	4 th	20 kg	600g	400g			580g
2	Do you apply fertilizers 15-45 cm away from the trunk?				7	5.83	
3	Do you spray 0.2% Polymax or Multiplex for micronutrients?				2	1.7	
F	IRRIGATION AND ORCHARD MANAGEMENT						
1	Do you weed your orchard monthly?				120	100	
2	Do you use paddy straw or plastic mulch for weed control?				88	73.33	
3	Do you irrigate during dry spells?				120	100	
G	INTERCROPPING						
1	Do you grow seasonal vegetables and leguminous crops as intercrop?				21	17.50	
2	Do you grow betelnut, pineapple, papaya as intercrop?				38	31.66	
H	PEST AND DISEASE MANAGEMENT						
1	For leaf miner do you use monocrotophos @ 2.5 g/l of water or Rogor@ 1.5 ml/l of water?				7	5.83	
2	For Trunk borer/ Bark eating caterpillar do you Prune and burn affected shoots and inject petrol in holes and plug with mud or cotton web?				92	76.66	
3	For Anthracnose/Dieback/Twig blight do you cut affected portion and paste Bordeaux paste?				19	15.83	
I	HARVESTING						
1	Do you harvesting criteria is when attain full size and develop attractive green to little yellow colour				99	82.50	
2	Do you know that Assam lemons are ready for harvesting from June to July and December to January?				95	17.50	

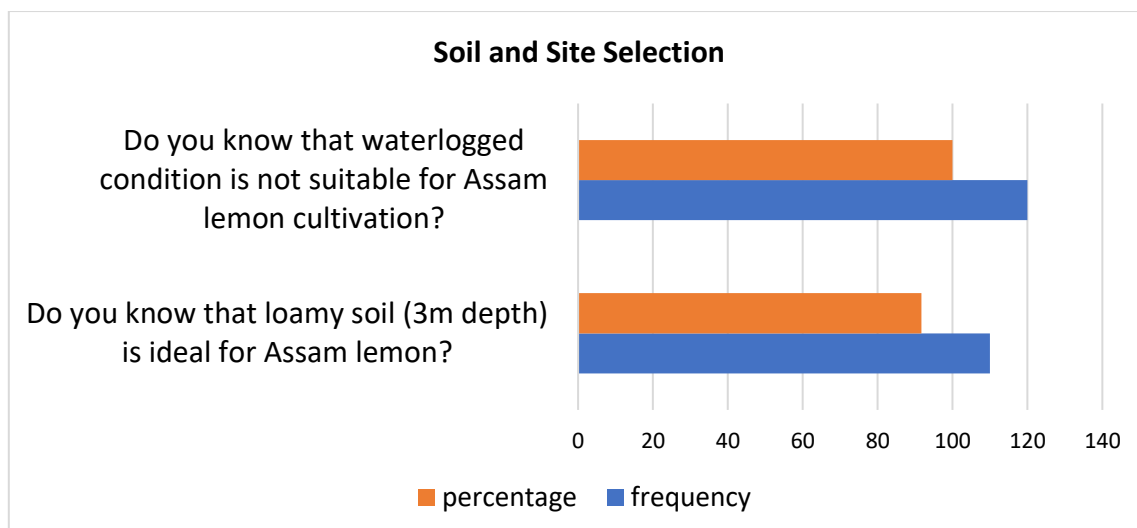


Fig 1. Distribution of respondents according to their knowledge on soil and site selection

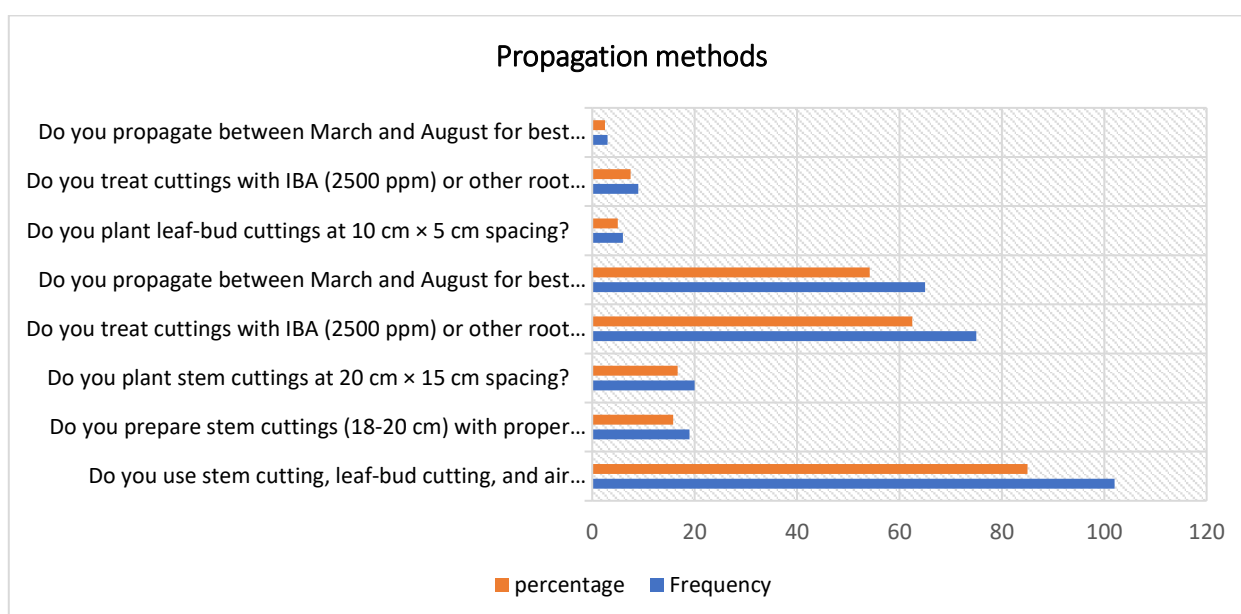


Fig 2. Distribution of respondents according to their knowledge on Propagation Methods

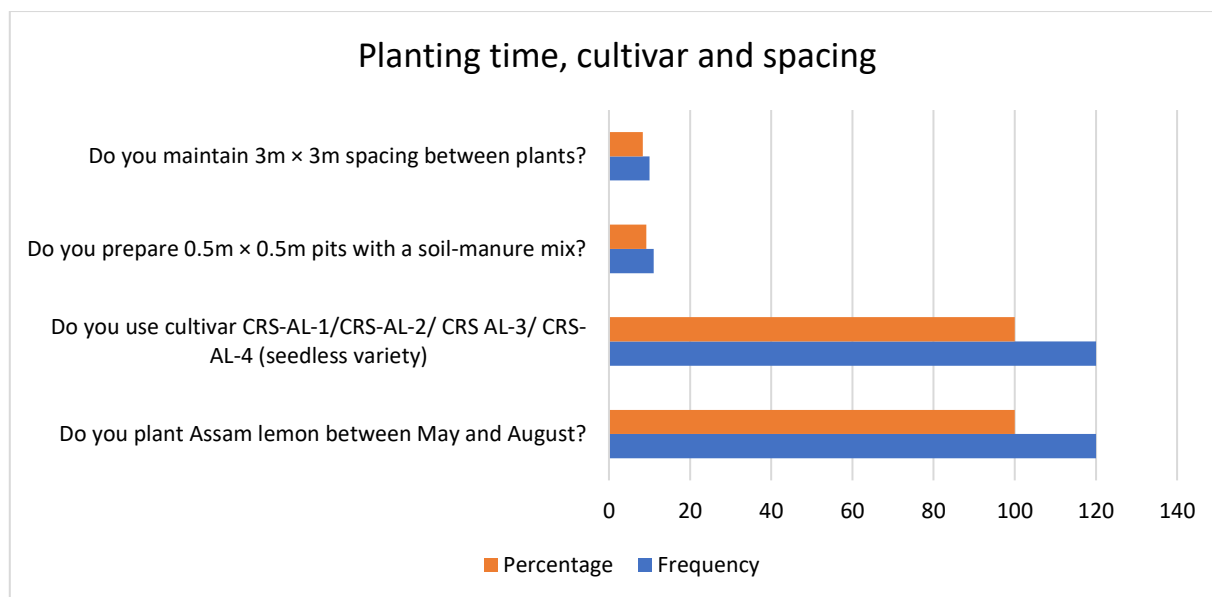


Fig 3. Distribution of respondents according to their knowledge on planting time, cultivar and spacing

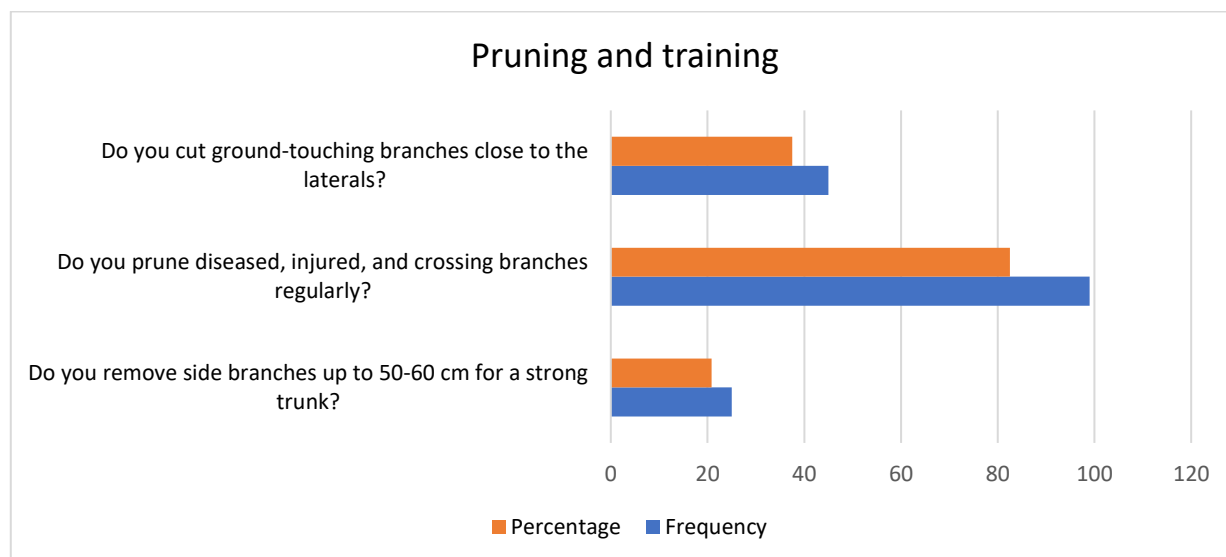


Fig 4. Distribution of respondents according to their knowledge on Pruning and training

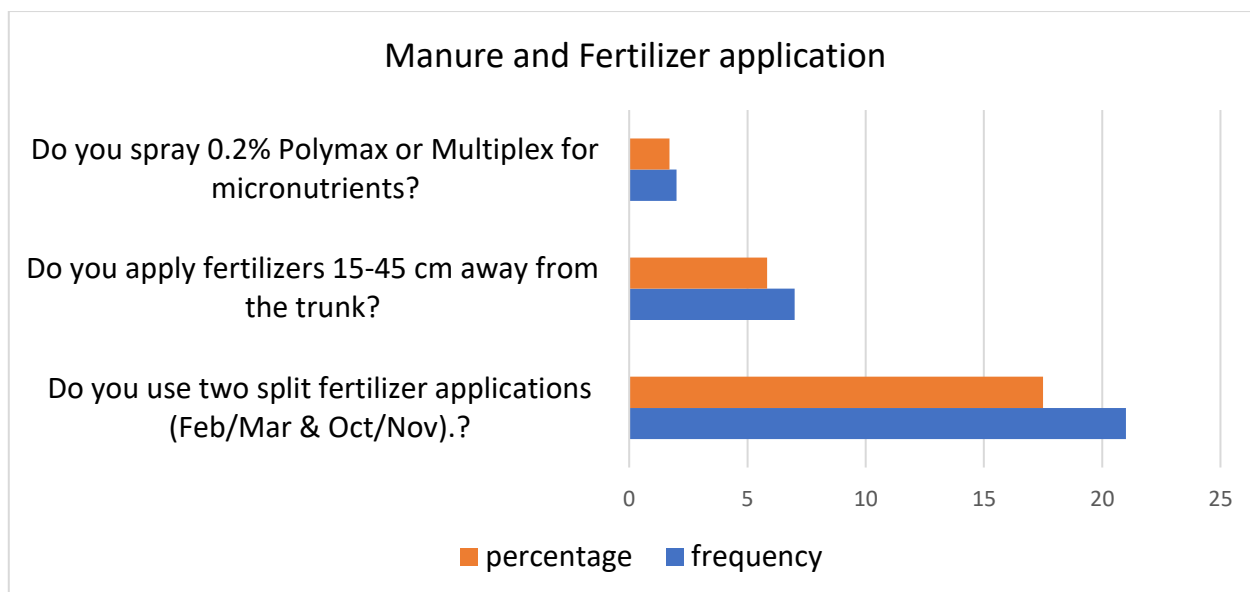


Fig 5. Distribution of respondents according to their knowledge on Manure and Fertilizer application

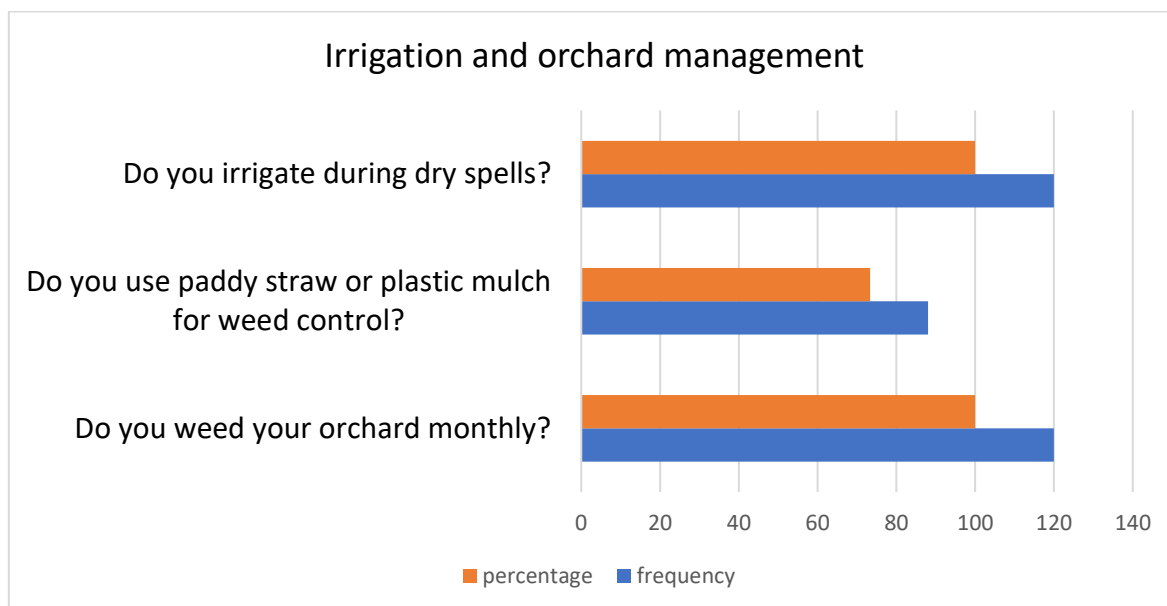


Fig 6. Distribution of respondents according to their knowledge on Irrigation and orchard management.

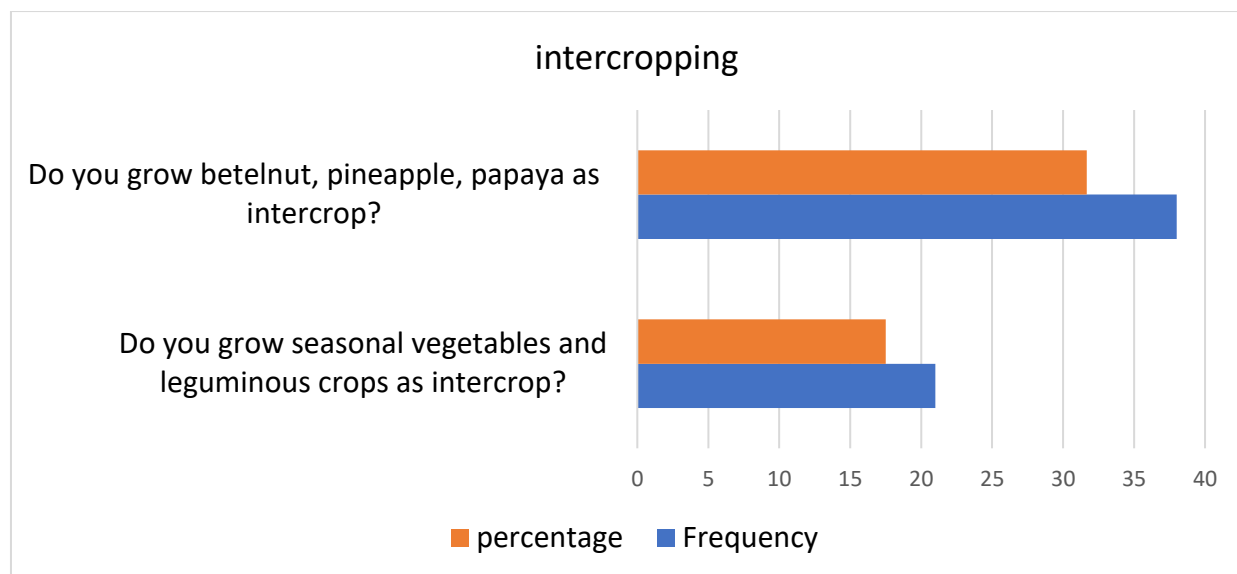


Fig 7. Distribution of respondents according to their knowledge on intercropping

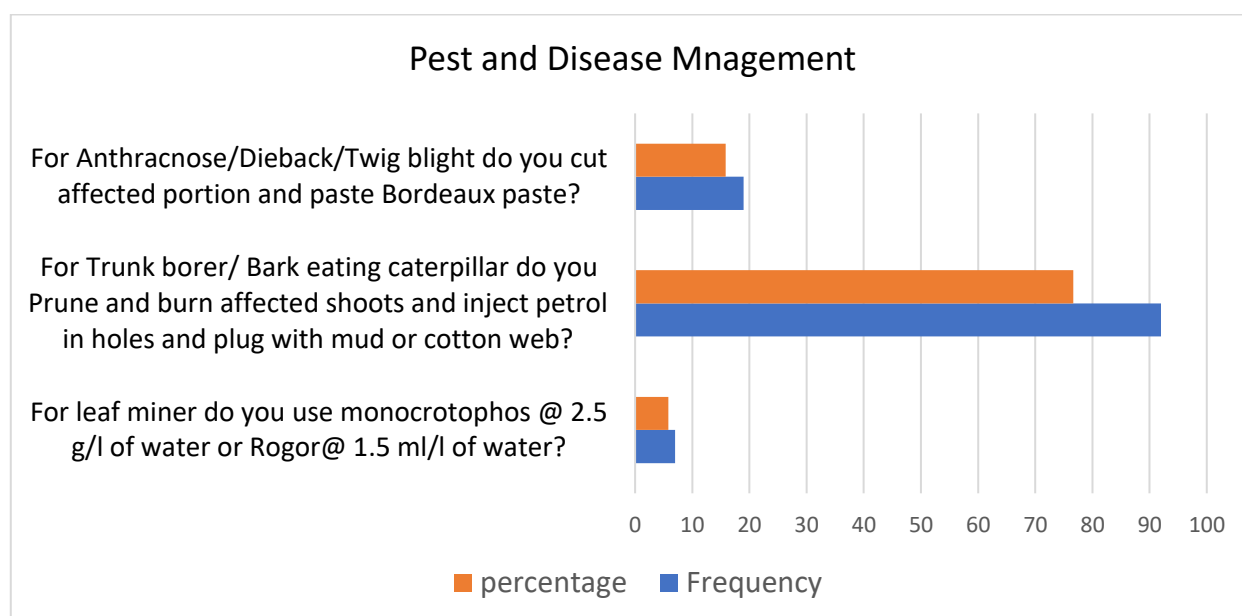


Fig 8. Distribution of respondents according to their knowledge on Pest and Disease Management

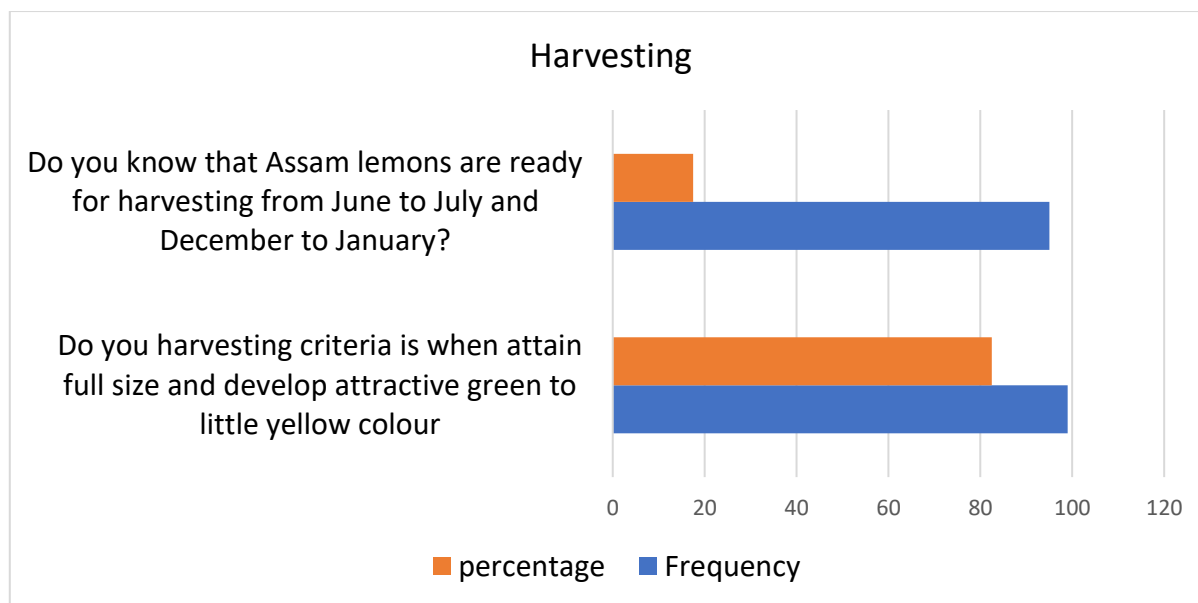


Fig 9. Distribution of respondents according to their knowledge on harvesting of Assam lemon

IV. DISCUSSION

A. Soil and Site Selection

Table 1 reflects that all 91.67 percent of the respondents were aware that loamy soil with a depth of about three meters is ideal for Assam lemon cultivation and 100 per that waterlogged conditions are unsuitable. This reflects a strong base of traditional and experiential knowledge among farmers, as soil suitability and drainage are easily observed in practice. The reason for this high awareness is that generations of farmers have experienced better growth and survival of lemon in well-drained soils, while losses under waterlogged conditions have made the disadvantage evident.

B. Propagation Methods

As depicted from table 1 and fig 2, it is noted that a majority of Assam lemon farmers (85.00%) in the sampled area knew about common propagation methods such as stem cutting, leaf-bud cutting, and air layering. However, when it came to technical details, adoption was very low. Only 15.83% prepared stem cuttings of 18–20 cm length with proper nodes, and just 16.67% followed the recommended spacing of 20 × 15 cm. Around 62.5% used IBA or root stimulants, while 54.17% were aware of the correct propagation season (March–August). In contrast, adoption of leaf-bud cutting recommendations was very poor—only 5% followed proper spacing, 7.5% treated with IBA, and 2.5% propagated during the right season. The probable reason behind this gap is lack of technical training and nursery management skills. Farmers generally depend on simple indigenous methods that are easier to apply, while

more scientific techniques require inputs, skill, and guidance, which are not easily available in rural areas.

C. Planting Time, Cultivar, and Spacing

From table 1 and fig 3, it can be found excellent awareness regarding planting time and improved varieties, with all respondents reporting that Assam lemon should be planted between May and August and that CRS-AL seedless varieties are desirable. However, very few followed proper pit preparation (9.17%) and spacing recommendations (8.33%). This may be attributed due to the fact that pit preparation requires more labour and resources, while spacing is often compromised due to small landholdings, where farmers prefer high-density planting to maximize the number of trees per unit area. This reflects a tendency to prioritize short-term yield over long-term orchard sustainability.

D. Pruning and Training

Data presented in table 1 and fig 4 reveals that most of the farmers (82.5%) practiced pruning of diseased and crossing branches, indicating that visible disease management is well understood. However, only 20.83% removed side branches up to 50–60 cm to develop a strong trunk, and 37.5% removed ground-touching branches. This shows that while farmers are proactive in addressing immediate health issues of the tree, they neglect systematic training practices that are essential for orchard structure and long-term productivity. The reason behind is that training techniques are more technical in nature, requiring scientific understanding, whereas disease-pruning provides quick and visible benefits that farmers are more inclined to adopt.

E. Manure and Fertilizer Application

Table 1 and fig 5 discloses that manure and fertilizer application practices showed very poor adoption. Only 17.5% of farmers applied fertilizers in two splits (February/March and October/November), and a mere 5.83% applied fertilizers at the correct distance from the trunk. Practices such as micronutrient application through foliar spray were seen to be very low i.e only 1.7 per cent of the respondents were found to use and have knowledge about micronutrients. The reasons include the high cost of fertilizers and micronutrients along with limited awareness of balanced nutrition, lack of extension services, and the traditional dependence on farmyard manure (FYM), which is more accessible and affordable compared to chemical inputs.

F. Irrigation and Orchard Management

Irrigation and orchard management practices were found to be well adopted. All respondents (100%) reported irrigating during dry spells and carrying out monthly weeding. Additionally, 73.33% practiced mulching with paddy straw or plastic as depicted in table 1 and fig 6. The high adoption of these practices can be attributed to their visible and immediate impact on plant survival, moisture conservation, and weed suppression. Farmers recognize these benefits through direct observation, which encourages them to consistently implement these practices without requiring much technical guidance.

G. Intercropping

Table 1 and fig 7 reveals that intercropping was found to be limitedly practiced by Assam Lemon growers of the sampled area. Only 17.50% of the farmers grew vegetables and legumes as intercrops, whereas 31.66% were seen to cultivate crops like betelnut, pineapple, and papaya along with Assam lemon. The probable reason behind this trend is that many farmers fear nutrient and moisture competition between crops and lemon trees, leading to hesitation in adopting intercrops. At the same time, crops like betelnut and pineapple are more popular because they are high-value cash crops and are considered compatible with lemon cultivation, thus providing additional income security to the growers.

H. Pest and Disease Management

The adoption of pest and disease management practices among farmers was found to be uneven as noted in Table 1 and fig 8. Only 5.83% of respondents reported using chemicals such as monocrotophos or Rogor for controlling leaf miner, showing very poor awareness of recommended chemical measures. In contrast, a large proportion (76.66%) managed trunk borer or bark-eating caterpillar by pruning and burning affected shoots and

injecting petrol in holes before plugging them with mud or cotton. This reflects a preference for practical and locally known methods over chemical control. For diseases such as anthracnose, dieback, and twig blight, only 15.83% of farmers followed the practice of cutting the affected portion and applying Bordeaux paste. The reason for such variation is that most farmers rely on simple physical or indigenous measures that they have learned traditionally, while technical recommendations like chemical sprays or Bordeaux paste are less popular due to lack of knowledge, high input cost, and limited accessibility to plant protection chemicals in rural areas.

J. Harvesting

As presented in table 1 and fig in terms of harvesting practices, 82.50% of respondents were aware of the correct maturity indicators, i.e., harvesting when fruits attain full size and develop an attractive green to slightly yellow colour. Similarly, 79.16% knew the correct harvesting seasons, which are June–July and December–January. This indicates that most farmers possess adequate knowledge about when and how to harvest Assam lemon. The probable reason for this high awareness is that harvesting is a visible and practical activity, and farmers have learned proper timing and criteria through continuous experience in the field. Unlike technical practices such as pruning or fertilizer application, harvesting is straightforward and directly linked to market quality and price, which makes farmers more conscious of following proper guidelines.

V. CONCLUSION

The study indicated that farmers showed strong awareness of fundamental practices such as appropriate soil and site selection, suitable planting seasons, use of recommended varieties, irrigation, and basic orchard maintenance. Nevertheless, notable gaps were observed in the adoption of more technical practices, including scientific propagation methods, pit preparation, proper spacing, canopy management through pruning, and balanced fertilizer application. In terms of pest and disease control, growers mostly relied on traditional or indigenous measures, while the use of recommended chemical treatments and micronutrient sprays was found to be minimal. Harvesting practices reflected comparatively higher knowledge levels, likely because they are straightforward and directly observable in the field. Overall, the findings suggest that although farmers' traditional experience has supported the continuation of Assam lemon cultivation, limited adoption of scientific practices remains a barrier to achieving higher yields and profitability. Therefore, enhancing farmer capacity through training,

ensuring timely access to quality inputs, and strengthening extension support are essential steps toward bridging these gaps and promoting Assam lemon as a sustainable and commercially rewarding fruit crop.

ACKNOWLEDGEMENT

The authors are grateful to all the sampled Assam lemon Growers of Tinsukia District for their invaluable participation primary data collection. We are also extremely thankful to the Directorate of Horticulture and Food processing Department for their cooperation in providing adequate secondary information on Assam lemon scenario of the sampled area and the state. We would also like to thank Dept. of Extension Education, FA, AAU and Dept. of Agricultural Statistics, FA, AAU for their constant support while conducting the study.

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Strategic Dissemination for Flour Mill Worker's Safety: Mitigating Health Hazards through Informed Practices

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Received: 02 Aug 2025; Received in revised form: 30 Aug 2025; Accepted: 04 Sep 2025; Available online: 13 Sep 2025

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Abstract— Amongst the hum of machinery and the ceaseless production of a dietary staple, the occupational health hazards confronting flour mill workers demand attention. This research undertook a comprehensive examination, shedding light on the intricate interplay between the working conditions and the problems faced by the workers within the confines of flour mills. In the heart of grain processing facilities, the health and safety of flour mill workers emerged as critical concerns. This research elucidates the complicated terrain of occupational health risks, highlighting the day-to-day struggles experienced by employees in this vital but frequently disregarded sector. This study was conducted on a sample of 100 respondents randomly selected from 20 flour mills of Ludhiana city. Ergonomic scales like OWAS and WERA were used to assess the work related postural discomfort experienced by the respondents. Results of postural analysis showed that for postures like full forward bending, half forward bending and standing with raised hands corrective measures need to be recommended in the near future and for postures like side bending and lifting immediate change is required. Factors contributing to worker's declined health were working in filthy conditions without using any personal protective equipment leading to respiratory issues whereas injuries like dislocations, slips, cuts, falls were also reported due to carrying heavy load on slippery floor and due to unguarded machines. Remedial measures like head cum face mask and information dissemination in the form of booklet were suggested for better workplace environment, reducing the musculoskeletal problems and injuries among the workers of the flour mills.



Keywords— Occupational health hazards, Flour dust, Flour mill workers, Postural discomfort, Indoor environment.

I. INTRODUCTION

Flour mills, integral to the global food production landscape, are not only the crucibles of sustenance but also environments where the well-being of the workforce faces nuanced challenges. As essential components in the grain processing chain, these facilities provide a cornerstone for society's dietary needs. However, within the intricate machinery and rhythmic processes lies a less visible concern – the occupational health hazards that cast shadows over the daily lives of flour mill workers. Since the 19th century, flour mills have been regarded as a boon for the purpose of turning cereals and grains into fine flour for

consumption. Previously, a fully mechanical process known as stone milling was used to grind the grains into a fine powder. This process used circular weights with a hole in the centre and a wooden handle at the periphery to rotate the upper stone disc weight. The upper stone's opening, known as the runner, was used to load grain, which was then spread across the lower stone, known as the sleeper, before being turned around by the wooden handle. Between the two substantial stone discs, the action of the stones smashes the entire grain, and the flour is gathered. As a result, preparing flour using conventional machinery takes a lot of time and work. On the onset of machines consisting shafts, belts and

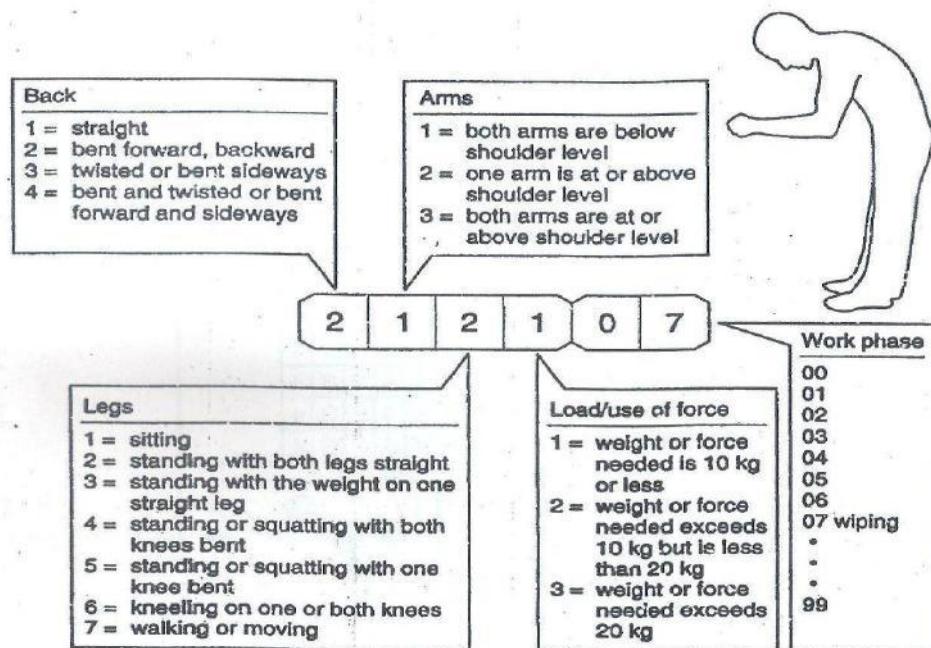
gears that are intended to reduce physical work and additional labour while increasing production. However, loading and unloading grain into the machine still needed manual labour, which involved putting the bulky grain sacs into the machine. Workers must lift the bulky bags over their shoulders to load them into the grinder or machine, pack the flour that has been produced, and then reload it or deliver it to customers. Additionally, employees at flour mills are more likely to have negative health impacts when exposed to flour dust and other unfavourable environmental factors, such as high temperatures, high relative humidity, loud noises, and inadequate lighting. Along with the dangers already described, there is another category of health risks that coexists with the other occupational risk factors. These risks are linked to the employees' inappropriate postures, which can result in the onset of work-related musculoskeletal illnesses. Loading, unloading, lifting, and packing are a few of the manual material-handling duties that cause workers to experience acute postural pain at work and an increased risk of occupational health risks. The main contributing factors for the onset of job-related musculoskeletal problems include recurrent forceful effort, bad posture, and forceful labour. This research embarks on a thorough exploration of the multifaceted occupational health challenges faced by those working in flour mills. The goal is not only to identify and understand the risks but, crucially, to develop informed strategies for intervention and knowledge dissemination. The choice of ergonomic scales, specifically the OWAS and WERA systems, allowed for a nuanced evaluation of the working conditions, offering insights into the postural stresses endured by mill workers. Beyond analysis, this research extended its scope to practical solutions, culminating in the creation of a comprehensive booklet and a head cum face mask. These interventions served a dual purpose: disseminating crucial knowledge among workers and providing tangible remedial measures for musculoskeletal disorders. As we delve deeper into the following sections, the focus will shift from the identification of occupational health hazards to the

formulation of informed interventions. By acknowledging the pivotal role of knowledge dissemination and practical measures, this study aspires to contribute not only to the academic discourse but, more importantly, to the well-being and safety of the dedicated individuals who form the backbone of the flour milling industry.

II. METHODOLOGY

The goal of this study was to compile and assess data on work related musculoskeletal disorders as well as the occupational health risks that workers in the city of Ludhiana's flour mills must deal with. The study was designed to be carried out in a certain order, starting with data collection, compilation, analysis, and ultimately data explanation. For the purpose of the mentioned study, 100 flour mill workers of Ludhiana city were selected for the study of prevalence of musculoskeletal disorders. Using the personal contact approach and the self-observation methodology, a field study of a sample of flour mill workers was undertaken. An interview schedule was created, pretested and utilised to collect information from the respondent's in-person. The use of relevant tools, subjective evaluation methods and objective assessment techniques was done in relation to the study of identifying work-related risk factors encountered by the workers. For assessing extent of postural discomfort ergonomic scales were used which are as follows:

- a. **OWAS method:** Ovako Working Position Analysis System, as provided by Karhu et al (1981). It defines the three primary areas—arms, back, and legs—where labour postures are most frequently used. The weight of the cargo carried is taken into account while calculating points. With the aid of these body components, a four-digit code is employed to depict the posture of the entire body. Four action types that were gleaned from the sources can be used to determine the necessity for ergonomic modifications. "Snapshot" observations were taken at regular intervals.



Achieving at Grand Score to identify the Action Level

Back	Arms	1			2			3			4			5			6			7			Legs
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	1	1	1	1	1	1	Use of force
	2	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	1	1	1	1	1	1	
	3	1	1	1	1	1	1	1	1	1	2	2	3	2	2	3	1	1	1	1	1	2	
2	1	2	2	3	2	2	3	2	2	3	3	3	3	3	3	3	2	2	2	2	3	3	Use of force
	2	2	2	3	2	2	3	2	2	3	3	4	4	3	4	4	3	3	4	2	3	4	
	3	3	3	4	2	2	3	3	3	3	3	4	4	4	4	4	4	4	4	2	3	4	
3	1	1	1	1	1	1	1	1	1	2	3	3	3	4	4	4	1	1	1	1	1	1	Use of force
	2	2	2	3	1	1	1	1	1	2	4	4	4	4	4	4	3	3	3	1	1	1	
	3	2	2	3	1	1	1	2	3	3	4	4	4	4	4	4	4	4	4	1	1	1	
4	1	2	3	3	2	2	3	2	2	3	4	4	4	4	4	4	4	4	4	2	3	4	Use of force
	2	3	3	4	2	3	4	3	3	4	4	4	4	4	4	4	4	4	4	2	3	4	
	3	4	4	4	2	3	4	3	3	4	4	4	4	4	4	4	4	4	4	2	3	4	

Action categories

- 1 no corrective measures
- 2 corrective measures in the near future
- 3 corrective measures as soon as possible
- 4 corrective measures immediately



Load / Force Use:

1= </= 10 Kg

2= 10 – 20 Kg

3= > 20 Kg

- b. **WERA method:** Workplace Ergonomic Risk Assessment, or WERA, was created by Rahman et al (2011). It is a tool for observation that offers a fast way to check working activities for exposure to physical risk factors linked to occupational musculoskeletal illnesses. This instrument addresses the five primary body areas of the shoulder, wrist, back, neck, and leg as well as the six risk variables of posture, repetition,

forceful, vibration, contact stress, and work length. It contains a score system and action levels that serve as a reference to determining the danger level. There are five stages involved in utilising this tool. Task observation is followed by task selection for assessment, task scoring, exposure calculation, and evaluation of action levels.

WORKPLACE ERGONOMIC RISK ASSESSMENT (WERA)

PHYSICAL RISK FACTOR		RISK LEVEL			SCORING SYSTEM																	
		LOW	MEDIUM	HIGH																		
1. Shoulder	1a. Posture				<table border="1"> <tr><td>Max</td><td>LOW</td><td>MED</td><td>HIGH</td></tr> <tr><td>Score</td><td>2</td><td>3</td><td>4</td></tr> <tr><td>W/O</td><td>3</td><td>4</td><td>5</td></tr> <tr><td>W/O</td><td>4</td><td>5</td><td>6</td></tr> </table>		Max	LOW	MED	HIGH	Score	2	3	4	W/O	3	4	5	W/O	4	5	6
	Max	LOW	MED	HIGH																		
Score	2	3	4																			
W/O	3	4	5																			
W/O	4	5	6																			
1b. Repetition				Score 1																		
2. Wrist	2a. Posture				<table border="1"> <tr><td>Max</td><td>LOW</td><td>MED</td><td>HIGH</td></tr> <tr><td>Score</td><td>2</td><td>3</td><td>4</td></tr> <tr><td>W/O</td><td>3</td><td>4</td><td>5</td></tr> <tr><td>W/O</td><td>4</td><td>5</td><td>6</td></tr> </table>		Max	LOW	MED	HIGH	Score	2	3	4	W/O	3	4	5	W/O	4	5	6
	Max	LOW	MED	HIGH																		
Score	2	3	4																			
W/O	3	4	5																			
W/O	4	5	6																			
2b. Repetition				Score 2																		
3. Back	3a. Posture				<table border="1"> <tr><td>Max</td><td>LOW</td><td>MED</td><td>HIGH</td></tr> <tr><td>Score</td><td>2</td><td>3</td><td>4</td></tr> <tr><td>W/O</td><td>3</td><td>4</td><td>5</td></tr> <tr><td>W/O</td><td>4</td><td>5</td><td>6</td></tr> </table>		Max	LOW	MED	HIGH	Score	2	3	4	W/O	3	4	5	W/O	4	5	6
	Max	LOW	MED	HIGH																		
Score	2	3	4																			
W/O	3	4	5																			
W/O	4	5	6																			
3b. Repetition				Score 3																		
4. Neck	4a. Posture				<table border="1"> <tr><td>Max</td><td>LOW</td><td>MED</td><td>HIGH</td></tr> <tr><td>Score</td><td>2</td><td>3</td><td>4</td></tr> <tr><td>W/O</td><td>3</td><td>4</td><td>5</td></tr> <tr><td>W/O</td><td>4</td><td>5</td><td>6</td></tr> </table>		Max	LOW	MED	HIGH	Score	2	3	4	W/O	3	4	5	W/O	4	5	6
	Max	LOW	MED	HIGH																		
Score	2	3	4																			
W/O	3	4	5																			
W/O	4	5	6																			
4b. Repetition				Score 4																		
5. Leg	5a. Posture				<table border="1"> <tr><td>Max</td><td>LOW</td><td>MED</td><td>HIGH</td></tr> <tr><td>Score</td><td>2</td><td>3</td><td>4</td></tr> <tr><td>W/O</td><td>3</td><td>4</td><td>5</td></tr> <tr><td>W/O</td><td>4</td><td>5</td><td>6</td></tr> </table>		Max	LOW	MED	HIGH	Score	2	3	4	W/O	3	4	5	W/O	4	5	6
	Max	LOW	MED	HIGH																		
Score	2	3	4																			
W/O	3	4	5																			
W/O	4	5	6																			
5b. Repetition				Score 5																		

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PHYSICAL RISK FACTOR

PHYSICAL RISK FACTOR		RISK LEVEL			SCORING SYSTEM																	
		LOW	MEDIUM	HIGH																		
6. Forceful	Lifting the load				<table border="1"> <tr><td>Max</td><td>LOW</td><td>MED</td><td>HIGH</td></tr> <tr><td>Score</td><td>2</td><td>3</td><td>4</td></tr> <tr><td>W/O</td><td>3</td><td>4</td><td>5</td></tr> <tr><td>W/O</td><td>4</td><td>5</td><td>6</td></tr> </table>		Max	LOW	MED	HIGH	Score	2	3	4	W/O	3	4	5	W/O	4	5	6
	Max	LOW	MED	HIGH																		
Score	2	3	4																			
W/O	3	4	5																			
W/O	4	5	6																			
6b. Repetition				Score 6																		
7. Vibration	Using of vibration tool				<table border="1"> <tr><td>Max</td><td>LOW</td><td>MED</td><td>HIGH</td></tr> <tr><td>Score</td><td>2</td><td>3</td><td>4</td></tr> <tr><td>W/O</td><td>3</td><td>4</td><td>5</td></tr> <tr><td>W/O</td><td>4</td><td>5</td><td>6</td></tr> </table>		Max	LOW	MED	HIGH	Score	2	3	4	W/O	3	4	5	W/O	4	5	6
	Max	LOW	MED	HIGH																		
Score	2	3	4																			
W/O	3	4	5																			
W/O	4	5	6																			
7b. Repetition				Score 7																		
8. Contact stress	Using of tool handle Or wearing hand gloves				<table border="1"> <tr><td>Max</td><td>LOW</td><td>MED</td><td>HIGH</td></tr> <tr><td>Score</td><td>2</td><td>3</td><td>4</td></tr> <tr><td>W/O</td><td>3</td><td>4</td><td>5</td></tr> <tr><td>W/O</td><td>4</td><td>5</td><td>6</td></tr> </table>		Max	LOW	MED	HIGH	Score	2	3	4	W/O	3	4	5	W/O	4	5	6
	Max	LOW	MED	HIGH																		
Score	2	3	4																			
W/O	3	4	5																			
W/O	4	5	6																			
8b. Repetition				Score 8																		
9. Task duration	Task/hr/day				<table border="1"> <tr><td>Max</td><td>LOW</td><td>MED</td><td>HIGH</td></tr> <tr><td>Score</td><td>2</td><td>3</td><td>4</td></tr> <tr><td>W/O</td><td>3</td><td>4</td><td>5</td></tr> <tr><td>W/O</td><td>4</td><td>5</td><td>6</td></tr> </table>		Max	LOW	MED	HIGH	Score	2	3	4	W/O	3	4	5	W/O	4	5	6
	Max	LOW	MED	HIGH																		
Score	2	3	4																			
W/O	3	4	5																			
W/O	4	5	6																			
9b. Repetition				Score 9																		

FINAL SCORE

Job/Task : _____

Date : _____

Observer : _____

Action Level		Risk (V)	
Risk Level	Final Score	Task is acceptable	
LOW	18-27	☐	
MED	28-44	☐	
HIGH	45-54	☐	

Based on WERA: An observational tool developed to investigate the physical risk factor associated with WERA, Shahrul Huzairi Abdul Rahuman, et al. (2011) and right United Network, Journal of Human Ergology, 2011, 40(2), 19-40

- c. Some statistical techniques such as frequency and percentages, mean scores and standard deviation were used to analyze the collected data.
- d. Also for investigating the comfort and satisfaction level of the developed interventions data was collected through scoring as per the table given below:

For comfort level

Response	Score
Extremely comfortable	
Comfortable	
Uncomfortable	

Scores: 3-Extremely comfortable, 2- Comfortable, 1- Uncomfortable

For satisfaction level

Response	Score
Highly satisfied	
Satisfied	
Moderately satisfied	

Scores: 3- Highly satisfied, 2- Satisfied, 1- Moderately satisfied

III. RESULTS

When the data collected was analysed it was observed that according to the socio personal profiles of the chosen respondents, 35% of the respondents were between the ages of 26 and 35 and majority of the respondents (65%), according to their employment profiles, put in 10 to 12 hours a day at their jobs with just 1.5 to 2 hours of rest break. There were 12 percent such respondents who used to cover a distance of 3.5 to 5 km daily to reach their workplace. The remuneration obtained by the workers in exchange of performing such hazardous work was also not very satisfactory, 68% of the respondents were getting paid less than Rs.15,000 per month. It has been observed during survey that the main tasks performed at flour mills were loading, unloading, stacking grain bags, cleaning the workplace, moving grain bags to the grinder machine, packaging the flour into bags, and distributing it to clients. Along with the aforementioned tasks, some workers were also involved in the operation of machinery installed at flour mills, such as the weighing scale used to measure grain and flour before and after grinding, the trolley frequently used to move grain bags, and the wheelbarrow used to guarantee the continuous operation of the grinding machine. Every action was carried out by every respondent at some point, according to observations.

Table 1: Job profile of the respondents n=100

Job profile	Frequency	Percentage (%)
Mill job		
Owner cum worker	08	08.00
Worker	92	92.00
Distance from home to workplace (km)		
Less than 1.5 km	45	45.00
1.5 to 3 km	43	43.00
3.5 to 5 km	12	12.00
Work duration		
8-9 hours	30	30.00
10 -12 hours	65	65.00
More than 12 hours	05	05.00
Monthly income of respondent (Rs) from the mill job		
Less than 15,000	68	68.00
15,000-20,000	24	24.00
More than 20,000	08	08.00
Average	Rs 12530/-	
Years of working in flour mill		
1 to 5 years	50	50.00
6 to 10 years	44	44.00
11 to 15 years	06	06.00
Reasons to take up mill worker job		
To inherit family business	08	08.00
Sole bread earner of the family	47	47.00
Near to home	35	35.00
Other	10	10.00

During the research survey it has been observed that it was not only the extreme environmental conditions responsible for ruining the health of the workers, but the other factor that was contributing in deteriorating the health of the respondents was the prevalence of musculoskeletal disorders. Every activity performed in the flour mill requires a lot of exertion and typical body postures which results in development of many problems immediately or gradually. Majority of the selected respondents being illiterate were unaware regarding adoption of correct body postures during work to minimize their exertion. They were informed about the activities to be performed according to their convenience without being aware about the adoption

of wrong postures. They face the difficulty as such and accepted that there is only one way to perform that particular activity. When asked the selected respondents about which activity they find most difficult to perform, multiple responses were observed depicted by the table below. As results reported by Petit *et al* (2016) are also in line with these findings who reported that half of the unskilled employees are exposed to Manual Material Handling (MMH) leading to chronic low back pain (LBP) and work impairment. Occupational injuries, limb musculoskeletal problems, and cardiovascular disease are all caused by manual material handling.

Table 2: Distribution of respondents as per difficulty in performing different activities
n=100

Activities	Frequency	Percentage (%)
Loading	56	56.00
Lifting	54	54.00
Cleaning	43	43.00
Unloading	40	40.00
Delivery	45	45.00

Injury or discomfort in the muscles, ligaments, and joints that support the limbs, neck, and back are referred to as MSDs (musculoskeletal diseases). A quick physical effort (such as carrying a heavy object), repetitive strain, or recurrent exposure to force, vibration, or an uncomfortable posture can all lead to MSDs [3]. As a result, respondents' musculoskeletal issues were evaluated using ergonomic scales. Two objective scales were used to assess the postures adopted by the respondents while carrying out their regular tasks at workplace.

Five of the most uncomfortable and common positions were chosen in order to analyse respondents' working postures while engaging in various tasks at work. As described in the methodology, the low cost posture analysis techniques OWAS (Ovako Work Assessment system) and WERA (Work Ergonomic Risk Assessment) were utilised to examine the chosen postures and have been discussed below

from table 2 to table 6.



Fig. 1: Full forward bending

Table 3: Analysis of posture I: Full forward bending

OWAS						
Posture	Back	Arms	Legs	Load/Effort	Final score	Action category
Score	2	1	2	1	2	Corrective measures in the near future

WERA											
Postur e	Shoulder	Wrist	Back	Neck	Leg	Forceful	Vibration	Contact stress	Task duration	Final score	Action level
Score	4	2	6	5	4	5	2	4	5	37	Task in need to further investigate and required change



Fig 2: Half forward bending

Table 4: Analysis of posture II: Half forward bending

OWAS						
Posture	Back	Arms	Legs	Load/Effort	Final score	Action category
Score	2	1	3	1	2	Corrective measures in the near future

WERA											
Posture	Shoulder	Wrist	Back	Neck	Leg	Forceful	Vibration	Contact stress	Task duration	Final score	Action level
Score	3	4	4	2	4	3	4	5	3	32	Task in need to further investigate and required change



Fig 3: Standing with raised hands

Table 5: Analysis of posture III: Standing with raised hands

OWAS						
Posture	Back	Arms	Legs	Load/Effort	Final score	Action category
Score	2	3	2	3	2	Corrective measures in the near future are required

WERA											
Posture	Shoulder	Wrist	Back	Neck	Leg	Forceful	Vibration	Contact stress	Task duration	Final score	Action level
Score	6	5	3	4	3	2	6	6	3	38	Task in need to further investigate and required change



Fig 4: Side bending

Table 6: Analysis of posture IV: Side bending

OWAS						
Posture	Back	Arms	Legs	Load/Effort	Final score	Action category
Score	3	1	4	1	3	Corrective measures as soon as possible are required

WERA											
Posture	Shoulder	Wrist	Back	Neck	Leg	Forceful	Vibration	Contact stress	Task duration	Final score	Action level
Score	5	6	6	4	5	4	6	6	3	45	Task is not accepted, immediately change



Fig 5: Lifting

Table 7: Analysis of posture V: Lifting

OWAS						
Posture	Back	Arms	Legs	Load/Effort	Final score	Action category
Score	3	1	4	1	3	Corrective measures as soon as possible are required

WERA											
Posture	Shoulder	Wrist	Back	Neck	Leg	Forceful	Vibration	Contact Stress	Task duration	Final score	Action level
Score	3	4	5	4	4	4	6	5	5	37	Task is not accepted, immediately change

As per the scores shown by the tables above, the most dangerous postures, according to the Ovako Working Posture Analysis System (OWAS), were lifting, side bending, half forward bending, and front bending. These postures required immediate correction. However, Work Ergonomic Risk Assessment (WERA) found that the side-bending position was unacceptable and needed to be changed right now. Based on the aforementioned findings, it is inferred that workers at flour mills put in a lot of overtime in challenging conditions for little pay. The risk factors for accidents and musculoskeletal problems, as well as various safety and security issues, were all presented to the respondents. The responders' postures were also the most hazardous and needed immediate attention, according to objective ratings. As a result, the user group should be offered suitable solutions to lessen the suffering. In order to prevent compromises with the company's productivity and health, one should be vigilant about adhering to the fundamental requirements connected to the job and the workplace.

Use and type of Personal Protective Equipment preferred by the respondents while working in the flour mills.

Personal Protective Equipment (PPE) aids in the protection of workers' health from hazardous working circumstances. During the current investigation, because of the prevalence of contagious viral infection, it was discovered that overall personal protective equipment (PPE) kit was used by a small number of workers at workplace while performing usual tasks, recommended by the customers to ensure safety and protection. Hence, some flour mill owners provided their workers the overall PPE kit. As Table 7 indicates that only 34 percent agreed with the using of PPE while 66 percent didn't bother about using any kind of PPE while working in the flour mill during viral infection. However, due to the abundance of flour dust and workers' close proximity with the flour grinding machine makes it a pre requisite to use PPE for their safety. Personal protective equipment was substantially connected with chronic respiratory health complications among flour mill industrial workers; therefore workers used pieces of cloth

instead of respirators/dust masks. It has been concluded that, workers in flour mills who wore pieces of cloth were less likely to acquire long term respiratory issues than those who did not. Along with this they found that the use of respiratory safety equipment among flour mill workers was quite low. Even though the majority of workers recognized

the need of wearing PPE to protect themselves from wheat dust particles, the mill owners did not provide workers with PPE. Following are some of the PPE discussed along with the percentage used by the workers of the flour mills in order to protect them from the flour dust and other debris to some extent.

Table 8: Use and type of PPE preferred by the workers in the flour mills.

n=100

PPE	Always		Sometimes		Never	
	Frequency	Percentage (%)	Frequency	Percentage (%)	Frequency	Percentage (%)
Overall PPE kit	34	34.00	-	-	66	66.00
Other PPE						
Head gear	06	06.00	56	56.00	38	38.00
Mask	49	49.00	35	35.00	16	16.00
Apron	-	-	-	-	100	100.00
Gloves	-	-	08	08.00	92	92.00

Score: 3-always, 2-sometimes, 1-never

Reasons for not using any PPE while working

On observation it has been seen that workers were all covered in flour dust while working and not provided with any kind of PPE by the owners. Because of low finances they were not able to afford buying proper PPE for themselves. It has been seen that few workers tied a piece of cloth on their head and mouth to safeguard them against the flour dust. Even after knowing that it is beneficial to wear PPE, the flour mill workers were seen without wearing one, it is understandable that the activities performed by the flour mill workers requires a lot of frequent movement that may be restricted by wearing another layer of fabric. But one can use proper head gear, masks and apron just to minimize the adherence of flour dust on their body parts. The reasons given by the workers for not wearing PPE are discussed as follows.

Uncomfortable: When enquired for the reason of not using PPE, data revealed that more than half of the respondents (57%) found it uncomfortable wearing any kind of PPE while working at their workplace.

Unaffordable: It has been observed through the data collected that 40 percent of the respondents agreed with the statement that using PPE is useful for them but they can't afford purchasing good quality PPE. Due to their limited

wages and family expenses they could not afford of purchasing PPE of adequate quality.

Don't know how to use it: It has been concluded through the field study that due to the onset of pandemic only few owners provided their workers with PPE kits because of the pressure build by the customers on them for checking the viral infection. But the problem was that they didn't give any information regarding how to use that PPE kit and how to maintain it further. Subsequently, as per the data of Table 8, it was concluded that 85 percent of the respondents actually did not know the correct ways of using proper PPE kit.

Creates hindrance while working: Concerning about hindrance created to the workers by wearing PPE while working, it was found that 43 percent of respondents agreed with the statement that PPE does provide a hindrance in body movements, impacting their work effectiveness.

Never been informed to use PPE: When the selected respondents were enquired about having knowledge about the use of PPE, then it has been revealed that only five percent of the respondents knew about the PPE and their use, whereas 95 percent were not having any awareness regarding PPE use and its maintenance.

Table 9: Distribution of respondents as per the reasons for not using PPE

n=100

Reasons	Frequency	Percentage (%)
Uncomfortable	57	57.00
Unaffordable	40	40.00
Don't know how to use it	85	85.00
Creates hindrance while working	43	43.00
Never been informed to use PPE	95	95.00

DEVELOPING INTERVENTION AND PREPARATION OF GUIDELINES.

On the basis of results of field survey and suggestions given by the flour mill workers an intervention was designed i.e Head Cover cum Face Mask. This intervention was designed and tested on selected respondents to protect them from excessive inhaling of flour dust. Concerning to minimize the extent of risk factors faced by the workers engaged in flour mills, significant remedial and corrective measures in the form of booklet including various exercises and curative postures were suggested for the workers of the flour mills.

Head Cover Cum Face Mask: To protect the workers from excessive inhaling of flour dust, which was the main health problem they were facing in flour mills, a suitable head cover cum face mask was designed. This mask is made from soft cotton fabric which is breathable and comfortable to wear. It has two soft fabric bands on either side with Velcro fastened at their ends that help it fit properly according to

the wearer's comfort. This intervention was introduced to the workers for assessing its comfort and satisfaction level among them while performing flour mill activities. This intervention was introduced to them and their comfort and satisfaction level was assessed while performing different flour mill activities. The comfort and satisfaction level of flour mill workers have been discussed in Fig 6 and 7 respectively.

Comfort level of the workers while using the developed intervention

Regarding comfort Fig.6 indicates that 70 percent of the respondents felt extremely comfortable while using head cover cum face mask as it completely fit to their head and face and their exposure to excessive flour dust was minimized, whereas 30 percent of the respondents find the intervention comfortable. However, none of the respondents felt that it was uncomfortable to use while performing various activities.

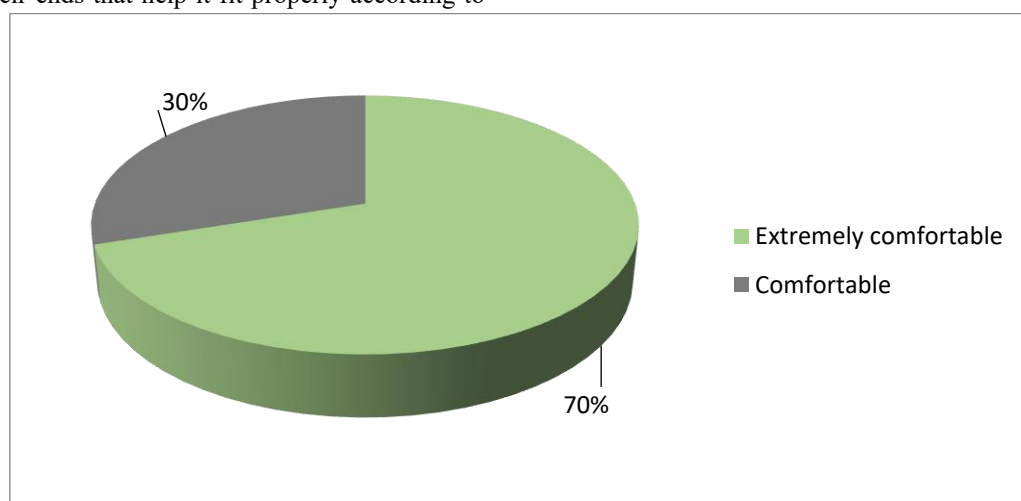


Fig 6: Distribution of the respondents as per comfort level achieved with the intervention

Satisfaction level of the workers while using the developed intervention

Regarding satisfaction Fig.7 indicates that 80

percent of the respondents were highly satisfied while using head cover cum face mask, whereas 10 percent of the respondents found the intervention satisfactory. Further, moderate satisfaction was reported by 10 percent of the

respondents as they were not use to of wearing any kind of

PPE while performing various activities.

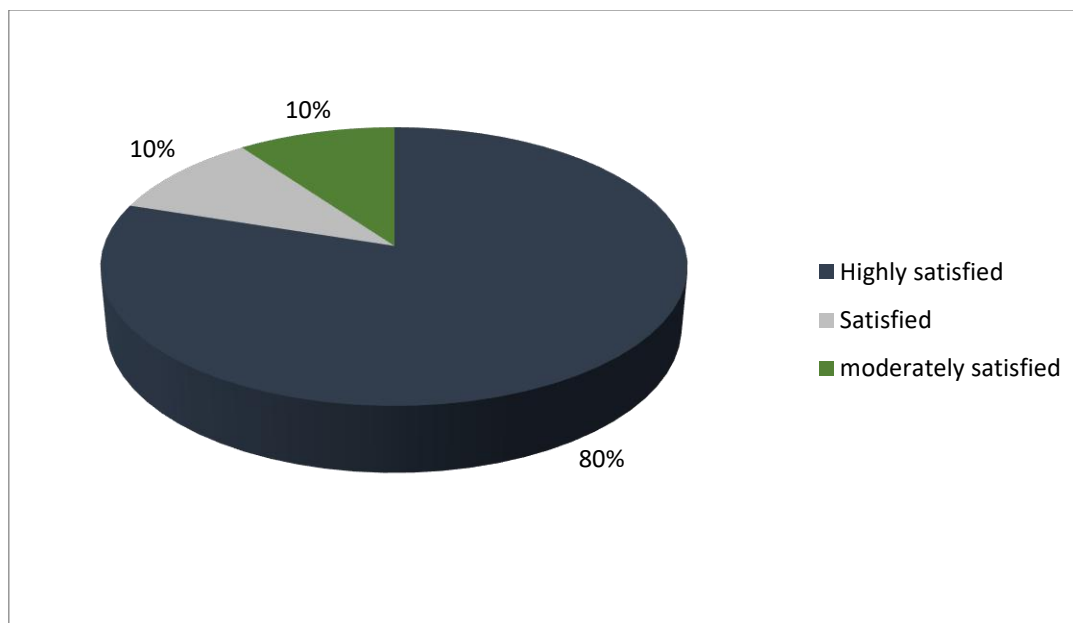


Fig. 7: Distribution of respondents as per satisfaction level after using head cover cum face mask

A small booklet constituting the guidelines for minimising the health risks among flour mill workers was also developed. It included certain instructions to be considered for workplace environment with some modifications in techniques like anti slip flooring and safe load carrying. Also there were some suggested postures to reduce the injuries and musculoskeletal problems of the workers for seated as well as for the standing tasks. Then there mentioned some yoga exercises like tadasana, shoulder shrugs, balasana and some exercises to strengthen neck upper back, shoulders, arms ,wrist and hand muscles and to prevent future injuries at workplace.

IV. CONCLUSION

A pleasant workplace atmosphere encourages employees to be productive enough to complete the tasks. For occupational health services to be provided in the flour mill's workplace in a practical and efficient manner, both the owners and managers of the company must be attentive of occupational health and safety. Additionally, it's important to be aware of the numerous hazards connected to adopting poor postures and to properly occupy the workplace with hazard management measures. This will improve the productivity of the company, lower the chance of accidents, and undoubtedly improve the health of the employees. Investment in ergonomic treatments is highly necessary to reduce deaths and health risks associated with working in a dangerous workplace. Only by carefully taking into account the physical, mental, and social factors while

building a workplace can the efforts be optimised. Small processing facilities reportedly lack these crucial factors, and the employees go about their business without taking any safety precautions. When picking and implementing a technology, planning and creating any workspace in line with ergonomic principles is essential since it creates compatibility between the worker and their environment. Only by incorporating ergonomics into workplace design can one accurately balance the needs of the task with the peculiarities of the personnel.

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Antifungal Activity of Endophytic *Nigrospora* Species Isolated from *Pluchea* Plants against Some Fungal Phytopathogens

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Received: 05 Aug 2025; Received in revised form: 04 Sep 2025; Accepted: 08 Sep 2025; Available online: 15 Sep 2025

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Abstract— Endophytic fungi represent promising biocontrol agents due to their ecological compatibility and production of diverse bioactive metabolites. In this study, two endophytic *Nigrospora* species *N. sphaerica* and *N. osmanthi* were isolated from healthy leaves of *Pluchea dioscoridis* and evaluated for their antagonistic activity against eight phytopathogenic fungi, including foliar pathogens (*Alternaria* sp., *Stemphylium* sp. and *Myrothecium verrucaria*) and soilborne pathogens (*Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Sclerotium rolfsii*). Dual culture assays revealed that *N. sphaerica* exhibited superior inhibitory effects, achieving up to 60% inhibition against *Alternaria* sp. and 53% against *M. verrucaria*, alongside moderate suppression of soilborne pathogens. Correspondingly, filtrate assays showed significant biomass reduction, particularly in foliar fungi, suggesting the presence of potent diffusible antifungal metabolites. GC–MS profiling of *Nigrospora sphaerica* and *N. osmanthi* culture extracts revealed the predominance of phenol, 2,4-bis (1,1-dimethylethyl)-, along with several key antifungal metabolites. *N. sphaerica*, in particular, exhibited a broader chemical spectrum, producing α -linolenic acid, arachidonic acid, lactic acid, citric acid, 2-butenedioic acid, glycerol, and aromatic hydrocarbons compounds with well-documented antifungal properties. This highlights the value of isolating safe endophytic fungi capable of naturally synthesizing potent bioactive metabolites for sustainable fungal disease management. These findings highlight *N. sphaerica* as a strong candidate for further development in sustainable plant disease management. However, additional *in vivo* and field-based investigations are essential to validate the practical application of endophytic *Nigrospora* spp. as biological control agents against fungal phytopathogens.

Keywords— *Pluchea dioscoridis*, *Nigrospora sphaerica*, biocontrol, GC–MS, antifungal activity, foliar pathogens, endophytic fungi, culture filtrates.



I. INTRODUCTION

Pluchea dioscoridis (L.) DC., commonly known as “Barnûf,” is a perennial wild shrub in the family *Asteraceae*, naturally distributed across arid and semi-arid regions of North Africa and the Middle East, including the Nile Valley, Delta, and desert peripheries of Egypt. In addition to its extensive use in traditional medicine for treating ailments such as colds, rheumatism, gastric ulcers, and epilepsy, it holds notable ecological value. This species contributes to soil stabilization, desertification control, and

phytoremediation of heavy metal contaminated soils, making it important for both biodiversity conservation and environmental restoration (Youssef and Diatta, 2023). Its adaptability to harsh climates and ability to host diverse microbial endophytes further enhance its potential as a model for plant microbe interaction studies under stress conditions.

significant threat to agricultural productivity, especially in tropical and subtropical regions. Among foliar pathogens, *Stemphylium* spp., *Alternaria* spp., and *Myrothecium*

verrucaria are well-known for causing leaf spots and blight symptoms, which compromise photosynthetic efficiency and reduce overall plant growth and yield (Fravel et al., 2003). Meanwhile, soilborne fungal pathogens continue to jeopardize root health in many economically important crops. Notably, species of *Fusarium*, such as *F. oxysporum* and *F. solani*, are well-established causal agents of vascular wilt, characterized by xylem colonization and systemic blockage that ultimately leads to plant death. *Macrophomina phaseolina* is a common cause of charcoal rot, particularly under heat and drought stress conditions, while *Rhizoctonia solani* and *Sclerotium rolfsii* are responsible for damping-off and stem/collar rots in a wide host range (Fontana et al., 2021).

Although chemical fungicides have long been the mainstay of disease management, their excessive use health risks to humans and non-target organisms. These challenges have spurred the development of sustainable alternatives, with biological control emerging as a promising strategy (Fontana et al., 2021).

In this context, endophytic fungi microorganisms that colonize internal plant tissues without causing visible disease to have gained increasing attention for their ability to produce diverse bioactive secondary metabolites with antifungal properties (Manganyi, 2020). These fungi can suppress pathogens via competition for nutrients and space, mycoparasitism, production of inhibitory metabolites, or by triggering systemic resistance in host plants. Among the most promising endophytes, species of the genus *Nigrospora* have attracted attention due to their biochemical diversity and antagonistic potential. For example, *Nigrospora* sp. produces phomalactone, a broad-spectrum antifungal compound active against several major phytopathogens (Ramesha et al., 2020). More recently, other metabolites such as nigrosphaeritriol and nigrosphaerilactol have been identified from *N. sphaerica*, showing potent antifungal activity and potential for development as eco-friendly fungicides (Salvatore et al., 2024). Importantly, endophytes isolated from the same host plant are often ecologically pre-adapted, enhancing their compatibility and persistence, which supports their utility as long-term biological control agents (Manganyi, 2020).

Given these considerations, the present study aims to isolate and characterize endophytic *Nigrospora* species from healthy *P. dioscoridis* plants and evaluate their antagonistic activity against a spectrum of fungal phytopathogens isolated from diseased *P. dioscoridis*, soil, and other crops. The goal is to identify ecologically compatible endophytes with strong biocontrol potential, contributing to sustainable crop protection while reducing dependence on synthetic fungicides and supporting environmentally sound agricultural practices.

II. MATERIALS AND METHODS

1. Plant Material Collection and Taxonomic Verification

Field surveys were conducted during the active growing season of *Pluchea dioscoridis* (L.) DC. Both symptomatic plants showing typical foliar disease symptoms (necrotic spots, chlorotic halos, and irregular lesions) and asymptomatic, apparently healthy plants were targeted. From each site, representative samples including leaves, stems, and roots were collected in sterile polyethylene bags and transported to the laboratory under cooled conditions. Plant specimen was identified by Dr. Omran Ghaly, Herbarium of Desert Research Center (CAIH) with Identification Code: CAIH-1350-R.

2. Isolation of Fungal Pathogens from Diseased Plants and Soil

Isolation procedures aimed to recover the full spectrum of fungal pathogens associated with *P. dioscoridis*. Diseased tissues were first washed under running tap water to remove debris, surface-sterilized in 1% sodium hypochlorite for 2 minutes, rinsed thrice with sterile distilled water, and blotted dry on sterile filter paper. Tissue segments (5 mm²) from lesion margins were plated onto Potato Dextrose Agar (PDA; Difco, USA) supplemented with streptomycin (100 mg/L) to suppress bacterial contamination. Plates were incubated at 25 ± 2°C in darkness for 5–7 days, and emerging fungal colonies were purified by hyphal-tip or single-spore isolation (Leslie and Summerell, 2006).

To broaden the pathogen panel, additional isolates were obtained from rhizosphere soils and infected tissues of other crops grown in proximity to *P. dioscoridis*, including faba bean, citrus, peanut, and alfalfa. Soil samples were processed using soil dilution plating, while plant tissues followed the same surface sterilization and plating protocol.

3. Morphological Identification of Pathogens

Purified isolates were transferred to fresh PDA and incubated under controlled conditions to induce sporulation. Colony morphology, pigmentation, and growth rate were documented, while microscopic examination of conidia, hyphae, and reproductive structures was performed using a compound microscope (Olympus BX51). Identification to genus level relied on morphological keys and descriptions (Barnett and Hunter, 1998), and representative isolates were retained for molecular identification.

4. Pathogenicity Testing

Pathogenicity of each isolate was confirmed using detached leaf assays (Bashyal et al., 2022). Healthy leaves of *P. dioscoridis* were surface-sterilized and placed on moist filter paper in sterile Petri dishes. Mycelial plugs (5 mm diameter) from actively growing cultures were placed on the leaf surfaces; controls received sterile PDA plugs. Plates

were incubated at 25 °C under a 12 h photoperiod, and lesion development was assessed over 5–7 days. Pathogens were re-isolated from symptomatic leaves and compared morphologically to the original cultures to satisfy Koch's postulates.

5. Isolation of Endophytic Fungi from Healthy Plants

Endophytic fungi were isolated from symptom-free tissues of *P. dioscoridis* collected from the same sites as diseased plants. Leaves and stems were surface sterilized by immersion in 1% sodium hypochlorite for 2 minutes, followed by 70% ethanol for 30 seconds, then rinsed in sterile distilled water. After blotting dry, tissue segments (5 mm²) were plated on PDA supplemented with streptomycin. Plates were incubated at 25 °C for up to 10 days, with daily monitoring for emerging fungal colonies from internal tissues. Colonization frequency (%) was calculated as the proportion of tissue segments yielding fungal growth relative to the total plated segments (Pavithra *et al.*, 2020).

6. Molecular Identification of Endophytes and Selected Pathogens

Genomic DNA was extracted from pure fungal cultures using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1990). The internal transcribed spacer (ITS) region of rDNA was amplified by PCR using primers ITS1 and ITS4. PCR reactions were performed in a 25 µL mixture containing 1× PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.4 µM of each primer, 1 U Taq DNA polymerase, and 50 ng template DNA. Thermal cycling conditions included initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 7 min.

PCR products were purified and sequenced commercially. Sequences were aligned and analyzed using BLASTn against the NCBI GenBank database. Phylogenetic trees were constructed in MEGA X to confirm species-level identification.

7. In Vitro Antagonistic Activity Assays

Two complementary approaches were used to evaluate the antagonistic potential of endophytes against the isolated phytopathogens:

- a) Dual culture assay following Skidmore and Dickinson (1976), 5 mm mycelial plugs of the pathogen and endophyte were placed 6 cm apart on PDA plates. Plates were incubated at 25 °C, and radial growth was measured after 7 days. Percentage inhibition was calculated relative to pathogen growth on control plates.
- b) Culture filtrate assay endophytes were grown in Potato Dextrose Broth (PDB) at 120 rpm and 25 °C for 14 days. Culture broth was filtered through Whatman No.1 paper, then sterilized using a 0.22 µm membrane filter. The sterile

filtrate was incorporated into PDA at final concentrations of 5%, 10%, and 15% (v/v). Pathogen mycelial plugs were placed centrally, and radial growth was measured after incubation.

8. Extraction and GC–MS Analysis of Bioactive Metabolites

The ethyl acetate extracts of the culture filtrates obtained from liquid-grown endophytic fungi were subjected to GC–MS analysis. Specifically, sterile culture filtrates of *Nigrospora osmanthi* and *Nigrospora sphaerica* grown in potato dextrose broth (PDB) were extracted with ethyl acetate in a 1:1 (v/v) ratio under vigorous shaking for 30 minutes. The organic layers were separated, dried over anhydrous sodium sulphate to remove residual moisture, and subsequently concentrated under reduced pressure using a rotary evaporator to yield the crude extract containing secondary metabolites.

The extracts were analysed using gas chromatography mass spectrometry (GC–MS) to identify volatile and semi-volatile components. Metabolites were tentatively identified by comparing their mass spectral fragmentation patterns and retention times with reference spectra from the NIST (National Institute of Standards and Technology) Mass Spectral Library. This method is widely accepted for metabolite profiling of fungal endophytes due to its sensitivity and reproducibility (Devi *et al.*, 2010; Kaur and Arora, 2009; Strobel and Daisy, 2003).

Such an approach enables the characterization of complex metabolite mixtures and facilitates the identification of bioactive compounds that may be involved in antifungal or antimicrobial activities (Saranraj and Sivasakthi, 2014; Narvaez-Barragan *et al.*, 2020).

9. Statistical Analysis

All experiments were conducted in triplicate following a completely randomized design. Data were analyzed by one-way ANOVA using SPSS software. Mean comparisons were performed using Tukey's HSD test at a significant level of $p < 0.05$.

III. RESULTS AND DISCUSSION

1. Isolation and Identification of Pathogenic Fungi

Field surveys of *Pluchea dioscoridis* plants showing foliar and stem lesions resulted in the isolation of two consistent pathogenic fungi directly associated with symptomatic tissues: *Alternaria* sp., producing olive-green to black colonies with beaked conidia, and *Stemphylium* sp., which exhibited slow-growing, dark brown to black colonies with multicellular muriform conidia. Both isolates successfully reproduced characteristic leaf spot and blight symptoms on

detached leaves under controlled pathogenicity assays, confirming their pathogenic role on *P. dioscoridis*.

To extend the relevance of the antifungal screening, a collection of agriculturally important soilborne fungi was established from rhizospheric soils or infected plant debris in nearby fields cultivated with crops such as alfalfa, peanut, citrus, and faba bean. The isolated pathogens included *Fusarium oxysporum*, *F. solani*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotium rolfsii*, and all of which are well-documented causal agents of root rots,

vascular wilts, charcoal rot, and damping-off diseases, also *Myrothecium verrucaria* as Foliar/stem blight causal agent in a wide range of crops.

This pathogen panel, comprising host-specific foliar pathogens and ecologically relevant soilborne fungi, was selected to support a comprehensive assessment of potential biocontrol agents, focusing on their antagonistic capabilities against diverse phytopathogens of both medicinal and economic significance.



Fig. 1: Morphological appearance of *Pluchea dioscoridis* plants collected during field survey. (A, B) Healthy plant showing normal foliage and stem development. (C–F) Naturally infected plants exhibiting foliar and stem symptoms caused by fungal pathogens isolated in this study.

Table 1. Pathogenic fungi isolated from *P. dioscoridis*, their source, morphology, and growth rate on PDA

Pathogen	Source of Isolation	Colony Morphology on PDA	Key Microscopic Features	Growth Rate (mm/day) $\pm$ SE	Disease Type
<i>Alternaria</i> sp.	Diseased leaves	Olive green to black	Muriform, beaked conidia	5.6 ± 0.3	Foliar (leaf spot)
<i>Stemphylium</i> sp.	Diseased leaves	Greyish, compact	Branched conidiophores, clusters of conidia	3.3 ± 0.2	Foliar (leaf spot)
<i>Myrothecium verrucaria</i>	Diseased stems	Whitish with green sporodochia	Cylindrical conidia in sporodochia	5.8 ± 0.2	Foliar/stem blight
<i>Fusarium oxysporum</i>	Diseased roots	Cottony white, pinkish reverse	Sickle-shaped macroconidia, microconidia in false heads	6.9 ± 0.2	Vascular wilt

<i>Fusarium solani</i>	Soil (peanut field)	Cream to pale brown	Elliptical macroconidia, abundant chlamydospores	6.5 ± 0.3	Root rot, stem canker
<i>Macrophomina phaseolina</i>	Alfalfa roots	Dark grey, fluffy	Numerous black microsclerotia	7.0 ± 0.2	Charcoal rot
<i>Rhizoctonia solani</i>	Soil (citrus orchard)	Brown, web-like mycelium	Right-angled branching hyphae	8.2 ± 0.3	Root rot, damping-off
<i>Sclerotium rolfsii</i>	Peanut stem base	White mycelium, abundant sclerotia	Tan to brown sclerotia	7.7 ± 0.2	Stem base rot, wilt

2. Isolation and Preliminary Identification of Endophytic Fungi

Endophytic fungal isolates were successfully recovered from surface-sterilized tissues of *Pluchea dioscoridis* plants that appeared asymptomatic in the field. Initial morphological and microscopic examinations revealed that the dominant isolates belonged to the genus *Nigrospora*. The colonies exhibited rapid growth with a characteristic cottony to fluffy aerial mycelium, initially white turning grayish-black with age. Microscopically, the isolates were identified by their dark, globose to subglobose, single-celled conidia, typically borne laterally on short, hyaline conidiophores (Fig. 2). The conidia measured approximately 10–15 μm in diameter, consistent with taxonomic descriptions of *Nigrospora* spp. (Ellis, 1971; Kirk, P.M., 1991; Barnett and Hunter, 1998.).

The vegetative mycelium consisted of branched, septate, and hyaline hyphae that later turned pigmented, a common

feature of dematiaceous fungi. No signs of pathogenicity were observed on host tissues at the time of sampling, supporting their endophytic nature.

To confirm their identity and differentiate between closely related taxa, molecular characterization was subsequently performed using ITS-rDNA sequencing. This molecular approach provided accurate species-level identification, confirming the isolates as *Nigrospora osmanthi* and *Nigrospora sphaerica*. The molecular data were further supported by phylogenetic analyses aligning the isolates within established *Nigrospora* clades.

These results underscore the importance of integrating morphological and molecular tools for the accurate identification of endophytic fungi and support the potential of *Nigrospora* spp. as candidates for biocontrol applications.



Fig. 2: Morphological and cultural characteristics of endophytic *Nigrospora* isolates from *Pluchea dioscoridis*; (A) Colony morphology and microscopic features of *Nigrospora osmanthi*, showing septate hyphae and typical black, single-celled conidia; (B) Colony morphology and microscopic features of *Nigrospora sphaerica*, displaying branched hyphae and globose to ellipsoid conidia.

3. Pathogenicity Testing

Given the biological and ecological divergence between foliar and soilborne pathogens, pathogenicity assessment was selectively applied only to the isolates suspected of causing foliar diseases in *Pluchea dioscoridis*. These included *Alternaria* sp. and *Stemphylium* sp., all of which were originally recovered from symptomatic leaves and stems of naturally infected *P. dioscoridis* plants.

3.1. Detached Leaf Assay for Foliar Pathogens:

Pathogenicity of the three foliar isolates was tested using the detached leaf assay. Healthy, surface-sterilized leaves of *P. dioscoridis* were placed on moist filter paper in sterile Petri dishes and inoculated with mycelial plugs from 7-day-old cultures of each fungal isolate. Control leaves received sterile PDA plugs. Inoculated leaves were incubated at $25 \pm 2^\circ\text{C}$ under a 12-hour photoperiod for 10 days, during which symptoms were monitored and lesion development recorded. The results, summarized in Table 2, showed clear variation in virulence among the tested isolates. *Alternaria* sp. caused the largest mean lesion diameter (15.1 ± 0.8 mm), followed closely by *Myrothecium verrucaria* (13.6 ± 0.7 mm), both producing lesions within six days post-inoculation. *Stemphylium* sp. induced significantly smaller

lesions (9.6 ± 0.5 mm) and exhibited a slightly longer incubation period of seven days. Based on these observations, the pathogenicity ranking among foliar isolates was (*Alternaria* sp.; *Myrothecium verrucaria*; *Stemphylium* sp.). This ranking aligns with known pathogenic behaviour of these fungi in various host plants, where *Alternaria* species are typically aggressive necrotrophs (Kusaba and Tsuge, 1995; Shoemaker and Babcock, 1992).

3.2. Rationale for Excluding Root Pathogens from Pathogenicity Assay:

In contrast, the five soilborne fungi isolated from rhizospheric soil and nearby crops (*Fusarium oxysporum*, *F. solani*, *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Sclerotium rolfsii*) were not subjected to detached-leaf assays. This was due to their fundamentally different infection biology, which involves root colonization and systemic vascular invasion, rendering leaf-based assays ineffective for evaluating their virulence. Moreover, the pathogenicity of these fungi is well-established in the literature, with multiple studies confirming their role in causing wilting, root rot, and damping-off symptoms in various economically important crops (Leslie and Summerell, 2006; Kaur *et al.*, 2012).

Table 2. Pathogenicity of foliar pathogens on detached *P. dioscoridis* leaves

Pathogen	Mean Lesion Diameter (mm) $\pm$ SE	Incubation Period (days)	Pathogenicity Rank
<i>Alternaria</i> sp.	15.1 ± 0.8	6	2
<i>Myrothecium verrucaria</i>	13.6 ± 0.7	6	2
<i>Stemphylium</i> sp.	9.6 ± 0.5	7	3

4. Antagonistic Activity of Endophytic *Nigrospora* Isolates

4.1 Dual Culture Assay

To assess the antagonistic efficacy of endophytic *Nigrospora* isolates against phytopathogenic fungi, a dual culture assay was employed. The test included three foliar pathogens (*Alternaria* sp., *Stemphylium* sp. and *Myrothecium verrucaria*) and soilborne pathogens (*Fusarium oxysporum*, *F. solani*, *Sclerotium rolfsii*, *Macrophomina phaseolina*, and *Rhizoctonia solani*). Each isolate of *N. sphaerica* and *N. osmanthi* was evaluated for its ability to suppress the radial growth of these pathogens. The results revealed that *Nigrospora sphaerica* exhibited superior antagonistic activity compared to *N. osmanthi* against all tested pathogens, indicating a species dependent difference in biocontrol efficacy. Notably, foliar pathogens showed higher susceptibility to both endophytes, particularly to *N. sphaerica*, which achieved inhibition rates exceeding 60% in *Alternaria* sp. and 53.9% in *Myrothecium*

verrucaria. This supports earlier findings that foliar endophytes are more competitive in the phyllosphere due to niche overlaps with foliar pathogens (Strobel and Daisy, 2003; Mousa and Raizada, 2013; Deshmukh *et al.*, 2018). In contrast, root infecting fungi such as *Sclerotium rolfsii*, *Macrophomina phaseolina* and *Rhizoctonia solani* exhibited markedly lower inhibition 32.3%, 24.2%, and 25.3% (Table 3), which could be due to their aggressive colonization strategies and physical resilience, such as sclerotia formation (Dean *et al.*, 2012). The intermediate response observed in *Fusarium* species suggests that endophyte-derived metabolites may partially limit their growth. Although initial inhibition zones were observed around the endophytic isolates, further incubation revealed that the endophytes continued to grow, progressively occupying the space and suppressing pathogen development through competitive exclusion. This suggests that inhibition was not only due to static antagonism but also involved active overgrowth and space competition (Fig. 3).

These findings underscore the ecological relevance of endophyte-host interactions and support the use of *N. sphaerica* as a potential biocontrol candidate, particularly

for foliar disease management in *Pluchea dioscoridis* and possibly other hosts.

Table 3. Radial Growth Inhibition of Pathogenic Fungi by *Nigrospora* spp. in Dual Culture Assays

Pathogen	Growth Alone (mm/day)	Growth with <i>N. osmanthi</i>	Inhibition %	Growth with <i>N. sphaerica</i>	Inhibition %
<i>Alternaria</i> sp.	9.6 ± 0.3	6.3 ± 0.2	34.4%	4.2 ± 0.3	56.3%
<i>Myrothecium verrucaria</i>	8.9 ± 0.4	5.8 ± 0.2	34.8%	4.1 ± 0.3	53.9%
<i>Stemphylium</i> sp.	7.8 ± 0.4	5.9 ± 0.2	24.4%	3.8 ± 0.3	51.3%
<i>Fusarium oxysporum</i>	10.1 ± 0.4	7.6 ± 0.3	24.8%	5.7 ± 0.4	43.6%
<i>Fusarium solani</i>	9.8 ± 0.3	7.3 ± 0.2	25.5%	5.6 ± 0.3	42.9%
<i>Sclerotium rolfsii</i>	9.3 ± 0.3	7.7 ± 0.2	17.2%	6.3 ± 0.4	32.3%
<i>Macrophomina phaseolina</i>	9.5 ± 0.4	8.0 ± 0.3	15.8%	7.2 ± 0.2	24.2%
<i>Rhizoctonia solani</i>	8.7 ± 0.3	7.4 ± 0.3	14.9%	6.5 ± 0.3	25.3%

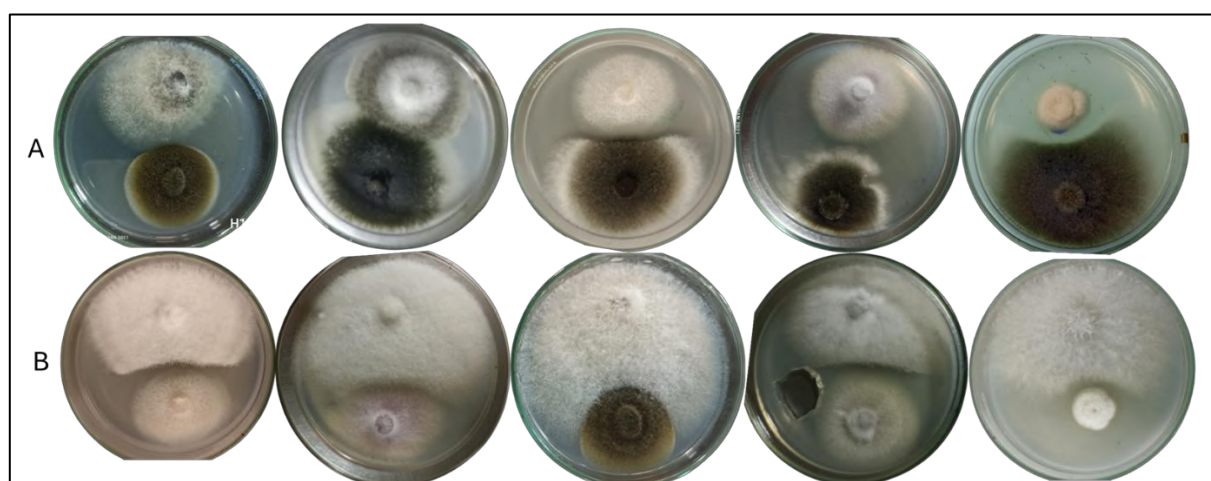


Fig. 3: Dual culture assay showing antifungal activity of endophytic *Nigrospora* isolates against phytopathogenic fungi. (A) *Nigrospora osmanthi*; (B) *Nigrospora sphaerica*. Although initial inhibition zones were observed around the endophytic isolates, further incubation revealed that the endophytes continued to grow, progressively occupying the space and suppressing pathogen development through competitive exclusion. This suggests that inhibition was not only due to static antagonism but also involved active overgrowth and space competition.

4.2 Effect of Culture Filtrates of *Nigrospora* spp. on Growth of Pathogens

To further assess the antagonistic efficacy of *Nigrospora osmanthi* and *Nigrospora sphaerica*, culture filtrate assays were conducted to evaluate the extracellular inhibitory potential of these endophytes against a range of phytopathogens. Filtrates obtained from liquid cultures of each isolate were incorporated into PDA medium at standardized concentrations. The radial growth of each pathogen was then recorded and compared to controls after 7 days of incubation. Table 4 summarizes the mean radial

growth rates and percentage inhibition values for each pathogenic fungus in response to the culture filtrates, along with Duncan's multiple range test letters indicating statistically significant differences ($P \leq 0.05$).

The results presented in Table 4 show clear differences in the antifungal activity of culture filtrates between *N. sphaerica* and *N. osmanthi*. In all tested pathogens, filtrates from *N. sphaerica* demonstrated significantly stronger inhibitory effects compared to *N. osmanthi*, suggesting a superior production of bioactive metabolites by this isolate. This intra-genus variability in antagonistic capacity is

consistent with earlier studies, which highlighted that secondary metabolite production among endophytes can vary markedly depending on strain and environmental adaptation (Kharwar *et al.*, 2011; Strobel, 2003).

Foliar pathogens such as *Alternaria* sp., *Myrothecium verrucaria*, and *Stemphylium* sp. were among the most susceptible to culture filtrates, with inhibition rates exceeding 40% in the case of *N. sphaerica*. This is likely due to the shared ecological niche between these pathogens and the endophytes, which may facilitate competitive exclusion and higher local accumulation of antifungal compounds (Schulz and Boyle, 2005; Ghorbanpour *et al.*, 2018).

In contrast, soilborne pathogens such as *Macrophomina phaseolina* and *Rhizoctonia solani* showed the lowest levels of growth inhibition, not exceeding 16% even under *N. sphaerica* filtrate treatment. These fungi are known for their robust sclerotial structures and tolerance to environmental stress, which may reduce the efficacy of diffusible

antifungal compounds (Dean *et al.*, 2012; Khaledi and Taheri, 2016).

Intermediate suppression was observed for *Fusarium* species, which are vascular pathogens with systemic colonization behavior. The moderate inhibition (~30–35%) suggests that while the endophytes metabolites interfere with hyphal development, they may not fully suppress more aggressive soilborne pathogens. Such partial inhibition aligns with previous findings in which *Fusarium* spp. showed variable responses to fungal filtrates, depending on the bioactive profile of the antagonistic strain (Palaniyandi *et al.*, 2013). Overall, the filtrate assay confirms the antagonistic potential of *Nigrospora* endophytes, particularly *N. sphaerica*, against a range of phytopathogens. These findings reinforce their biocontrol capacity and ecological compatibility with the aerial parts of *Pluchea dioscoridis*, making them promising candidates for further development as biological control agents.

Table 4. Effect of Culture Filtrates of *Nigrospora* spp. on Radial Growth of Pathogenic Fungi (mm/day) and Growth Inhibition (%).

Pathogenic Fungi	Control Growth (mm/day)	N. osmanthi Filtrate (mm/day)	Inhibition (%)	N. sphaerica Filtrate (mm/day)	Inhibition (%)
<i>Alternaria</i> sp.	8.0 a	5.2 c	35.0	4.4 d	45.0
<i>Myrothecium verrucaria</i>	7.6 a	5.3 c	30.3	4.5 d	40.8
<i>Stemphylium</i> sp.	7.4 a	5.5 c	25.7	4.8 d	35.1
<i>Fusarium solani</i>	8.2 a	6.1 bc	25.6	5.3 c	35.4
<i>Fusarium oxysporum</i>	8.1 a	6.3 bc	22.2	5.6 c	30.9
<i>Sclerotium rolfsii</i>	7.8 a	6.6 b	15.4	6.2 bc	20.5
<i>Rhizoctonia solani</i>	8.0 a	7.0 ab	12.5	6.7 b	16.3
<i>Macrophomina phaseolina</i>	7.9 a	7.1 ab	10.1	6.8 b	13.9

Effect of Fungal Culture Filtrates of *Nigrospora* spp. on Dry Biomass of Phytopathogenic Fungi

To explore the metabolite-based antagonistic potential of the endophytic *Nigrospora* isolates (*N. osmanthi* and *N. sphaerica*), culture filtrate assays were conducted, where the dry biomass of various phytopathogenic fungi served as a quantitative measure of growth inhibition. This approach distinguishes the chemical inhibitory effects mediated by secondary metabolites from direct mycelial competition.

As illustrated in Fig. 4, both isolates significantly reduced fungal biomass compared to the control. *N. sphaerica* consistently exhibited a greater inhibitory effect across all tested pathogens, corroborating its superior performance in dual culture assays. The most pronounced suppression was

observed against foliar pathogens such as *Alternaria* sp. and *Myrothecium verrucaria*, with their dry weights dropping to approximately 0.29 g/plate and 0.26 g/plate, respectively. This supports the hypothesis that foliar-derived endophytes may biosynthesize metabolites specifically adapted to target aerial pathogens, consistent with findings by Amin *et al.* (2021) and Gakuubi *et al.* (2022).

Conversely, soilborne pathogens like *Rhizoctonia solani* and *Macrophomina phaseolina* showed more moderate reductions (dry weights 0.51–0.55 g/plate), which could be attributed to their well-documented resilience and enzymatic detoxification systems (Khaledi *et al.*, 2016; Singh and Yadav, 2020).

Intermediate responses were recorded for *Fusarium oxysporum* and *F. solani* (0.41–0.45 g/plate), indicating a partial susceptibility likely involving disruption of cellular processes such as membrane integrity or secondary metabolism (Larran *et al.*, 2016).

These results suggest that while *N. sphaerica* exhibits broader antifungal metabolite diversity and potency, its effectiveness is more apparent against foliar pathogens. The reduced biomass levels confirm that the secreted bioactive compounds contribute substantially to fungal inhibition and reinforce the potential use of these endophytes in integrated disease management strategies.

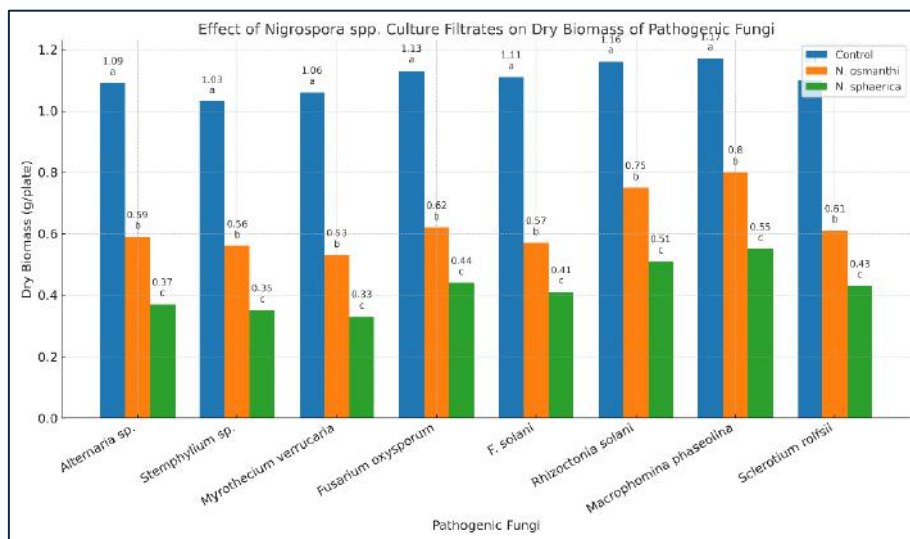


Fig. 4: Effect of Culture Filtrates of *Nigrospora* spp. on Dry Biomass of Phytopathogenic Fungi (g/plate)

4.3 Molecular Identification of Endophytic *Nigrospora* Isolates

To confirm the taxonomic identity of the two endophytic fungal isolates obtained from healthy *Pluchea dioscoridis* leaves, molecular identification was carried out using internal transcribed spacer (ITS) rDNA sequencing. Genomic DNA was extracted from actively growing mycelia, and amplification of the ITS region was performed using the universal primers (ITS1: 5'-TCC GTA GGT GAA CCT GCG G-3'; ITS4: 5'-TCC TCC GCT TAT TGA TAT GC-3'). The amplified PCR products were purified and sequenced bidirectionally. The resulting sequences were analyzed using the BLASTn algorithm against the NCBI GenBank database to determine species-level similarity (Fig. 5).

The first isolate, designated H1, produced a high-quality sequence of 592 base pairs. BLASTn analysis revealed a 99% identity (567/571 bp) with *Nigrospora osmanthi* (GenBank accession number MH645207.1). The query coverage was 100%, confirming a high-confidence match. Phylogenetic placement aligned H1 within the *Trichosphaeriaceae* family under the order

Trichosphaeriales, and genus *Nigrospora*. These results affirm the identification of H1 as *Nigrospora osmanthi*.

The second isolate, designated H2, yielded a 575 bp ITS sequence, which showed 99% identity (549/552 bp) with *Nigrospora sphaerica* (GenBank accession number MG669225.1), also with 100% coverage. The isolate was taxonomically assigned to *Nigrospora sphaerica* based on sequence similarity, supporting its classification within the same taxonomic lineage as H1.

Interpretation and Relevance, the high sequence identity and coverage of both isolates to their respective type strains support their accurate species-level identification. These findings align with previous studies demonstrating the utility of ITS sequencing for delimiting *Nigrospora* species (Wang *et al.*, 2018; Gomes *et al.*, 2013). Importantly, both isolates were recovered from asymptomatic *P. dioscoridis* tissues and did not produce symptoms under pathogenicity assays, confirming their endophytic. The confirmed identity of these isolates allows for reliable attribution of the observed biocontrol effects in subsequent antagonism and filtrate assays. Moreover, it supports future work exploring metabolite profiling or gene expression patterns related to antifungal activity (Zhao *et al.*, 2011).

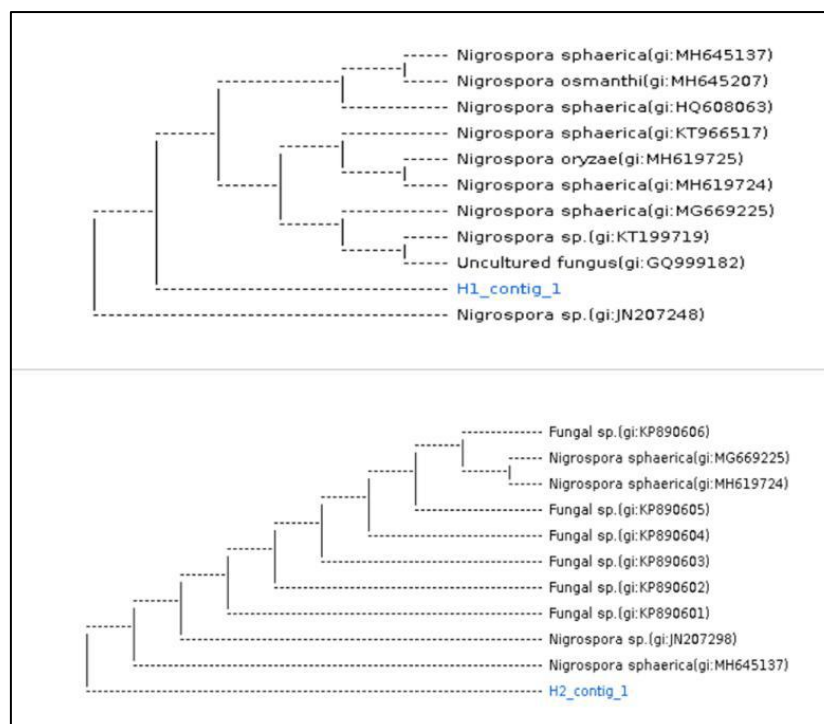


Fig. 5: Phylogenetic dendrograms illustrating the molecular identification of two endophytic fungal isolates from *Pluchea dioscoridis* based on ITS region sequencing. (A): Isolate H1 clustered with *Nigrospora osmanthi* (GenBank accession no. MH645207.1) with 99% sequence identity. (B): Isolate H2 clustered with *Nigrospora sphaerica* (GenBank accession no. MG669225.1) with 99% sequence identity. The trees were constructed using standard BLASTn alignment and similarity clustering NCBI database sequences. Bootstrap values indicate the confidence of clustering for each node.

4.4 GC–MS-Based Metabolomic Profiling of Endophytic *Nigrospora* spp. Culture Filtrates

Nigrospora species, GC–MS analysis was performed on ethyl acetate extracts of culture filtrates from *Nigrospora osmanthi* and *N. sphaerica*. The analysis enabled identification of several volatile and semi-volatile compounds, many with reported antimicrobial functions, providing a chemical basis for the observed variation in biocontrol activity.

4.4.1 Metabolite Profile of *Nigrospora osmanthi*

The GC–MS chromatogram of *N. osmanthi* revealed 24 peaks with retention times ranging from 8.447 to 37.687 minutes. The chemical composition was dominated by a single compound at $R_t = 34.722$ min (48.48%), identified as phenol, 2,4-bis(1,1-dimethylethyl)-, a compound known for its broad-spectrum antimicrobial and antioxidant properties (Kaur and Arora, 2009). Other minor compounds included 1-monopalmitin ($R_t = 29.397$ min, 10.09%) and citric acid ($R_t = 22.874$ min, 35.89%), (Fig. 6).

Although a relatively high number of peaks was detected, the predominance of a single compound indicates a metabolically biased output concentrated along a narrow biosynthetic pathway. While this major phenolic metabolite may possess antifungal potential, the overall lack of

chemical diversity and the low representation of complementary bioactive compounds likely contribute to the isolate's moderate antifungal efficacy. Phenol derivatives such as 2,4-di-tert-butylphenol have been shown to inhibit fungal spore germination and growth by disrupting membrane (Devi *et al.*, 2010), but their efficacy may depend on synergistic interactions with other metabolites, which appear minimal in this strain.

4.4.2 Metabolite Profile of *Nigrospora sphaerica*

In contrast, GC–MS profiling of *N. sphaerica* revealed a total of 15 distinct peaks, reflecting a notably richer chemical diversity and more balanced distribution of metabolite intensities. The predominant compound detected was phenol, 2,4-bis(1,1-dimethylethyl)- ($R_t = 34.773$ min, 100%), followed by benzene, 1,3-bis(1,1-dimethylethyl)- ($R_t = 35.789$ min, 49.8%), both of which are lipophilic aromatic compounds known for their membrane-disruptive properties. Beyond these major constituents, the isolate also produced a range of bioactive metabolites including lactic acid, which is recognized for its acidifying effect that creates unfavourable conditions for pathogen survival, and glycerol, a metabolite often associated with cellular stress responses and osmotic balance. Organic acids such as citric acid and 2-butenedioic acid, along with β -D-(+)-

xylopyranose, were also present and may enhance antifungal activity through mechanisms involving metal ion chelation, pH reduction, or interference with pathogen metabolism. Additionally, the detection of α -linolenic acid and arachidonic acid fatty acids known to inhibit biofilm formation and compromise fungal membranes further supports the potential of *N. sphaerica* as a multifaceted source of antifungal compounds.

The presence of multiple moderately to highly abundant compounds, spanning both polar and non-polar classes, suggests that *N. sphaerica* harbors a broad-spectrum metabolic. These compounds likely act synergistically, targeting various aspects of fungal physiology such as membrane permeability, enzymatic activity. This chemical richness is consistent with the enhanced antifungal activity observed for *N. sphaerica* in both dual culture and filtrate assays.

Comparative Interpretation: Although *N. osmanthi* exhibited a greater number of chromatographic peaks, its metabolite distribution was heavily dominated by a single phenolic compound, which accounted for over 98% of the total peak area. In contrast, the chemical profile of *N. sphaerica* was more balanced and functionally diverse, with several bioactive metabolites contributing substantially to the overall composition of the extract. This functional chemical diversity likely underpins the superior biocontrol efficacy of *N. sphaerica*, as confirmed in bioassays. The results highlight that not only the number of metabolites, but their relative abundance and functional complementarity are critical in determining antifungal potential.

The observed variation in metabolite expression between the two fungal strains may stem from inherent genetic differences in their secondary metabolic pathways, as well as disparities in host-endophyte interactions during the initial isolation process. Additionally, environmental and culture conditions are known to influence metabolic flux and can significantly shape the profile of secondary metabolites produced (Strobel, 2003; Kusari *et al.*, 2012). These findings underscore the importance of integrating metabolomic profiling with antifungal bioassays to guide the selection of promising fungal isolates for further development in biological control programs. The detection of known antifungal compounds in *N. sphaerica* highlights

its strong potential for incorporation into integrated pest management (IPM) strategies.

Bioactive Metabolites and Antifungal Potential: The GC-MS profiles of both *Nigrospora osmanthi* and *N. sphaerica* revealed the presence of several volatile and semi-volatile metabolites with reported antifungal properties. Most notably, 2,4-di-tert-butylphenol (also known as Phenol, 2,4-bis(1,1-dimethylethyl)-) was identified as the major compound in both extracts, with a relative abundance exceeding 98% in *N. osmanthi* and 100% in *N. sphaerica*. This phenolic compound has been widely documented for its antimicrobial and antifungal properties, attributed to its membrane-disrupting effects and interference with ergosterol synthesis in fungal cells (Devi *et al.*, 2010; Huang *et al.* 2007; Kaur and Arora, 2009; Ghorbanpour, *et al.*, 2018).

Additional metabolites detected exclusively or at higher intensity in *N. sphaerica* include Benzene, 1,3-bis(1,1-dimethylethyl)-, which has been associated with antifungal activity via disruption of lipid bilayers (Saranraj and Sivasakthi, 2014), and lactic acid, a known inhibitory agent that lowers extracellular pH and impairs fungal respiration (Magnusson and Schnürer, 2001). The detection of citric acid and glycerol in both isolates may also contribute to antifungal efficacy through osmotic stress induction and chelation of essential metal ions (Jung *et al.*, 2003; Hallsworth and Magan, 1995).

Furthermore, *N. sphaerica* produced fatty acid derivatives such as α -linolenic acid and arachidonic acid, both of which are recognized for their fungitoxicity and ability to disrupt membrane integrity in phytopathogens (Walters *et al.*, 2004; Narvaez-Barragan *et al.*, 2020). These metabolites, especially when present in combination, may account for the enhanced and broader-spectrum antifungal activity exhibited by *N. sphaerica* in bioassays. The presence and distribution of these metabolites not only explain the superior biocontrol potential of *N. sphaerica* but also highlight the utility of metabolomic profiling in guiding the selection of promising endophytes for antifungal applications. Future work involving compound purification and structure activity relationship (SAR) studies is warranted to confirm the specific roles of these bioactives in pathogen inhibition.

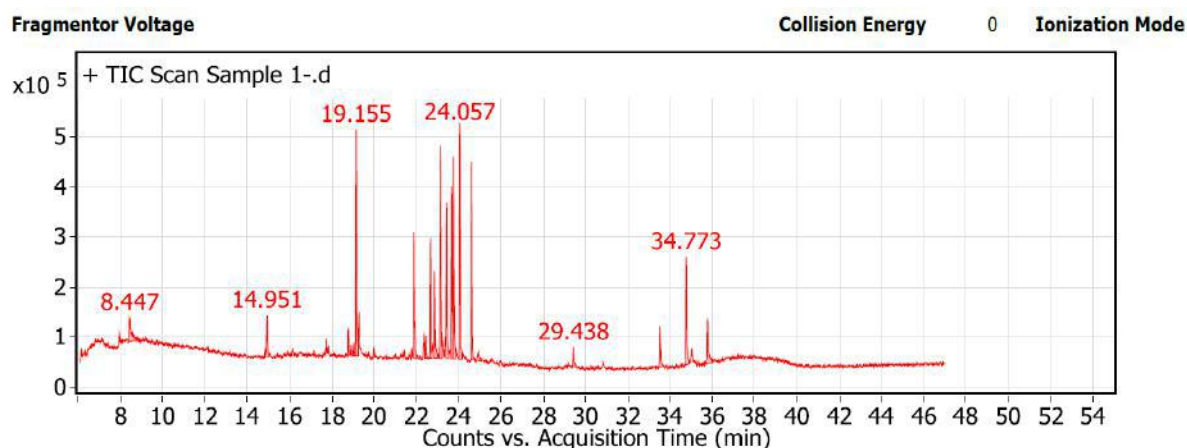


Fig. 6: GC–MS chromatograms of ethyl acetate extracts from culture filtrates of endophytic *Nigrospora osmanthi* showing 24 major peaks

Table 5. GC–MS Detected Peaks in Culture Filtrate of *N. osmanthi*

Peak	RT	Name	Formula	Area	Area Sum %
1	8.447	Lactic Acid, 2TMS derivative	C ₉ H ₂₂ O ₃ Si ₂	324397.63	2.77
2	14.951	Glycerol, 3TMS derivative	C ₁₂ H ₃₂ O ₃ Si ₃	257515.76	2.2
3	18.783	Propylene glycol, 2TMS derivative	C ₉ H ₂₄ O ₂ Si ₂	133172.22	1.14
4	19.041	Arachidonic acid, TMS derivative	C ₂₃ H ₄₀ O ₂ Si	58312.6	0.5
5	19.155	L-5-Oxoproline, 2TMS derivative	C ₁₁ H ₂₃ NO ₃ Si ₂	1330542.5	11.36
6	19.997	Succinic acid (tms)	C ₁₀ H ₂₂ O ₄ Si ₂	51776.68	0.44
7	21.014	2,4-Hexadien-1-ol	C ₆ H ₁₀ O	29310.13	0.25
8	21.895	Xylitol, 5TMS derivative	C ₂₀ H ₅₂ O ₅ Si ₅	637209.12	5.44
9	22.464	Glyceric acid, 3TMS derivative	C ₁₂ H ₃₀ O ₄ Si ₃	104564.7	0.89
10	22.684	.beta.-Arabinopyranose, 4MS derivative	C ₁₇ H ₄₂ O ₅ Si ₄	524338.61	4.48
11	22.874	Citric acid, 4TMS derivative	C ₁₈ H ₄₀ O ₇ Si ₄	477565.38	4.08
12	23.147	Methyl .alpha.-D-glucufuranoside, 4TMS derivative	C ₁₉ H ₄₆ O ₆ Si ₄	1306674.3	11.16
13	23.42	2-Butenedioic acid, (Z)-, 2TMS derivative	C ₁₀ H ₂₀ O ₄ Si ₂	828003.87	7.07
14	23.663	D-Galactose, 5TMS derivative	C ₂₁ H ₅₂ O ₆ Si ₅	942605.46	8.05
15	23.761	.beta.-D-(+)-Xylopyranose, 4TMS derivative	C ₁₇ H ₄₂ O ₅ Si ₄	1090960.2	9.32
16	24.057	Ribitol, 5TMS derivative	C ₂₀ H ₅₂ O ₅ Si ₅	1121786.3	9.58
17	24.611	β-D-glucose 5-TMS derivative	C ₂₁ H ₅₂ O ₆ Si ₅	825202.82	7.05
18	24.93	α-Linolenic acid, TMS derivative	C ₂₁ H ₃₈ O ₂ Si	109200.24	0.93
19	29.438	1-Monopalmitin, 2TMS derivative	C ₂₅ H ₅₄ O ₄ Si ₂	134196.3	1.15
20	33.521	Phenol, 2,6-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂ O	324724.18	2.77
21	34.773	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	C ₄₂ H ₆₃ O ₃ P	645042.4	5.51
22	35.782	Benzene, 1,3-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂	395442.75	3.38
23	37.231	Silicic acid, diethyl bis(trimethylsilyl) ester	C ₁₀ H ₂₈ O ₄ Si ₃	26890.61	0.23
24	37.785	1,4-Benzenediol, 2,6-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂ O ₂	28083.68	0.24

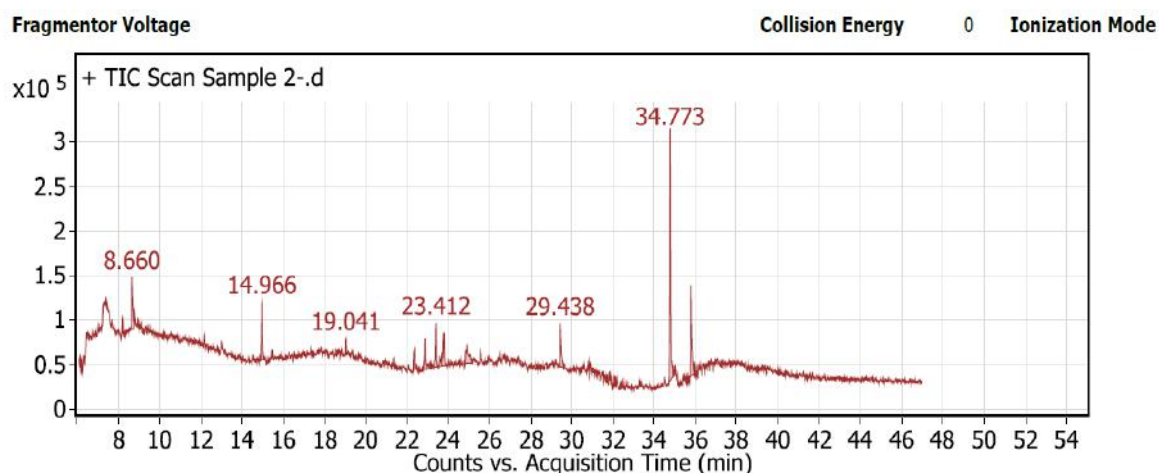


Fig. 7: GC–MS chromatograms of ethyl acetate extracts from culture filtrates of endophytic *Nigrospora sphaerica* displaying 15 major peaks

Table 6. GC–MS Detected Peaks in Culture Filtrate of *N. sphaerica*

Peak	RT	Name	Formula	Area	Area Sum %
1	8.66	Lactic Acid, 2TMS derivative	C ₉ H ₂₂ O ₃ Si ₂	205574.63	7.94
2	14.966	Glycerol, 3TMS derivative	C ₁₂ H ₃₂ O ₃ Si ₃	192181.08	7.43
3	19.041	Arachidonic acid, TMS derivative	C ₂₃ H ₄₀ O ₂ Si	36773.52	1.42
4	21.371	2,4-Hexadien-1-ol	C ₆ H ₁₀ O	25933.24	1
5	22.873	Citric acid, 4TMS derivative	C ₁₈ H ₄₀ O ₇ Si ₄	110045.65	4.25
6	23.116	Methyl α-D-glucofuranoside, 4TMS derivative	C ₁₉ H ₄₆ O ₆ Si ₄	22363.69	0.86
7	23.412	2-Butenedioic acid, (Z)-, 2TMS derivative	C ₁₀ H ₂₀ O ₄ Si ₂	146200.52	5.65
8	23.685	D-Galactose, 5TMS derivative	C ₂₁ H ₅₂ O ₆ Si ₅	68840.04	2.66
9	23.792	β-D-Xylopyranose, 4TMS derivative	C ₁₇ H ₄₂ O ₅ Si ₄	149211.9	5.77
10	24.019	Ribitol, 5TMS derivative	C ₂₀ H ₅₂ O ₅ Si ₅	20109.93	0.78
11	24.9	α-Linolenic acid, TMS derivative	C ₂₁ H ₃₈ O ₂ Si	184475.29	7.13
12	29.438	1-Monopalmitin, 2TMS derivative	C ₂₅ H ₅₄ O ₄ Si ₂	203564.46	7.87
13	34.773	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	C ₄₂ H ₆₃ O ₃ P	798110.29	30.84
14	35.789	Benzene, 1,3-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂	397456.94	15.36
15	36.23	Cyclobarbitol	C ₁₂ H ₁₆ N ₂ O ₃	26928.39	1.04

IV. CONCLUSION

This study provides compelling evidence for the antagonistic potential of endophytic *Nigrospora* species isolated from *Pluchea dioscoridis* against a spectrum of foliar and soilborne phytopathogens. Two isolates *Nigrospora osmanthi* and *N. sphaerica* were recovered from symptomatic leaf tissues and molecularly confirmed via ITS-rDNA sequencing. *In vitro* dual culture and culture filtrate bioassays revealed that *N. sphaerica* consistently

exhibited stronger antifungal activity, particularly against foliar pathogens such as *Alternaria* sp. (inhibition rate >60%) and *Myrothecium verrucaria* (54%), whereas *N. osmanthi* showed moderate inhibition across all tested fungi.

endophytic fungi. *N. sphaerica* exhibited a chemically diverse and functionally rich profile, encompassing a broad spectrum of volatile and semi-volatile metabolites. These included phenolic compounds such as 2,4-di-tert-

butylphenol, organic acids like lactic and citric acids, sugar alcohols, and polyunsaturated fatty acids including α -linolenic and arachidonic acids all of which are known for their pronounced antifungal and antimicrobial activities. In contrast, the chemical profile of *N. osmanthi* was markedly unbalanced, being dominated by a single phenolic metabolite that constituted over 98% of the total detected compounds. The dominance of a single metabolite in profile of *N. osmanthi* likely contributes to its reduced spectrum of antifungal activity relative to *N. sphaerica*.

The antifungal efficacy of *N. sphaerica* is likely attributed to the synergistic action of its chemically diverse metabolites, as opposed to reliance on a single dominant compound. These findings underscore the importance of metabolite diversity and functional complementarity in effective biocontrol, and highlight the value of combining bioassays with metabolomic data for the strategic selection of biocontrol agents.

In conclusion, *Nigrospora sphaerica* and *N. osmanthi* represent a promising candidate for development as a broad-spectrum biocontrol agent, particularly for tested pathogens. Future research should focus on purification and structure activity studies of its bioactive metabolites, alongside in plant trials, to validate its efficacy under field conditions and explore its integration into sustainable pest management strategies.

ACKNOWLEDGMENT

The authors would like to express their sincere gratitude to Dr. Omran N. Ghaly, plant taxonomist and Director of the Herbarium of Desert Research Center (DRC), for his valuable contribution in the accurate identification of the plant specimens. The identification was carried out following the standard taxonomic verification protocols adopted by the herbarium.

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Impact of Elevated CO₂ and Temperature on Growth, Physiology and Yield of Black Gram (*Vigna mungo* L. Hepper) Genotypes

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Received: 03 Aug 2025; Received in revised form: 02 Sep 2025; Accepted: 06 Sep 2025; Available online: 19 Sep 2025

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Abstract— Climate change significantly impacts crop production and productivity, particularly in leguminous crops like black gram, which are primarily cultivated under rainfed conditions. The present study evaluated the response of four black gram genotypes (*Vigna mungo* L. Hepper) to elevated CO₂ (eCO₂) and elevated temperature (eT) using the Carbon dioxide and Temperature Gradient Chamber (CTGC) facility at ICAR-CRIDA. The experimental conditions included ambient temperature (aT), eCO₂ (550 ± 50 ppm), and three gradients of elevated temperature (eT1 = aT+1.5°C, eT2 = aT+3.0°C, eT3 = aT+4.5°C) individually and in combination with eCO₂. Results revealed that eCO₂ significantly improved biomass accumulation, photosynthetic rate, and yield traits of all the black gram genotypes, while temperature at higher gradients negatively impacted plant growth and yield. The combined treatment of eCO₂ + eT had a mitigating effect, particularly at eT1+eCO₂, where plants exhibited improved photosynthetic rate, water-use efficiency, and biomass accumulation. However, this amelioration effect declined at eT2+eCO₂ and became negligible at eT3+eCO₂. The negative effects of elevated temperature counteracted the advantage of elevated CO₂. However, at eT3, the negative effects of temperature stress outweighed the benefits of eCO₂, leading to reduced yield. Among the genotypes, PLU-826 exhibited the highest photosynthetic rate (Anet) under eCO₂, while PSRJ-95016 showed improved performance under eT1+eCO₂. Yield parameters such as pod number and seed weight significantly declined under eT3, highlighting the importance of selecting climate-resilient genotypes to sustain black gram productivity under changing environmental conditions.



Keywords— Black gram (*Vigna mungo* L. Hepper), climate change, elevated CO₂, genotypes, temperature, yield.

I. INTRODUCTION

Climate change, characterized by rising atmospheric CO₂ levels and increasing global temperatures, presents a substantial threat to agricultural productivity, especially for rainfed crops like black gram (*Vigna mungo* L. Hepper). As a C₃ legume, black gram is generally responsive to elevated CO₂ (eCO₂), with reported improvements in photosynthetic capacity, biomass accumulation, and water-use efficiency [1-2]. However, these benefits are often constrained by the concurrent rise in temperature, which can accelerate phenological development, reduce grain filling, and increase oxidative stress and respiration losses [3-4].

While the individual effects of eCO₂ and elevated temperature (eT) have been well characterized in cereal crops like rice and wheat [5], the combined impact of these climate factors on leguminous crops remains underexplored. Recent studies suggest that legume responses to eCO₂ and eT are highly genotype-specific, necessitating the identification of cultivars with resilience to climate extremes [6-8]. Black gram, in particular, lacks robust experimental data under controlled conditions simulating future climate scenarios.

Given that atmospheric CO₂ concentrations are expected to reach ~550 ppm by 2050 [9], it becomes critical to

understand the interactive effects of eCO₂ and eT on the physiological and yield performance of black gram genotypes. Moreover, studies in crops like soybean and mungbean have demonstrated significant genotype-by-environment interactions under eCO₂ and heat stress [10-11], highlighting the need to identify stable and high-performing black gram genotypes under these evolving conditions.

The present study was aimed to assess the growth, morpho-physiological, yield and yield components response of four black gram genotypes under eCO₂, eT, individually and in combination in CTGC facility. These findings contribute in identifying better performing genotype/s of this important rainfed short duration leguminous crop in predicted future climatic conditions.

II. METHODOLOGY

2.1 Plant material and experimental conditions

Four black gram genotypes- IPU-06-02, PLU-826, PSRJ-95016 and IPU-94-1 were received from ICAR-IIPR, Kanpur and which are popular released varieties in India. The growth and yield responses of black gram genotypes were assessed at elevated CO₂ (eCO₂) of 550ppm and at three gradients of elevated temperature both individually and in combination with eCO₂ in Carbon dioxide and Temperature Gradient Chamber (CTGC) facility during summer season at Central Research Institute for Dryland Agriculture (ICAR-CRIDA, 17°27'N latitude, 78°35'E

longitude, and approximately 515 meters above sea level) Hyderabad, Telangana, India.

The CTGC facility consists of eight chambers with 30 meters length, 6 meters width and 4 meters height at the centre [12]. These 8 chambers categorised as- two chambers were maintained at ambient temperature (aT); two chambers are with elevated CO₂ (eCO₂ – 550 ± 50ppm); two chambers are with elevated temperature (eT) with three gradients (eT1- aT+1.5°C; eT2- aT+3.0°C; eT3- aT+4.5°C); two chambers are with elevated temperature at three gradients over aT with the combination of eCO₂ (eT1+eCO₂; eT2+eCO₂; eT3+eCO₂). This facility is equipped with advanced SCADA (Supervisory Control and Data Acquisition) software linked with PLC, which facilitates monitoring and controlling set environmental parameters like temperature, humidity and CO₂.

The land within the chambers were ploughed thoroughly and black gram genotypes were sown with 30 X 10 cm spacing. The recommended doses of fertilizers (N @ 20 kg ha⁻¹ and P @ 40 kg ha⁻¹ and K @ 20 kg ha⁻¹) were applied and maintained uniform irrigation at regular intervals along with standard plant protection measures to control pests and diseases throughout the study period.

2.2 Weather conditions

The weather parameters during crop growth period were presented in Fig 1. The maximum air temperature during crop growth period ranged from 30°C to 39.8°C with an average of 36.46°C, while minimum temperature ranged from 23.2°C to 29.2°C with an average value of 26°C.

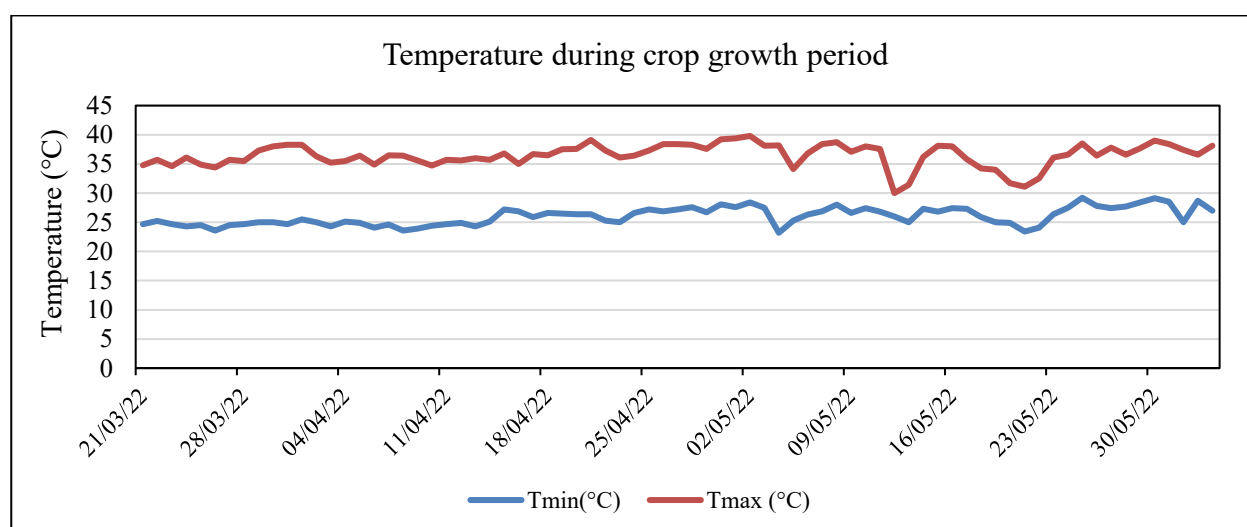


Fig.1. Minimum and maximum temperatures during crop growth period

The data on morphological, physiological, biomass and yield traits of all the genotypes under different treatments were recorded in three replications.

2.3 Physiological parameters

Physiological observations were recorded in all the treatments during pre-flowering stage on the fully expanded leaf of each genotype in three replicates. The net photosynthetic rate (A_{net}), stomatal conductance (g_s) and transpiration rate (Tr) were recorded between 10:00 and 12:00 h using portable photosynthesis system (LI-6400, LI-COR, Nebraska, USA) with irradiance set at $1200 \mu\text{mol m}^{-2}\text{s}^{-1}$ under different treatments by setting the respective CO₂ level and temperature of CTGC. The water use efficiency (WUEi) was calculated as the ratio of A_{net} and Tr using the formula $WUE = A_{net}/Tr$.

2.4 Morphological parameters

Morphological observations were recorded at harvest by carefully uprooting the plants in three replications from each treatment for each genotype. Plants were divided into stems, leaves, roots and pods. The roots were cleaned gently with water. Root and shoot lengths were measured in cm with the measuring scale, and root volume was recorded as displacement of water and expressed in ml.

2.5 Biomass and yield parameter

The harvested plant parts were subjected to drying in a hot air oven set at 60°C and allowed to dry until the plant samples reached constant weight for determination of dry weights. The dry weights of the leaf, stem and root was measured using scientific weighing balance and expressed as gram per plant. The yield characteristics, including pod number/pl, pod weight (g/pl), and 100 seed weight (g), total biomass (g/pl), vegetative biomass (g/pl), and the HI (harvest index) were determined from the collected data sets. Harvest index was calculated as $HI = (\text{Seed yield}/\text{Total biomass}) \times 100$ and expressed in percentage.

2.6 Statistical analysis

The analysis of variance (ANOVA) of replicated data was performed using STAR software ver. 0.1 to assess the significant differences among the genotypes, treatments, and their interaction.

III. RESULTS AND DISCUSSION

3.1 Physiological traits

The ANOVA results (Table 1) revealed that physiological parameters of A_{net} , g_s , Tr and WUE were highly significant ($p < 0.01$) for Genotypes, Treatments and their interaction. The results demonstrated significant ($p <$

0.01) effects of elevated CO₂ (eCO₂) and elevated temperature (eT) on physiological parameters across black gram genotypes. The net photosynthetic rate (A_{net}) increased under eCO₂, with overall mean enhancement of 43.44% which ranged from 40.17% (IPU-94-1) to 50.11% (PSRJ-95016) as compared with ambient conditions, indicating improved carbon assimilation. These findings align with recent studies in black gram showing a 22.3% increase in photosynthesis and a 41.3% increase in yield under elevated CO₂ combined with high day temperature (+3 °C), indicating improved CO₂ assimilation and reproductive performance [4] (Guna et al., 2023).

The A_{net} of all black gram genotypes was maintained up to eT2 even when the temperatures are above optimum for the crop selected, however at eT3 it was impacted negatively, which supports the work of [13] Hatfield et al. (2011), this drop-in photosynthesis at eT3 is consistent with findings in kidney bean, where elevated temperature beyond 34 °C reduced reproductive success despite enhanced CO₂ [14]. Among the four genotypes, PSRJ-95016 recorded negative response for A_{net} at all three elevated temperature gradients (eT1 to eT3) over aT with highest reduction at eT3 (-7.71%).

However, these adverse effects of eT were alleviated in presence of eCO₂ (eT+ eCO₂) across all genotypes with an average increase over aT ranged from 21.72% (eT3+ eCO₂) to 37.46% (eT1+ eCO₂). This reduction in temperature-induced stress due to CO₂ enrichment is consistent with findings by [2] Leakey et al. (2009), who observed similar alleviatory effects of elevated CO₂ in various crops. Although overall increased A_{net} was recorded in individual eCO₂ compared to in combination with eT, PLU-826 exhibited genotypic variability by showing better performance than eCO₂ ($42.23 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) at eT1+ eCO₂ ($45.40 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and eT2+ eCO₂ ($42.93 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$).

Stomatal conductance (g_s) exhibited mixed response among treatments and genotypes. In contrast to A_{net} , higher g_s was recorded with eT1 (35.05%) than eCO₂ (17.57%) but genotypic difference observed with PSRJ-95016 with higher g_s value recorded at eCO₂ (28.31%). Even though all the genotypes showed low g_s at eT2 and eT3 compared to eT1, but IPU-06-02 showed difference response to elevated temperature gradient with increasing g_s values at eT3(42.41%) and eT2(45.56%) respectively. The highest g_s was observed in PLU-826 at eT1 (2.55 cm s^{-1}), which represented a 56.44% increase over aT. However, extreme temperature stress (eT3) reduced g_s across all genotypes, with PLU-826 experiencing the most pronounced decline (-16.97%). This reduction was compensated in presence of eCO₂ (eT2+eCO₂, eT2+eCO₂)

except in PSRJ-95016. These findings align with reports of [15] Sage & Kubien, (2007) indicating that moderate temperature elevations enhance gs, but prolonged exposure reduces stomatal function.

Transpiration rate (Tr) also exhibited a mixed response to treatments, with eCO₂ generally leading to reductions in with eT. The Tr was increased as the temperature gradient was increased with the highest Tr recorded in IPU-94-1 at eT3 (16.77 mmol H₂O m⁻² s⁻¹), reflecting a 16.17% increase over aT. In contrast, PSRJ-95016 recorded the largest decline in Tr at eT3 (-12.72%), suggesting a strong stomatal limitation under heat stress. These results support findings from pprevious studies on groundnut [16], which reported reduced Tr under elevated CO₂. This is likely due to the stomatal closure mechanisms induced by CO₂ enrichment, which reduces water loss but also limits CO₂ uptake.

The response of Water-use efficiency (WUE) clearly indicating eT impacted more of Anet than Tr, leading to lower values of WUE in eT conditions. Interestingly, an average increase in WUE (47.87%) was recorded under eT1+eCO₂, compared to eCO₂ (44.60%) over aT across all genotypes. The exception was IPU-06-02, which exhibited a significantly higher increase in WUE under eCO₂ (68.17%) than eT1+eCO₂ (46.80%). Similarly, PSRJ-95016 showed a 49.72% increase in WUE under eCO₂, but only 43.04% with eT1+eCO₂. The alleviation effect of eCO₂ on temperature-induced stress was evident in

WUE trends, as genotypes with better water-use strategies maintained photosynthetic efficiency even under heat stress. The water-saving effect under eCO₂ supports the CO₂ fertilization theory, where reduced stomatal aperture conserves transpiration without limiting photosynthesis [1].

The interactive effects of eCO₂ and eT suggest that elevated temperature gradients can be beneficial when coupled with CO₂ enrichment, but higher temperature stress offsets these advantages.

3.2 Morphological characteristics

ANOVA revealed that all the morphological parameters such as plant height, stem girth, branch number, root length, root volume, leaf number was highly significant (P<0.01) for genotypes (except for plant height), treatments, and interaction of genotypes × treatments (except with branch number) (Table 1).

The elevated temperature negatively impacted morphological traits, including plant height, stem girth, leaf number, and branch number, with noticeable variations among black gram genotypes except root parameters (Table 3). Whereas elevated CO₂, both individually and in combination with elevated temperature significantly and positively influenced these traits. The highest mean performance of plant height was recorded under eCO₂+eT1 over aT while lowest was under eT3 (Table 3). Among the genotypes, PSRJ-95016 recorded maximum reduction (-28.21%) of plant height under eT3.

Table 1. ANOVA of all parameters of black gram genotypes at ambient, elevated CO₂, three gradients of elevated temperature and its combination with elevated CO₂ conditions

ANOVA	G	T	G x T
A _{net}	37.7	353.2	10.1
gs	0.3	0.3	0.1
Tr	15.3**	2.0**	2.6**
WUE	0.3**	2.2**	0.1**
PH	3.12	2360	81.2
LN	364.1**	626.3**	46.0**
BN	12.5**	9.1**	0.3
SG	0.9**	1.4**	0.2**
RL	7.2**	21.4	3.8**
RV	1.4	1.2	0.5
LDW	60.7**	36.3	10.8**
SDW	191	96.0	27.3
RDW	2.1	0.4	0.4
PWT	83.5	231.8**	9.9
TBM	892.0**	868.5**	81.4
PN	2568	2794	28.0
SN	39803**	27506**	1403.6**
SWT	74.4**	196.7**	9.1
VBM	1014	307**	80.8
HI (%)	123	150	17.6

Error	3.34	0.00	0.6	0.03	11.9	6.2	0.2	0.0	1.2	0.0	1.5	2.9	0.0	3.1	8.2	12.4	85.9	3.0	5.6	6.9
CV (%)	5.1	2.8	5.3	7.4	3.9	7.3	8.1	4.0	7.3	6.5	10.9	10.5	9.7	6.9	5.3	4.3	2.1	81.0	10.3	9.7

(*Significant at 0.05%, **Significant at 0.01%); G-genotypes; T-treatments; GxT- genotypes x treatments; CV- coefficient of variation; A_{net}- photosynthetic rate; gs- stomatal conductance; Tr- transpiration rate; WUE- water use efficiency; PH- plant height; LN- leaf number; BN- branch number; SG- stem girth, RL- root length; RV- root volume; LDW-leaf dry weight; SDW- stem dry weight; RDW- root dry weight; PWT-Pod weight; TBM- total biomass; PN-pod number; SN- seed number; SWT- seed weight; VBM- vegetative biomass; HI- harvest index

Table 2. Physiological parameters of black gram genotypes at ambient, elevated CO₂, three gradients of elevated temperature and its combination with elevated CO₂ conditions

Photosynthetic rate (μmol CO ₂ /m ² /s)								
	aT	eCO ₂	eT1	eT2	eT3	eT1 +eCO ₂	eT2 +eCO ₂	eT3 +eCO ₂
IPU-06-02	31.50	44.50	31.13	32.10	31.47	42.47	39.87	37.83
PLU-826	29.70	42.23	35.07	30.53	31.43	45.40	42.93	38.23
PSRJ-95016	30.27	45.43	28.87	29.30	27.93	39.40	36.47	35.90
IPU-94-1	31.37	43.97	33.13	36.20	31.17	41.40	38.63	37.47
Mean	30.71	44.03	32.05	32.03	30.50	42.17	39.48	37.36
Stomatal conductance (cm/s)								
IPU-06-02	1.16	1.39	1.61	1.69	1.66	1.60	1.79	1.30
PLU-826	1.63	1.95	2.55	1.48	1.35	1.95	1.64	1.96
PSRJ-95016	1.48	1.90	1.76	1.55	1.82	1.86	1.79	1.55
IPU-94-1	1.49	1.54	1.89	1.73	1.70	1.80	1.84	1.73
Mean	1.44	1.70	1.95	1.61	1.63	1.81	1.77	1.64
Transpiration rate (mmol/m ² /s)								
IPU-06-02	14.13	11.83	13.70	14.10	15.10	12.93	14.00	14.93
PLU-826	15.10	16.37	15.63	15.37	14.83	14.43	16.30	15.33
PSRJ-95016	14.93	15.07	13.93	13.80	13.03	13.60	13.70	13.30
IPU-94-1	14.43	15.70	15.30	15.90	16.77	13.47	14.17	14.70
Mean	14.65	14.74	14.64	14.79	14.93	13.61	14.54	14.57
Water Use Efficiency (μmol CO ₂ /mmol H ₂ O)								
IPU-06-02	2.24	3.76	2.27	2.28	2.08	3.28	2.85	2.53
PLU-826	1.97	2.58	2.25	1.99	2.12	3.15	2.64	2.50
PSRJ-95016	2.03	3.03	2.07	2.12	2.15	2.90	2.67	2.71
IPU-94-1	2.17	2.81	2.18	2.29	1.86	3.07	2.73	2.56
Mean	2.10	3.05	2.19	2.17	2.05	3.10	2.72	2.58

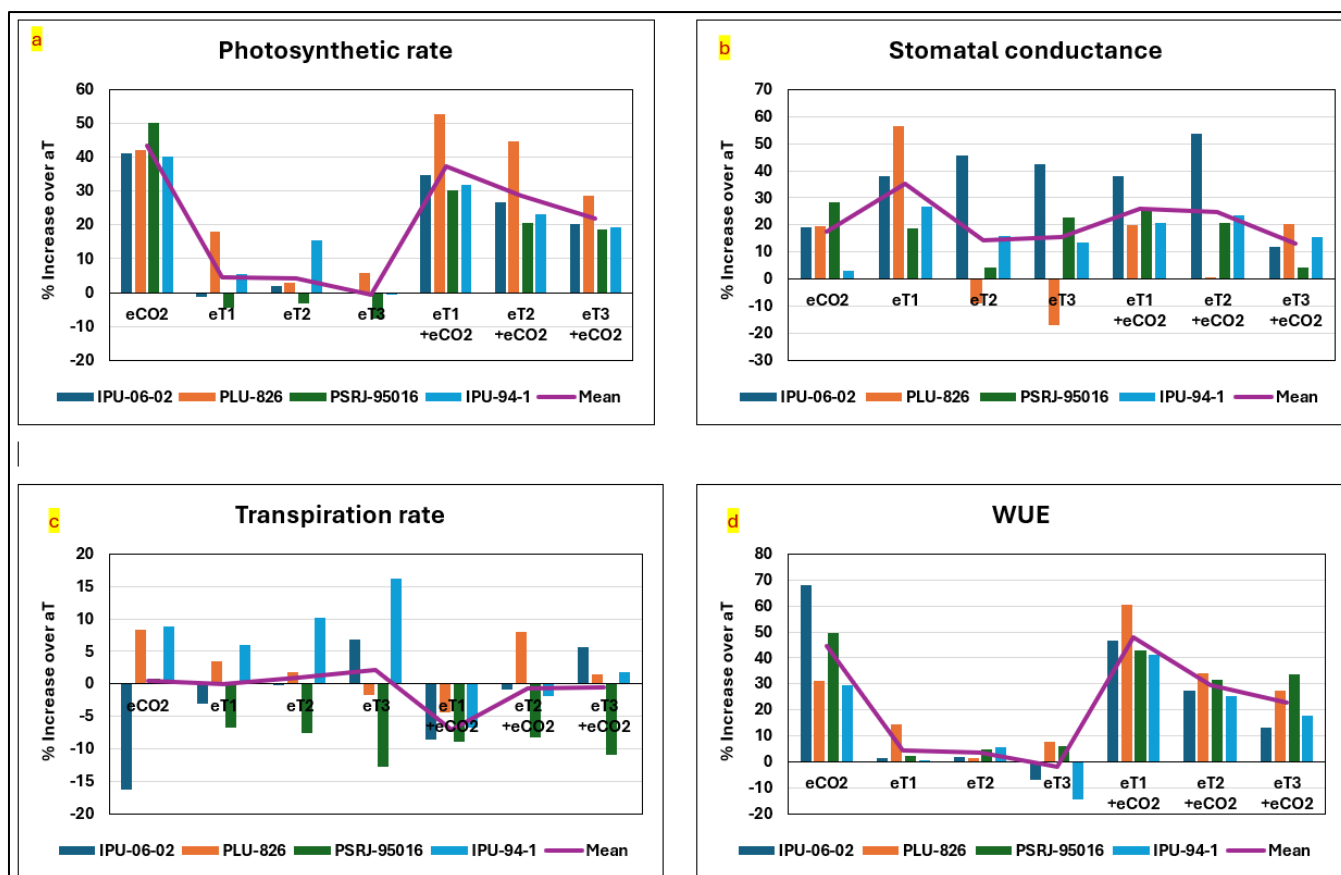


Fig.2. Physiological traits response of black gram at individual and in combination of elevated CO₂ and temperature over ambient condition

According to [17] Vanaja et al. (2024), elevated CO₂ enhanced plant height by increasing biomass allocation towards stem and petiole development, contributing to structural growth with improved carbon assimilation. Similar improvements in plant height and leaf number under eCO₂ have been observed in SPAR chamber experiments with black gram, showing 29-45% early vegetative growth increases [4].

Stem girth followed a similar trend, the stem girth decreased as the temperature gradient increases, which was ameliorated in the presence eCO₂, with a significant increase over aT. The highest stem girth was recorded under eCO₂+eT1 and lowest under eT3. There is a significance genotypic variation was observed among genotypes with highest amelioration of eCO₂ was observed with IPU-94-1 (eCO₂+eT1) and lowest with PSRJ-95016 (eCO₂+eT3).

A significant decrease in leaf number was observed across all black gram genotypes under elevated temperature, with the overall reduction ranging from eT1

(-2.84%) to eT3 (-25.58) (Table 3). However, this reduction was mitigated in the presence of eCO₂ (eCO₂+eT), particularly under eCO₂+eT1 conditions, where the leaf number surpassed that under individual eCO₂. While highest leaf number was recorded with PLU-826 under eCO₂ alone, the greatest improvement (over aT) under combined treatment (eCO₂+eT1) was observed/noted in IPU-06-02, highlighting the genotypic variation in response to these conditions.

Branch number followed a similar trend, showing a negative impact under elevated temperature at eT2 and eT3 across all genotypes, except for PSRJ-95016, which was adversely affected at all three temp gradients/levels. The decline in branch number, however, was lessened under eCO₂ across genotypes. The most notable improvement was observed in IPU-94-1 under eCO₂+eT1, with a 37.50% increase over aT. In contrast, PSRJ-95016 exhibited the least recovery, recording a -10.53% decrease under eCO₂+eT3.

Table 3. Mean performance of morphological traits of four black gram genotypes at aT, eCO₂, eT1, eT2, eT3, eT1 +eCO₂, eT2 +eCO₂ and eT3 +eCO₂

Genotype								
Plant height (cm)	aT	eCO₂	eT1	eT2	eT3	eT1 +eCO₂	eT2 +eCO₂	eT3 +eCO₂
<i>IPU-06-02</i>	81.00	90.33	81.67	78.00	66.00	111.00	102.67	97.33
<i>PLU-826</i>	83.00	107.00	80.67	77.67	66.33	100.33	98.00	90.00
<i>PSRJ-95016</i>	91.00	107.67	84.67	71.33	65.33	109.00	100.33	80.00
<i>IPU-94-1</i>	82.67	103.67	76.67	74.67	70.00	106.33	101.33	93.33
Mean	84.42	102.17	80.92	75.42	66.92	106.67	100.58	90.17
Stem girth (mm)								
<i>IPU-06-02</i>	6.03	6.53	5.97	5.67	5.47	6.53	6.17	6.07
<i>PLU-826</i>	5.60	6.60	6.33	6.50	5.77	6.93	6.67	6.57
<i>PSRJ-95016</i>	5.50	6.27	5.73	5.63	6.03	6.53	6.27	5.57
<i>IPU-94-1</i>	5.67	6.17	5.50	6.10	5.60	6.50	6.37	6.27
Mean	5.70	6.39	5.88	5.98	5.72	6.63	6.37	6.12
Leaf number/plant								
<i>IPU-06-02</i>	27.33	33.00	25.33	18.00	16.67	48.67	35.67	26.67
<i>PLU-826</i>	34.00	50.00	32.67	29.67	24.00	48.33	45.33	35.67
<i>PSRJ-95016</i>	34.00	41.33	31.00	29.00	24.33	37.00	35.67	30.67
<i>IPU-94-1</i>	30.67	39.33	33.33	33.33	29.00	44.00	43.33	39.33
Mean	31.50	40.92	30.58	27.50	23.50	44.50	40.00	33.08
Branch number								
<i>IPU-06-02</i>	6.67	8.33	6.67	6.33	5.67	8.67	7.67	7.33
<i>PLU-826</i>	5.33	6.67	5.33	5.00	4.33	7.00	5.33	5.00
<i>PSRJ-95016</i>	6.33	7.00	5.33	5.33	4.67	6.67	6.67	5.67
<i>IPU-94-1</i>	5.33	7.00	5.33	5.00	5.00	7.33	6.33	5.67
Mean	5.92	7.25	5.67	5.42	4.92	7.42	6.50	5.92
Root length (cm)								
<i>IPU-06-02</i>	13.67	16.33	14.00	14.67	15.67	13.67	15.00	15.13
<i>PLU-826</i>	13.67	16.67	15.05	15.33	15.83	16.00	13.33	13.33
<i>PSRJ-95016</i>	12.67	16.00	13.50	18.67	18.67	16.00	15.33	15.33
<i>IPU-94-1</i>	13.33	16.67	13.33	17.00	16.73	13.67	12.33	13.00
Mean	13.33	16.42	13.97	16.42	16.73	14.83	14.00	14.20
Root volume (ml)								
<i>IPU-06-02</i>	2.67	3.63	3.02	3.42	3.17	3.00	2.67	2.70
<i>PLU-826</i>	2.77	3.70	2.90	3.10	3.27	4.00	4.00	4.00
<i>PSRJ-95016</i>	2.30	3.67	2.50	3.00	3.40	3.27	2.80	2.53
<i>IPU-94-1</i>	2.63	3.00	3.27	3.00	3.13	3.97	3.93	3.50
Mean	2.59	3.50	2.92	3.13	3.24	3.56	3.35	3.18

3.3 Biomass and yield traits

ANOVA revealed highly significant ($P < 0.01$) variability in genotypes, treatments, and their interaction for biomass and yield traits (Table 1). Elevated CO₂ (eCO₂) positively influenced biomass accumulation, while elevated temperature (eT) had a negative impact. The degree of response varied among genotypes for root and stem characteristics.

Shoot dry weight (SDW) and leaf dry weight (LDW) showed notable improvements under eCO₂+eT1 compared to individual eCO₂ treatments, relative to ambient conditions (aT). Root dry weight (RDW) was slightly higher under individual eCO₂. Vegetative biomass (VBM) was highest under eCO₂+eT1, while the percentage increase in reproductive biomass (pod weight and seed weight) was greater under individual eCO₂ across most genotypes.

This differential response in biomass allocation suggests that eCO₂ may enhance carbon partitioning

towards reproductive structures, potentially improving yield potential. Similar trends were reported in black gram where eCO₂ combined with moderate heat stress increased photosynthesis, pod number (28%), and grain yield by ~41% [4]. Broader evidence also supports this trend. A meta-analysis showed that elevated CO₂ increased reproductive allocation in crops [18]. Similarly, in quinoa, seed dry mass increased by 12–44% while total biomass rose only ~10%, indicating preferential allocation to reproduction [19]. These findings reinforce our observation that eCO₂ can enhance reproductive investment, improving yield potential in black gram. Genotypic variation was observed, with IPU-94-1 showing a significant reduction in VBM under eCO₂, contrary to other genotypes. However, reproductive biomass increased under eCO₂ in IPU-94-1, indicating a possible shift in resource allocation favoring yield components. This variation in response highlights the importance of genotype selection for adapting to future climate scenarios.

Table 4. Mean performance of biomass of four black gram genotypes at aT, eCO₂, eT1, eT2, eT3, eT1 + eCO₂, eT2 + eCO₂ and eT3 + eCO₂

Stem Dry Weight (g/pl)								
	aT	eCO ₂	eT1	eT2	eT3	eT1 + eCO ₂	eT2 + eCO ₂	eT3 + eCO ₂
IPU-06-02	13.16	15.67	16.42	13.15	10.26	17.63	16.05	15.92
PLU-826	13.77	24.84	19.35	15.97	12.23	26.06	22.03	20.47
PSRJ-95016	10.65	15.84	11.84	13.68	9.06	19.12	13.80	12.15
IPU-94-1	18.52	15.25	14.75	19.21	21.20	26.50	15.98	13.22
Mean	14.02	17.90	15.59	15.50	13.19	22.32	16.96	15.44
Leaf Dry Weight (g/pl)								
IPU-06-02	8.45	14.01	11.40	10.83	8.20	11.63	11.39	11.25
PLU-826	9.56	14.14	11.72	13.19	8.08	14.19	12.43	10.28
PSRJ-95016	7.79	11.85	9.53	8.80	6.29	12.57	7.74	8.95
IPU-94-1	15.14	12.59	11.50	14.09	14.01	18.36	10.04	8.18
Mean	10.23	13.15	11.04	11.73	9.15	14.19	10.40	9.67
Root Dry Weight (g/pl)								
IPU-06-02	0.80	0.73	0.57	1.25	0.90	0.87	0.63	0.52
PLU-826	1.42	1.81	1.44	0.80	1.28	1.95	1.52	1.94
PSRJ-95016	0.84	1.85	1.65	1.44	1.55	1.38	0.85	1.02
IPU-94-1	1.56	1.33	1.35	1.24	1.89	1.42	1.12	0.91
Mean	1.16	1.43	1.25	1.18	1.40	1.40	1.03	1.10

Vegetative Biomass (g/pl)								
<i>IPU-06-02</i>	28.45	36.70	33.10	31.40	23.94	38.63	34.19	33.15
<i>PLU-826</i>	30.04	45.96	38.13	35.24	26.94	47.66	41.29	38.35
<i>PSRJ-95016</i>	24.71	35.83	28.69	29.31	22.48	39.38	28.43	28.45
<i>IPU-94-1</i>	40.71	35.99	34.12	41.08	42.52	52.98	33.71	28.75
Mean	30.98	38.62	33.51	34.26	28.97	44.66	34.41	32.18
Total Biomass (g/pl)								
<i>IPU-06-02</i>	46.09	64.07	50.97	47.40	36.37	62.12	54.07	49.69
<i>PLU-826</i>	48.72	71.12	55.68	50.13	42.27	75.49	62.61	59.36
<i>PSRJ-95016</i>	40.65	60.59	44.93	44.27	35.13	60.38	45.56	43.33
<i>IPU-94-1</i>	63.58	66.11	53.62	59.93	60.47	76.36	51.81	48.15
Mean	49.76	65.47	51.30	50.43	43.56	68.59	53.51	50.13

Yield parameters were significantly influenced by eCO₂ and temperature gradients, with notable variations across genotypes. A significant reduction in mean performance was observed under eT as temperature gradient increased. These parameters improved under eCO₂ alone and in combination (eCO₂+eT). Seed number and seed weight were notably higher under individual eCO₂, while husk weight was significantly higher in combination (eCO₂+eT), suggesting improved seed filling across black gram genotypes under individual eCO₂. Though not directly assessed in this study, elevated CO₂ combined with heat stress has been shown to reduce grain micronutrient concentrations—particularly iron and zinc—by approximately 9–18% in legumes [20]. These reductions are often associated with nutrient dilution effects under eCO₂. On average, pod number increased under eCO₂ (32.55%) and eCO₂+eT1 (31.13%) compared to aT. PSRJ-95016 showed the highest increase (33.58%), demonstrating a strong response to CO₂ enrichment. Pod weight was highest in PSRJ-95016 under eCO₂ (45.25% increase over aT), while IPU-06-02 recorded the steepest decline under eT3 (-28.24%), indicating its vulnerability to temperature stress.

Seed number and seed weight showed substantial increases under eCO₂, with PSRJ-95016 exhibiting the highest improvement among all genotypes over aT. However, PSRJ-95016 also showed reductions under all eT gradients, indicating sensitivity to temperature stress. Similar trends have been documented in mungbean and kidney bean, where extreme temperature nullified the benefits of elevated CO₂ by impairing reproductive development [10, 15]. IPU-06-02 recorded the highest significant reduction under eT3.

Harvest index (HI) and 100-seed weight were negatively impacted by eT but ameliorated by eCO₂. Interestingly, IPU-94-1 recorded the lowest VBM under eCO₂ but the highest HI, with the greatest amelioration under eCO₂+eT3. For 100-seed weight, PLU-826 showed the highest value under eCO₂+eT1, demonstrating the greatest amelioration among all genotypes.

These findings highlight the significant role of eCO₂ in improving yield traits, while eT leads to reductions. The results confirm that genotypic variation plays a crucial role in adapting to changing environmental conditions and emphasize the need for selecting stress-resilient cultivars to sustain productivity in future climates. The observed increases in biomass and yield components under eCO₂ align with previous studies on legumes and other C3 crops. The enhanced carbon assimilation and improved water-use efficiency under eCO₂ likely contribute to the observed improvements in growth and yield parameters. However, the negative impacts of elevated temperature, particularly at higher gradients, underscore the complex interactions between CO₂ and temperature in determining crop productivity.

The genotypic variations observed in this study emphasize the importance of breeding and selecting cultivars that can capitalize on the benefits of eCO₂ while maintaining resilience to temperature stress. These patterns align with recent reviews highlighting that crop genotypes with greater reproductive allocation and efficient resource use perform better under elevated CO₂ and warming scenarios [21]. Future research should focus on understanding the physiological and molecular mechanisms underlying these genotypic differences to inform breeding strategies for climate-resilient black gram varieties.

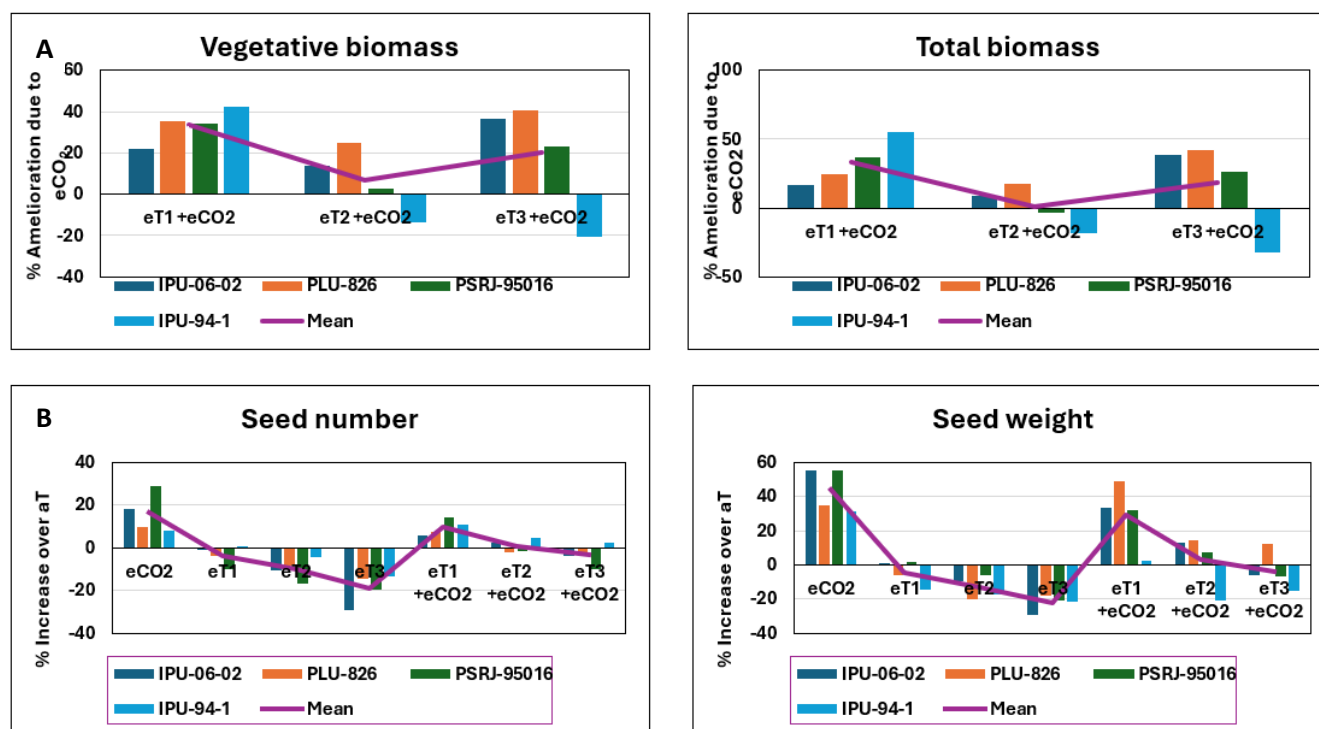


Fig.3. A. Amelioration of high temperature impact by eCO₂ on biomass; B. Impact of eCO₂ and elevated temperature in over yield traits of four black gram genotypes

Table 5. Mean performance of yield traits of four black gram genotypes at aT, eCO₂, eT1, eT2, eT3, eT1 + eCO₂, eT2 + eCO₂ and eT3 + eCO₂

Cluster no./pl								
	aT	eCO ₂	eT1	eT2	eT3	eT1 + eCO ₂	eT2 + eCO ₂	eT3 + eCO ₂
IPU-06-02	18.00	24.67	20.00	16.67	14.67	31.00	29.33	23.67
PLU-826	29.33	41.67	28.00	21.67	17.33	50.67	38.00	30.33
PSRJ-95016	26.00	42.00	23.67	20.33	18.33	36.67	30.67	30.00
IPU-94-1	25.67	31.00	21.33	20.33	18.67	36.33	30.33	22.00
Mean	24.75	34.83	23.25	19.75	17.25	38.67	32.08	26.50
Pod number/pl								
IPU-06-02	74.33	96.67	71.33	65.67	53.33	95.33	88.00	72.67
PLU-826	85.00	107.33	78.00	77.33	63.67	106.67	96.00	80.00
PSRJ-95016	65.33	91.67	64.00	62.67	51.00	93.00	75.00	60.33
IPU-94-1	90.33	120.67	81.33	79.67	78.33	116.00	97.67	86.67
Mean	78.75	104.08	73.67	71.33	61.58	102.75	89.17	74.92
Pod wt. (g/pl)								
IPU-06-02	23.69	33.67	22.58	22.17	17.00	32.00	26.00	22.00
PLU-826	23.98	30.33	23.17	20.18	20.67	33.29	26.63	26.68
PSRJ-95016	21.38	31.05	21.91	20.35	18.23	27.32	23.16	21.20

<i>IPU-94-1</i>	28.36	36.93	26.03	25.39	23.36	30.08	24.67	25.84
Mean	24.35	32.99	23.42	22.02	19.82	30.67	25.12	23.93
Husk wt. (g/pl)								
<i>IPU-06-02</i>	6.05	6.30	4.71	6.17	4.57	8.51	6.12	5.46
<i>PLU-826</i>	5.29	5.16	5.62	5.28	5.35	5.45	5.31	5.66
<i>PSRJ-95016</i>	5.44	6.29	5.67	5.39	5.58	6.31	6.03	6.33
<i>IPU-94-1</i>	5.49	6.81	6.52	6.54	5.41	6.70	6.58	6.44
Mean	5.57	6.14	5.63	5.85	5.23	6.74	6.01	5.97
Seed no.								
<i>IPU-06-02</i>	427.33	504.33	422.33	381.33	303.00	453.00	437.33	411.00
<i>PLU-826</i>	476.67	522.67	459.33	436.00	408.00	513.00	466.00	464.00
<i>PSRJ-95016</i>	408.00	526.33	367.67	340.33	327.67	465.67	400.67	368.00
<i>IPU-94-1</i>	481.00	521.00	482.33	461.33	416.33	532.33	503.33	493.67
Mean	448.25	518.58	432.92	404.75	363.75	491.00	451.83	434.17
Seed wt. (g/pl)								
<i>IPU-06-02</i>	17.64	27.37	17.87	16.00	12.43	23.49	19.88	16.54
<i>PLU-826</i>	18.69	25.16	17.55	14.90	15.33	27.84	21.32	21.01
<i>PSRJ-95016</i>	15.94	24.76	16.23	14.96	12.65	21.00	17.13	14.88
<i>IPU-94-1</i>	22.87	30.12	19.51	18.85	17.95	23.38	18.10	19.40
Mean	18.79	26.85	17.79	16.18	14.59	23.93	19.11	17.96
HI (%)								
<i>IPU-06-02</i>	38.24	42.70	35.05	33.79	34.14	37.79	36.75	33.35
<i>PLU-826</i>	38.35	35.42	31.51	29.79	36.23	36.87	34.00	35.44
<i>PSRJ-95016</i>	39.19	40.80	36.03	33.74	36.00	34.80	37.63	34.29
<i>IPU-94-1</i>	36.09	45.48	36.41	31.48	29.75	30.62	34.94	40.30
Mean	37.97	41.10	34.75	32.20	34.03	35.02	35.83	35.85
100 Seed wt. (g)								
<i>IPU-06-02</i>	4.13	5.43	4.23	4.20	4.10	5.19	4.54	4.03
<i>PLU-826</i>	3.92	4.81	3.82	3.42	3.75	5.42	4.58	4.53
<i>PSRJ-95016</i>	3.90	4.70	4.42	4.39	3.86	4.51	4.28	4.04
<i>IPU-94-1</i>	4.75	5.78	4.05	4.09	4.31	4.39	3.60	3.93
Mean	4.17	5.18	4.13	4.03	4.01	4.88	4.25	4.13

*HI = Harvest Index.

IV. CONCLUSION

Elevated CO₂ (eCO₂) significantly influenced black gram genotypes, enhancing photosynthetic efficiency, WUE, and biomass accumulation. PLU-826 and IPU-06-02 exhibited greater adaptability under eCO₂+eT1, with higher photosynthetic rate, WUE, and pod number, whereas PSRJ-95016 and IPU-94-1 were more sensitive to eT3, with reduced biomass and yield. Morphological traits, including

plant height and leaf number, improved under eCO₂, particularly in IPU-06-02 and PLU-826. Biomass accumulation increased significantly under eCO₂, with PSRJ-95016 and IPU-06-02 exhibiting enhanced carbon assimilation. Yield traits, including pod number, seed weight, and harvest index (HI), responded positively to eCO₂, with IPU-06-02 showing the highest seed weight under eCO₂ and IPU-94-1 experiencing the greatest

reduction under eT3. The interaction of eCO₂ and moderate temperature elevation (eT1) improved yield, while extreme heat stress (eT3) reduced these benefits.

These results emphasize the role of eCO₂ in alleviating temperature stress, improving productivity, and highlighting genotypic differences. Selecting climate-resilient black gram cultivars is crucial for future adaptation.

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L-Tryptophan Improves Germination and Early Growth of *Glycine max* Seedlings Subjected to Cold Test

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Received: 12 Aug 2025; Received in revised form: 10 Sep 2025; Accepted: 15 Sep 2025; Available online: 19 Sep 2025

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Abstract— The genetic potential of seeds does not inherently ensure uniform establishment and optimal stand quality in commercial crop fields. This challenge has led to the development of various agricultural inputs, often applied directly to seeds, including L-tryptophan. The present study was conducted using a completely randomized factorial experimental design with two factors and four replications. The first factor comprised two levels: the presence (CT) and absence (ACT) of a cold test. The second factor included four concentrations of L-tryptophan: 0, 0.02, 0.04, and 0.08 g kg⁻¹ of seeds. Soybean seeds were sown on Germitest® paper substrate and maintained in a germination chamber. Evaluations included germination rate (normal and abnormal seedlings, as well as hard or dead seeds), primary root length, shoot length and dry matter. The application of L-tryptophan exhibited a protective effect on soybean seeds subjected to cold stress, indicating its potential as an effective seed treatment for crops expected to encounter low temperatures and high soil moisture during germination.

Keywords— amino acid, cold test, germination test, soybean, seed vigor.



I. INTRODUCTION

Soybean is highlighted as the sixth most produced staple crop in the world and occupies the fourth largest area of cultivation (FAO, 2025). Brazil stands out as the World's largest producer of soybeans (USDA-FAS, 2025), with a production in the harvest season of 2024/2025 reaching approximately 164 million metric tons (IBGE, 2025). Nevertheless, the country faces big challenges and risks regarding the production of this crop (Da-Silva *et al.*, 2020).

Seed quality is one of the many factors that considerably affect soybean establishment and yield. Although, seeds bring with themselves high genetic potential, this potential does not guarantee uniform establishment and initial soybean stand quality in commercial fields (Mariano-da-Silva *et al.*, 2025; Mógór *et al.*, 2008). Hence, this has led to the emergence of various agricultural inputs designed to be applied directly to seeds with the aim of improving crop

establishment, as it is the case of the use of the essential amino acid L-tryptophan. These inputs have been associated with greater root development and stress resistance (Pessoa, 2021).

L-tryptophan is one of the 20 essential amino acids required for protein biosynthesis. This amino acid contains an amino group (basic), a carboxyl group (acidic), and a side chain containing an indole structure, making it an aromatic and nonpolar amino acid (Mariano-da-Silva, 2023). Plants synthesize tryptophan either from shikimic acid or anthranilic acid, and when degraded, it is responsible for producing auxin (Taiz *et al.*, 2021).

Ahemad & Kibret (2014) demonstrated that auxin levels increase in plant tissues following an exogenous application of L-tryptophan. It is well known that L-tryptophan is the main precursor in auxin biosynthesis pathways, a phytohormone that plays a fundamental role in cell elongation (Taiz *et al.*, 2021). In association with

gibberellin, jasmonic acid, salicylic acid, and abscisic acid, auxin positively influences the effect of PIN proteins, which regulate radicle protrusion (Mukherjee *et al.*, 2014; Wan *et al.*, 2018). On the other hand, the interaction between auxin and jasmonic acid promotes plant tolerance to abiotic stresses, such as drought, and biotic stresses caused by fungi or insects (Hussain, 2024; Wan *et al.*, 2018).

Vigor tests (stress conditions: water deficit, salinity, flooding, cold, controlled deterioration, and accelerated aging) were developed to identify differences in the physiological potential of seed batches (Krzyzanowski *et al.*, 2020). The cold test is probably the most studied vigor test, assessing the ability of seeds to germinate in cold and moist soils (Cicero *et al.*, 2020). Therefore, this test is expected to verify the protective effect of L-tryptophan.

These cold and humid conditions during sowing are commonly found in Southern states of Brazil, where, according to the Ordinance 886 of the Ministry of Agriculture and Livestock (MAPA, 2023), soybean sowing can begin as early as September 11 (Paraná State), October 01 (Rio Grande do Sul State), and October 02 (Santa Catarina State).

The objective of this study was to evaluate the effects of L-tryptophan on the germination process and seedling development of soybeans subjected to the cold test, using the parameters of germination, root length, shoot length, and dry matter.

II. MATERIAL AND METHODS

2.1 Experimental settings

The experiment was conducted at the Seed and Grain Laboratory located at the Federal University of Fronteira Sul, campus Chapecó. The soybean seeds used during the experiment were produced during the 2023/2024 harvest season by Limagrain Brasil S/A. These seeds are from the cultivar 60163IPRO (category S1) and present a germination potential of 80% and a purity of 99%.

2.2 Experimental design

The trial was conducted using a completely randomized factorial design with two factors and four replications (Ares & Granato, 2014; Pimentel-Gomes, 2000). The first factor consisted of two sub-factors: the presence (CT) or absence of the cold test (ACT). The second factor consisted of four different sub-factors: 0, 0.02, 0.04, and 0.08 g of tryptophan per kg⁻¹ of seed.

2.3 Treatment with L-tryptophan

The soybean seeds used in the trial were divided into eight batches of 200 g each and stored in transparent plastic

bags. L-tryptophan was previously diluted in water in Petri dishes, generating the four concentrations previously mentioned. Once the solution was added to the plastic bags, air was injected, and the bags were vigorously shaken until the distribution of the treatments was considered homogeneous. The batches were then placed to dry in the shade at an approximate temperature of 25 °C for 24 h (Marcos-Filho, 2015).

2.4 Cold and germination test

Each batch was divided into two sub-batches, where one sub-batch was allocated to the conventional germination test and the other to the cold test. Seeds designated for the cold test were sown on Germitest® paper substrate previously moistened with a volume of water corresponding to 3.0 times the dry weight of the paper. The rolls were then placed inside plastic bags, sealed with adhesive tape, and stored in a BOD chamber at 10 ± 1 °C for five days (Cicero *et al.*, 2020; Sá *et al.*, 2011). After this period, the bags were opened, and the germination test was conducted. Seeds designated for the conventional germination test were sown on Germitest® paper substrate, previously moistened with a volume of water equivalent to 2.5 times the dry weight of the paper. The paper rolls were maintained in a germinator at a constant temperature of 25 ± 1 °C (Brasil, 2025). Germination evaluations were performed on the fifth and eighth days after sowing, and the data were converted into percentage of normal seedlings (Brasil, 2025; Sá *et al.*, 2011).

2.5 Root and shoot length

With the aid of a millimeter ruler, the length of the primary root and shoot length were measured, and the average results were expressed in cm per seedling (Brasil, 2025; Sá *et al.*, 2011).

2.6 Dry matter

The determination of dry matter was performed on normal seedlings, quantifying the shoot and root portions. After weighing, the seedlings were placed in paper bags and dried in an oven with forced air circulation at a constant temperature of 80 ± 2 °C for 24 hours (Sá *et al.*, 2011). After drying, the samples were weighed again after several hours at room temperature, and the dry matter content was determined and expressed in grams per seedling.

2.7 Statistical analysis

All ANOVA assumptions were checked (normality, homogeneity and independence) for all data collected during the trials. When all assumptions were satisfied, ANOVA was used to analyze the variables (F-test at 95%). A multi comparison test (Tukey's HSD) or a regression analysis, both at 95% confidence level, was used to

compare means that were identified as significantly different (Pimentel-Gomes, 2000; Zimmermann, 2004).

III. RESULTS AND DISCUSSION

According to the analysis of variance (F-test), a significant interaction was observed between the CT/ACT factors and L-tryptophan doses for all tested variables, including the percentage of normal seedlings, percentage of abnormal seedlings, root length, shoot length, and dry matter content, indicating a dependency between these factors. By further analyzing the interaction effect through an additional analysis of variance (F-test), where the levels of the L-tryptophan dose factor were compared within each CT/ACT factor level (and vice versa), a significant effect was observed for the CT and ACT factors within all tested

L-tryptophan doses for all variables (Table 1). A significant effect was also observed for the L-tryptophan dose factor within each CT/ACT condition, specifically at the doses of 0, 0.02, 0.04, and 0.08 g kg⁻¹ of seed for all analyzed variables. This effect can be observed through the estimates of the second-order polynomial equations presented in Fig. 01 (A, B, C, D, E and F).

The results obtained demonstrated that increasing the L-tryptophan dose led to a higher percentage of germinated seeds, both in those subjected to cold treatment (CT) and those without it (ACT), indicating that L-tryptophan positively modulated seed germination. Additionally, L-tryptophan treatment reduced the percentage of hard and dead seeds compared to the control. However, this effect was accompanied by an increase in abnormal seedlings in the cold-treated group.

Table 1. Mean percentage of normal and abnormal seedlings, ungerminated seeds, root length, shoot length, and dry matter content of soybean seeds and seedlings with and without the application of the cold test and treated with different doses of L-tryptophan.

Variable	Treatment	L-tryptophan concentration (g kg ⁻¹ of seeds)			
		0.0	0.02	0.04	0.08
Normal seedlings (%)	ACT	77.3 a	81.4 a	85.3 a	85.8 a
	CT	60.3 b	74.0 b	75.0 b	76.0 b
CV = 2.741%					
Abnormal seedlings (%)	ACT	19.8 a	10.0 b	12.5 b	12.5 b
	CT	16.5 b	17.8 a	16.8 a	16.8 a
CV = 20.425%					
Ungerminated seeds (dead and hard seeds) (%)	ACT	3.0 b	8.6 ns	2.3 b	1.8 b
	CT	23.3 a	8.3 ns	8.4 a	7.3 a
CV = 20.233%					
Root length (cm seedling ⁻¹)	ACT	8.05 a	11.07 a	11.18 a	11.88 a
	CT	6.63 b	8.19 b	8.10 b	8.51 b
CV = 5.532%					
Shoot length (cm seedling ⁻¹)	ACT	0.93 a	1.33 a	1.47 a	1.68 a
	CT	0.40 b	0.57 b	0.54 b	1.03 b
CV = 12,068%					
Dry matter (g seedling ⁻¹)	ACT	0.3138 ns	0.8293 a	0.7793 a	0.7302 a
	CT	0.3163 ns	0.2927 b	0.5643 b	0.5918 b
CV = 10.200%					

Means followed by the same letter do not differ among themselves within the same column for the same variable, according to Tukey's HSD test at 95% confidence level.

ns – not significant according to the F-test (5%);

ACT – absence of the cold test;

CT – presence of the cold test;

CV – Coefficient of variance.

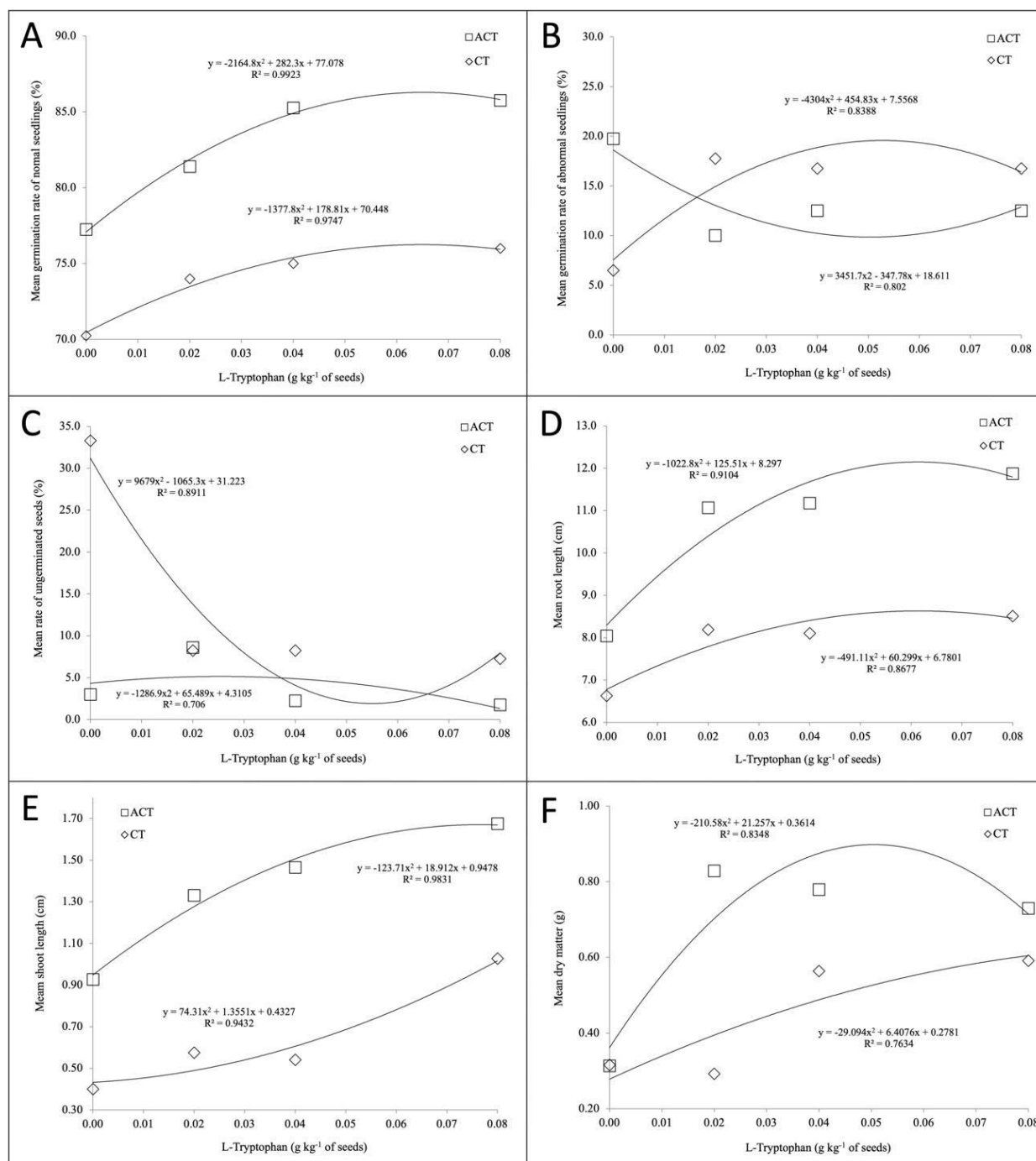


Fig. 1. Second-order polynomial equations referring to (A) mean germination rate of normal seedlings, (B) mean germination rate of abnormal seedlings, (C) ungerminated seeds, (D) mean root length of seedlings, (E) shoot length of seedlings and (F) dry matter of seedlings, of soybean (*Glycine max* L.), with (CT) and without (ACT) the application of the cold test and treated with different doses of L-tryptophan.

As the treatments combining CT and L-tryptophan resulted in more than 70% normal seedlings, it can be inferred that L-tryptophan effectively protected the seeds from the stress induced by the test (Cicero *et al.*, 2020).

This trend has already been reported by several authors. Korkmaz *et al.* (2020) demonstrated that pepper (*Capsicum annuum* L.) seeds treated with L-tryptophan

showed a significant improvement in germination rates under salt stress. Hussain *et al.* (2024), evaluating the effects of L-tryptophan on sunflower (*Helianthus annuus* L.) seed germination under subtoxic doses of cadmium, reported that the amino acid reduced the metal's adverse effects on seed germination rates. Queiroz *et al.* (2023) demonstrated that the exogenous application of L-

tryptophan in soybean (*Glycine max* L.) seeds significantly increased germination rates, concluding that the amino acid can serve as an important inducer of seed germination and vigor. Hanci *et al.* (2019) tested the effects of various doses of a combination of L-tryptophan and melatonin on the germination of onion (*Allium cepa* L. cv. Valenciana) seeds under low temperatures, observing significant increases in germination rates at 7 °C. Similarly, Abdelkader *et al.* (2023) reported a 10% increase in germination rates compared to the control in onion (*A. cepa* L. cv. Gaza) seeds treated with L-tryptophan.

The variables root length and shoot length were also affected by the exogenous application of L-tryptophan. Although both parameters showed decreases when subjected to the cold stress test, it was observed that higher doses of L-tryptophan resulted in greater root and shoot length.

Similar results can be found in the literature. Hussain *et al.* (2024) demonstrated that L-tryptophan treatment mitigated the deleterious effects of cadmium on root and shoot growth in sunflower (*Helianthus annuus* L.) seedlings. Abdelkader *et al.* (2023) reported that the greatest root lengths were obtained when onion (*Allium cepa* L. cv. Gaza) seeds were treated with L-tryptophan. El-Sayed *et al.* (2019) showed that L-tryptophan alleviated the toxic effects of cadmium on plant height and leaf mass in eucalyptus (*Eucalyptus gomphocephala*).

Analyzing the results, it is evident that increasing L-tryptophan doses led to an increase in dry matter content, both in the CT and ACT treatments. As previously observed, L-tryptophan treatment enhanced shoot and root length parameters, directly influencing the dry matter content.

Other authors have also reported this trend. Sadak & Ramadan (2021) tested the effects of various L-tryptophan doses on the fresh and dry weight of the shoot in white lupin (*Lupinus termis* L.) under different water stress conditions, observing that the amino acid increased these parameters compared to the control. Sudadi & Suryono (2015) and Sanada & Agahara (2023) showed that exogenous L-tryptophan enhanced dry matter content in both shoots and roots of soybean (*Glycine max* L.).

IV. CONCLUSION

L-tryptophan exhibited a protective effect on soybean seeds subjected to the cold test, improving germination percentage, dry matter accumulation, root length, and shoot length. This suggests that L-tryptophan is a promising alternative for seed treatment, particularly for

crops sown under low-temperature and soils with high moisture conditions.

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Social and personal characteristics of tribal farm women involved in sericulture

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Received: 09 Aug 2025; Received in revised form: 10 Sep 2025; Accepted: 17 Sep 2025; Available online: 24 Sep 2025

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Abstract— The study entitled “Social and Personal Characteristics of Tribal Farm Women Involved in Sericulture” was undertaken to examine the demographic and socio-economic profile of women engaged in sericulture. Dhemaji district of Assam was purposively selected for the study owing to its rich tradition of eri and muga silk rearing among tribal households. A total of 120 respondents were chosen using a multistage random sampling procedure across ten villages. Data were collected through personal interviews with the help of a structured schedule and analysed using frequency, percentage, mean, and standard deviation. The findings revealed that the majority of respondents (70.00%) belonged to the middle-aged group of 36–60 years, with most having educational attainment up to high school (33.34%). Nuclear families were predominant (78.34%) with medium family size (3–8 members, 86.66%), and most families combined sericulture with agriculture or allied activities (54.16%). A large proportion were marginal farmers (65.84%), with an average operational holding of 5.67 bighas under sericulture and a mean experience of 22.19 years. Annual income from sericulture averaged ₹2.56 lakhs, with Eri silk emerging as the dominant product marketed (96.66%), followed by growing interest in value-added products (70.00%). While extension contacts were moderate (77.50%), only 40.84% of respondents had received formal training, reflecting reliance on traditional knowledge. Marketing was mainly through local markets and fairs (71.60%), supplemented by direct sales to boutiques and designers (34.10%). The study concludes that sericulture is a vital livelihood activity for tribal women, contributing significantly to household income and cultural continuity. However, challenges persist in terms of small landholdings, low training exposure, and limited access to structured markets. Strengthening institutional support, capacity-building, and market linkages could enhance the sustainability and profitability of women-led sericulture in Assam.



Keywords— Sericulture, Tribal women, Muga, Eri, Dhemaji, Assam

I. INTRODUCTION

Sericulture is an important agro-based industry in India, deeply linked with rural livelihood, tradition, and socio-cultural practices. It plays a vital role in generating employment and strengthening the rural economy, particularly in the northeastern states where it serves as a key source of income (CSB, 2023). Assam enjoys a distinctive position in the country as it is the only state that

produces all four varieties of silk—mulberry, muga, eri, and tasar. Among these, muga silk, valued for its natural golden shine, and eri silk, often called the “fabric of peace,” are native to the state and symbolize its cultural identity (Bordoloi et al., 2020). Currently, sericulture in Assam is practiced across nearly 3.04 lakh hectares, involving around 2.6 lakh households in production activities (Statistical Handbook of Assam, 2023). Tribal communities, in

particular, have been closely associated with eri and muga culture, which are not only important for their income but also deeply embedded in their traditional way of life. Women contribute extensively at each stage of sericulture—ranging from rearing silkworms, collecting leaves, harvesting cocoons, to spinning and weaving. Their role is crucial not only for sustaining family earnings but also for preserving indigenous weaving practices within tribal society (Saikia & Kalita, 2019). The personal and social attributes of tribal farm women, such as their age, literacy, household structure, level of participation, and decision-making ability, strongly influence their performance and engagement in sericulture. These factors shape their capacity to utilize resources, adopt innovations, and maintain livelihood security through silk production. With this background, the present study has been carried out with the following objective

Objective: To examine the social and personal characteristics of tribal farm women engaged in sericulture in Dhemaji district of Assam.”

II. MATERIALS AND METHODS

The study was conducted in Dhemaji district of Assam, selected purposively for the research. A total of ten villages were chosen through random sampling, from which twelve respondents were randomly drawn in each village, constituting a sample size of 120. Data were collected through personal interviews using a pre-tested structured schedule. The respondents were identified following a multistage sampling procedure. For the analysis of data, statistical techniques such as mean, frequency, percentage, and standard deviation were used.

III. RESULTS

Table 1: Distribution of respondents according to their social and personal characteristics

n=120

SI No	Category	f	(%)	Mean	SD
1	Age				
	Below 36	18	15.00	48.05	12.02
	36-60	84	70.00		
	Above 60	18	15.00		
2	Education level				
	Illiterate	10	8.33		
	Signature literate	9	7.50		
	Primary school passed	12	10.00		
	Middle school passed	19	15.83		
	High school passed	40	33.34		
	Higher secondary passed	24	20.00		
	Graduate	6	5.00		
3	Family type				
	Nuclear	94	78.34		
	Joint	18	15.00		
	Extended	8	6.66		
4	Family size				
	Small (below 3)	2	1.67	5.42	2.59
	Medium (3-8)	104	86.66		
	Large (Above 8)	14	11.67		
5	Occupation of family				
	Only sericulture	41	34.16		

	Sericulture+Agriculture/Allied	65	54.16		
	Sericulture+Service(salaried)	6	5.00		
	Sericulture+Wage earner	4	3.34		
	Sericulture+Business	4	3.34		
6	Total annual income				
	Low (Below Rs 1 lakh)	10	8.33	Rs 3.74 Lakhs	Rs 2.74 lakhs
	Medium (Rs 1 lakh – Rs 6.48 lakhs)	95	79.17		
	High (Above Rs 6.48 lakhs)	15	12.5		
7	Operational land holding(ha)				
	Marginal farmer (less than 1 ha)	79	65.84		
	Small farmer (1 to 2 ha)	36	30		
	Semi medium farmer (2 to 4)	5	4.16		
8	Area under sericulture (bigha)				
	Low (below 2.67 bigha)	6	5.00	5.67 bigha	2.96 bigha
	Medium (2.67 bigha-8.59 bigha)	102	85.00		
	High (more than 8.59 bigha)	12	10.00		
9	Experience in sericulture (years)				
	Low (Below 10 years)	11	9.16	22.19	11.75
	Medium (10-34 years)	91	75.84		
	High (Above 34 years)	18	15.00		
10	Annual income from sericulture				
	Low (Below Rs 0.405 lakhs)	8	6.67	Rs 2.56 lakhs	2.16 lakhs
	Medium (Rs 0.405 lakhs-Rs 4.72 lakhs)	95	79.16		
	High (Above Rs 4.72 lakhs)	17	14.17		
10(a)	Annual income from Muga				
	Low (Below Rs 0.28 lakhs)	1	0.84	Rs 2.36 lakhs	Rs 2.08 lakhs
	Medium (Rs 0.28 lakhs-Rs 4.44 lakhs)	100	83.33		
	High (Above Rs 4.44 lakhs)	19	15.83		
10(b)	Annual income from Eri				
	Low (Below Rs 0.09 lakhs)	13	10.84	Rs 0.213 lakhs	Rs 0.123 lakhs
	Medium (Rs 0.09 lakhs-Rs 0.33 lakhs)	89	74.16		
	High (Above Rs 0.33 lakhs)	18	15.00		
11	Sources of labour				
	Own	96	80.00		
	Own+Hired	24	20.00		
12	Extension contacts				
	Low (Below 5)	8	6.67	7.69	2.37
	Medium (5-10)	93	77.50		
	High (Above 10)	19	15.83		

13	Training exposure				
	Yes	49	40.84		
	No	71	59.16		
14	Nature of product sale				
	Muga seed/silk cocoon	82	68.33		
	Eri seed cocoon	22	18.33		
	Eri silk cocoon	92	76.66		
	Muga silk	78	65.00		
	Eri silk	116	96.66		
	Value added products	84	70.00		
15	Selling points				
	Local markets and fairs	86	71.60		
	Government and cooperative organisations	3	2.50		
	Direct sales to boutiques and designers	41	34.10		
	Online platforms	8	6.60		
	Own shop	8	6.60		
	Others (Relatives/traders)	38	31.60		

IV. DISCUSSION

1. Age

The data presented in the table 1 reveals that sericulture is largely maintained by individuals belonging to the 36–60 years age group. At this stage of life, people generally prioritize stability, security, career advancement, and improved income opportunities. Farmers within this age bracket are found to be highly motivated and enthusiastic in performing productive activities that contribute to household earnings. Their active involvement and commitment in sericulture not only provide economic gains but also establish them as role models within their communities. In doing so, they inspire the younger generation to participate in sericulture and pursue self-employment.

2. Education level

According to the data in Table 1 most (33.34%) of the respondents were high school passed, followed by higher secondary passed (20.00%), middle school passed (15.83%), primary school passed (10.00%), illiterate (8.33%), signature literate (7.50%), graduate only few respondents (5.00%). These findings align with the finding of Sharma and Hussain (2018).

This indicates that women from rural areas and semi-literate backgrounds, where access to higher education is limited, are predominantly engaged in sericulture as a source of livelihood. The findings highlight the necessity of

implementing capacity-building programmes that enhance their skills, enabling them to become more empowered, improve their productivity, and achieve upward social mobility through sericulture.

3. Family type

As presented in Table 4.1.3, the majority of respondents involved in sericulture belonged to nuclear families, accounting for 78.34 percent of the total. In comparison, 15.00 percent were from joint family systems, while 6.66 percent were associated with extended families. This indicates that the nuclear family system is the predominant household structure in the study area. Such a pattern may be attributed to changing social dynamics, migration, and the preference for independent living. Despite having fewer members, nuclear families are able to effectively sustain sericulture, as most of the activities are carried out within the household. Smaller family units often allow for quicker decision-making, clearer division of roles, and greater participation of women in income-related activities. This structure proves advantageous as it promotes collective involvement while enhancing women's authority in managing sericulture operations.

4. Family size

The data in Table 1 reveal that a considerable majority (86.66%) of sericulture rearers lived in households with 3–8 members, indicating that medium-sized families are most prevalent in the study area. This was followed by 11.67

percent of respondents belonging to larger households with more than eight members. The average family size among those engaged in sericulture was around five to six members. Only a small proportion came from very small or very large families. These findings suggest that medium-sized families are better suited to managing sericulture, as they provide adequate manpower and mutual support for different activities.

5. Occupation of family

It was observed that 54.16 percent of respondents practiced sericulture alongside agriculture or allied activities, highlighting a strong reliance on integrated farming-based livelihoods. Sericulture served as the primary and exclusive occupation for 34.16 percent of the respondents. A relatively smaller proportion combined sericulture with salaried employment (5.00%), wage earning (3.34%), or business activities (3.34%), indicating the presence of occupational diversification among a few households.

6. Total annual income

Table 1 shows that 79.17 percent of the rearers reported an annual income between ₹1 lakh and ₹6.48 lakhs. A smaller proportion, 12.50 percent, earned more than ₹6.48 lakhs, while 8.33 percent of respondents belonged to the low-income category, with earnings below ₹1 lakh per year. The results indicate that over half of the respondents belong to the medium-income category, primarily because of their reliance on seasonal and small-scale activities such as sericulture and agriculture. Their earnings are further constrained by weak market linkages, limited alternative employment opportunities, and inadequate access to extension services. These outcomes are consistent with the observations reported by Mamatha and Chaya (2012).

7. Operational land holding(ha)

It was observed that 65.84% of the respondents were classified as marginal farmers making up the largest segment, while 30.00% were identified as small farmers. On the other hand, a very small portion, 4.16% fell under the semi-medium farmer category.

The prevalence of smaller landholdings can be attributed to the practice of family inheritance, wherein land is divided among heirs across generations, resulting in reduced plot sizes for individual farmers. This situation is further intensified by the scarcity of cultivable land in the region coupled with rising population pressure, which has left most farmers with only small or marginal holdings. Similar observations were reported by Sonam and Hans (2020) who also noted that a significant proportion of respondents possessed marginal landholdings.

8. Area under sericulture (bigha)

According to Table 1, 85.00% of the respondents owned land in the range of 2.67 to 8.59 bighas, followed by 10.00% of the respondents reported having more than 8.59 bighas under sericulture. On the other hand, only 5.00% of the respondents were found to have less than 2.67 bighas of land under sericulture. The table indicates that the majority of respondents possessed landholdings ranging between 2.67 bigha and 8.59 bigha, suggesting potential for sustainable income generation. A few respondents had very small holdings, underscoring the need for support measures such as improved inputs or collective initiatives. Farmers with relatively larger areas could be motivated to adopt commercial approaches and serve as lead farmers. The diversity in landholding size emphasizes the importance of providing scale-specific extension support.

***1 hectare= 7.46 bigha (Assam)**

9. Experience in sericulture

From the information presented in table 1, it can be observed that 75.84% of the individuals involved in sericulture possessed a moderate range of experience spanning 10 to 34 years. Those with more than 34 years of involvement accounted for 15.00%, while only 9.16% had been engaged in sericulture for under decade.

The findings indicate that the majority of respondents had long-standing involvement in sericulture, underscoring its traditional significance in the region. A considerable proportion with medium levels of experience reflects a strong base of knowledge and practical skills. The presence of highly experienced individuals may be attributed to generational transfer of practices and expertise. Conversely, the smaller share of respondents with limited experience suggests that fewer youth are entering sericulture, possibly due to alternative employment opportunities or inadequate institutional support. These results are consistent with the observations of Bhat (2022).

10. Annual income from sericulture

Data in Table 1 shows that a majority (79.16%) of the respondents had an income ranging between Rs 0.405 lakhs-Rs 4.72 lakhs from sericulture, whereas a smaller portion (14.17%) of the respondents earned above Rs 4.72 lakhs, indicating relatively higher success in sericulture-based livelihood. On the other hand, 6.67% of respondents earned below Rs 0.405 lakhs annually, reflecting poor returns likely due to limited resources, low productivity or weak market access. The high standard deviation indicates considerable variation in income levels, which may be attributed to differences in landholding size, input utilization, adoption of technology, and access to markets. Although the majority of respondents belong to the middle-

income group, the observed income disparity emphasizes the need for targeted interventions to support low-income farmers and ensure inclusive growth within the sericulture sector. This observation is consistent with the findings of Bhausahab (2012).

10(a) Annual income from Muga

From the information presented in table 1 it can be inferred that a large majority (83.33%) of respondents had annual earnings ranging from ₹0.28 lakhs to ₹4.44 lakhs, with a mean income of ₹2.36 lakhs and a relatively high standard deviation of ₹2.08 lakhs, indicating substantial income variation. In addition, 15.83 percent of respondents reported annual incomes exceeding ₹4.44 lakhs, demonstrating the profitability of muga sericulture when managed efficiently and supported by proper market access. By contrast, only a single respondent (0.84%) was found in the lowest income category, earning less than ₹0.28 lakhs annually.

The high variation in income, as reflected by the standard deviation, suggests differences in productivity, scale of operation, technological adoption, and access to markets. While most respondents are concentrated in the middle-income range, the existence of both very low and very high-income groups points to unequal returns from Muga rearing. Strengthening input support, extension services, and market linkages could help reduce this disparity and enhance income levels across all groups.

10(b) Annual income from Eri

Data in Table 1 depicted that (74.16%) of rearers earned between Rs 0.09 lakhs-Rs 0.33 lakhs followed by 15% earned above Rs 0.33 lakhs annually. Only 10.84% earned below Rs 0.09 lakhs annually. The data clearly indicates that average income from Eri is significantly lower as compared to Muga, primarily due to the lower market value of Eri silk products. Unlike Muga, which is highly valued for its sheen and exclusivity, Eri is commonly used for coarser and warmer fabric, resulting in reduced profit margins. Eri rearing is often practiced on a smaller scale, largely for household use or local sale, and lacks strong market linkages. As a result, for most respondents, Eri serves as a subsidiary source of income, rather than a primary livelihood activity.

11. Sources of labour

Majority of the rearers (80%) depend entirely on their own family labour for sericulture activities, whereas only 20% of respondents used a combination of both hired labour and family. On the other hand, none of the respondents depended solely on hired labour.

The data suggest that sericulture in the study area is predominantly a family-centered activity, carried out within

the household without reliance on hired labor. The lack of exclusive wage labor may indicate either financial constraints in affording additional workers or a preference to manage production within the family as a cost-saving strategy.

12. Extension contacts

Data in Table 1 clearly illustrates that 77.50% of the participants exhibited a moderate degree of interaction with extension personnel. In comparison, 15.83% reported a high frequency of contact, while a minimal share of 6.67% experienced limited engagement with extension personnel. The results indicate that the majority of women rearers belonged to the medium category, suggesting a moderate level of exposure to extension services. The limited proportion of respondents in the high-contact group points to the need for strengthening efforts to enhance engagement. A small share of women with low extension contact may be due to inaccessibility, lack of awareness, or socio-cultural barriers. Strengthening extension linkages would enable wider dissemination of technology, promote the adoption of improved practices, and ultimately enhance productivity and livelihood opportunities in sericulture. These findings are consistent with those of Singh (2022).

13. Training exposure

From Table 1 we can observe 59.16% of the rearers had not undergone any formal training for sericulture. On the other hand, only 40.84% had received training in sericulture. Out of 40.84 % respondents who had received training in sericulture, majority of them obtained from Department of Sericulture through field level training programmes and extension activities.

The findings suggest that the majority of respondents practiced sericulture relying primarily on traditional knowledge and informal learning. Limited awareness of existing training opportunities, coupled with challenges such as distance, transportation constraints, and inadequate extension outreach, may hinder their access to such programs. Additionally, low literacy levels and lack of self-confidence could further restrict their participation.

14. Nature of product sale

Data in Table 1 reveals that Eri silk was the most widely marketed product, with 96.66 percent of respondents involved in its sale. This was followed by Eri silk cocoons (76.66%) and value-added items (70.00%). Sales of muga seed/silk cocoons and muga silk were reported by 68.33 percent and 65.00 percent of respondents, respectively, reflecting a moderate level of participation. In contrast, only 18.33 percent engaged in the sale of Eri seed cocoons, indicating minimal involvement in seed production. Overall, Eri-based sericulture emerged as the dominant

activity, with an increasing inclination toward value addition and entrepreneurial ventures.

15. Selling points

Data presented in Table 1 highlights that 71.60% of rearers sold their products through local markets and fairs followed by 34.10% sold their products directly to boutiques and designers, 31.60% respondents sold their products to others which include relatives, middlemen and informal contacts. Whereas only small percentage of respondents used online platforms (WhatsApp and phone calls) and own shops (6.60%). On the other hand, only 2.50% sold their products through government or cooperative organisation.

The findings show that most producers rely on traditional outlets like local markets and fairs, while some are beginning to connect with urban buyers such as boutiques and designers for better returns. Personal networks and intermediaries also play a major role in sales. However, limited use of online platforms, own shops, and institutional channels highlights untapped potential. Expanding into digital and formal marketing avenues could significantly enhance income and market reach.

V. CONCLUSION

The study concludes that sericulture in the Dhemaji district of Assam is predominantly practiced by middle-aged women from nuclear and medium-sized families, relying mainly on household labour. Most respondents possess small or marginal landholdings and have education up to high school, reflecting limited access to formal training and resources. While sericulture is often combined with agriculture and allied activities, it remains a significant source of household income, with Eri silk dominating production and marketing. Income levels are moderate but show considerable disparities due to differences in landholding, productivity, technological adoption, and market access. Extension contacts and formal training are limited, resulting in a reliance on traditional knowledge and informal learning. Marketing is largely conducted through local markets, fairs, and personal networks, whereas digital platforms, own shops, and institutional channels remain underutilized. The growing interest in value-added products and entrepreneurial initiatives, however, indicates opportunities for income enhancement and skill development. To promote sustainability and profitability, targeted interventions are needed, including capacity-building programmes, improved access to quality inputs and credit, and the formation of producer groups. Strengthening extension services and structured marketing channels can enhance adoption of improved practices and expand market reach. Overall, sericulture offers significant

potential for women's empowerment, rural entrepreneurship, and socio-economic development, provided adequate institutional and policy support is available.

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Impact of Domestic waste on Groundwater Quality in Dianeguela (Commune 6, Bamako)

Impacts des Rejets Domestiques sur la Qualite des eaux Souterraines a Dianeguela (Commune 6, Bamako)

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Received: 09 Aug 2025; Received in revised form: 10 Sep 2025; Accepted: 17 Sep 2025; Available online: 24 Sep 2025

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Abstract— In Commune 6 of the Bamako district, groundwater is exposed to domestic pollution, particularly in the Dianéguela neighborhood. The objective of this study is to analyze the groundwater in Dianéguela and identify the negative impacts of domestic liquid waste on the population. The methodology was based on documentary research, field data collection, and laboratory water quality analysis. In situ parameters (pH, conductivity) were measured. GPS was used to georeference water points. Ion chromatography was used to analyze nitrates and nitrites. An automatic sampler was used to analyze bicarbonate. The main results show that bicarbonate levels vary from 7 mg/L to 1230 mg/L, nitrate levels range from 0.001 mg/L to 416.21 mg/L, and nitrite levels range from 0.001 mg/L to 33.490 mg/L. Human consumption of this water without treatment has a negative impact on consumers' health. Excess bicarbonate at levels above 200 mg/L gives the water a bitter taste. Excess nitrate at levels above 50 mg/L or nitrite at levels above 3 mg/L can cause methemoglobinemia in infants and gastric cancer in adults. Reverse osmosis can be used to correct these non-compliances. Local and national authorities will need to raise awareness among the populations of Dianéguela and, above all, carry out periodic monitoring of groundwater quality.



Keywords— Dianéguela, water, impact, discharge, physicochemical

Résumé— Dans la commune 6 du district de Bamako, les eaux souterraines sont exposées à la pollution domestique, notamment dans le quartier de Dianéguela. L'objectif de cette étude est d'analyser les eaux souterraines de Dianéguela et identifier les impacts négatifs des rejets liquides domestiques sur la population. La méthodologie s'est basée sur la recherche documentaire, la collecte de données de terrain et l'analyse de la qualité des eaux au laboratoire. Les paramètres in situ (pH, conductivité) ont été mesurés. Un GPS a été utilisé pour géoréférencer les points d'eau. La chromatographie ionique a été utilisée pour l'analyse des nitrates et nitrites. Le passeur automatique a été utilisé pour l'analyse du bicarbonate. Les principaux résultats montrent que les teneurs en bicarbonate varient de 7 mg/L à 1230 mg/L, celles des nitrates oscillent entre 0,001 mg/L et 416,21 mg/L et celles des nitrites entre 0,001 mg/L et 33,490 mg/L. La

consommation humaine de ces eaux sans traitement impacte négativement la santé des consommateurs. L'excès de bicarbonate à des teneurs supérieures à 200 mg/L donne un goût amer. L'excès de nitrate à des teneurs supérieures à 50 mg/L ou de nitrite à des teneurs supérieures à 3 mg/L peut provoquer la méthémoglobinémie chez le nourrisson, et le cancer gastrique chez l'adulte. L'osmose inverse permet de corriger ces non conformités. Les autorités locales et nationales devront accentuer la sensibilisation des populations de Dianéguela et surtout procéder au contrôle périodique de la qualité des eaux souterraines.

Mots-clés— *Dianéguela, eau, impact, rejet, physicochimique*

I. INTRODUCTION

Dans le monde, l'OMS estime qu'en 2022 que plus de 1,5 milliard de personnes ne disposent toujours pas de services d'assainissement de base tels que les toilettes privées ou des latrines et parmi elles 419 millions défèquent encore à l'air libre, dans les caniveaux, derrière les buissons ou dans les plans d'eaux [1]. Le chapitre 21 de l'agenda 21 adopté au sommet de la Terre à Rio en 1992 est entièrement consacré à la gestion des déchets pour un développement durable [2].

En Afrique l'eau et l'assainissement demeurent des défis majeurs, surtout pour les citoyens ruraux et pauvres. L'eau potable et l'assainissement sont essentiels pour la santé de tous les africains ainsi qu'au développement social et économique de leurs pays et pourtant des millions de personnes n'y ont pas accès, malgré l'engagement des gouvernements en faveur des Objectifs du Développement Durable des Nations Unies et de l'agenda 2063 de l'Union Africaine, la plupart des pays sont loin d'atteindre leur objectif consistant à garantir la disponibilité et la gestion durable de l'eau et de l'assainissement pour tous [3].

Au Mali, les problèmes d'assainissement se posent avec acuité, à l'instar de beaucoup d'autres pays d'Afrique, aussi bien dans les zones rurales que dans les zones urbaines. L'insalubrité, liée en partie à l'insuffisance d'ouvrages d'assainissement, entrave le développement socio-économique du pays à travers la dégradation de l'environnement, la propagation des maladies et diverses autres nuisances qui affectent la santé des populations en particulier. Le conflit connu par le Mali à partir de 2012 a sans aucun doute ralenti la mise en place des activités. Par conséquent, de nombreux objectifs n'ont pas été atteints. L'objectif concernant l'accès à un assainissement de base était fixé à 57 % pour 2015 et n'a qu'à peine atteint les 32% [4].

Au niveau national, l'assainissement a fait son apparition en tant que secteur à part entière et son niveau de priorité a progressivement augmenté, la constitution du 22 Juillet 2023 pose la pierre en stipulant dans son préambule que « le peuple souverain du Mali s'engage à assurer l'amélioration de la qualité de vie, la protection de

l'environnement ». Son article 22 ajoute « Toute personne a droit à un environnement sain et durable » [5].

Un manque d'assainissement de mauvaise qualité porte atteinte au bien être humain et au développement social et économique [6]. Le manque d'assainissement constitue un facteur de risque important pour la santé des Maliens. Il affecte les populations, dont plus de la moitié souffre en permanence de maladies liées au déficit d'accès à un assainissement adéquat [1]. La dissémination des eaux usées, ordures et déchets dangereux menace l'environnement en général, et la qualité des sols et des ressources en eau en particulier.

Le problème se pose avec beaucoup d'acuité en commune 6 du district de Bamako [7]. Le quartier de Dianéguela est une zone de très forte densité de la teinturerie artisanale. Des centaines de femmes pratiquent la teinte des tissus à travers tout le quartier, que ce soit dans les concessions, dans la rue ou au bord de la rivière du quartier. Les rejets liquides d'origines domestiques sont déversés directement dans les rues du quartier de Dianéguela par chaque famille, ces rejets stagnent et s'infiltrent dans le sous-sol [8].

En outre, le quartier ne dispose pas de plan d'évacuation des rejets liquides domestiques, ce qui constitue un risque d'une part, de pollutions du sol et des ressources en eaux surtout celles souterraines et d'autre part, de prolifération des maladies hydriques.

L'objectif de cette étude est d'analyser la qualité des eaux souterraines de Dianéguela et leurs impacts sur la population. Pour ce faire, nous avons procédé à l'analyse des paramètres physicochimiques des eaux de forages et des puits du quartier.

II. MATERIEL ET METHODES

2.1. Zone d'étude

Le quartier de Dianéguela est situé dans le sixième arrondissement du district de Bamako (figure 1) [9]. Il est limité au nord par Missabougou, au sud par Magnambougou, à l'est par le fleuve Niger, à l'ouest par Sokorodji. Il est traversé par le fleuve Niger et une rivière appelée par la population « Babla ». Il compte une population de 32 196 habitants [10] et une superficie de

0,68 km² [11]. La forte densité de la population accentue les rejets des eaux domestiques.

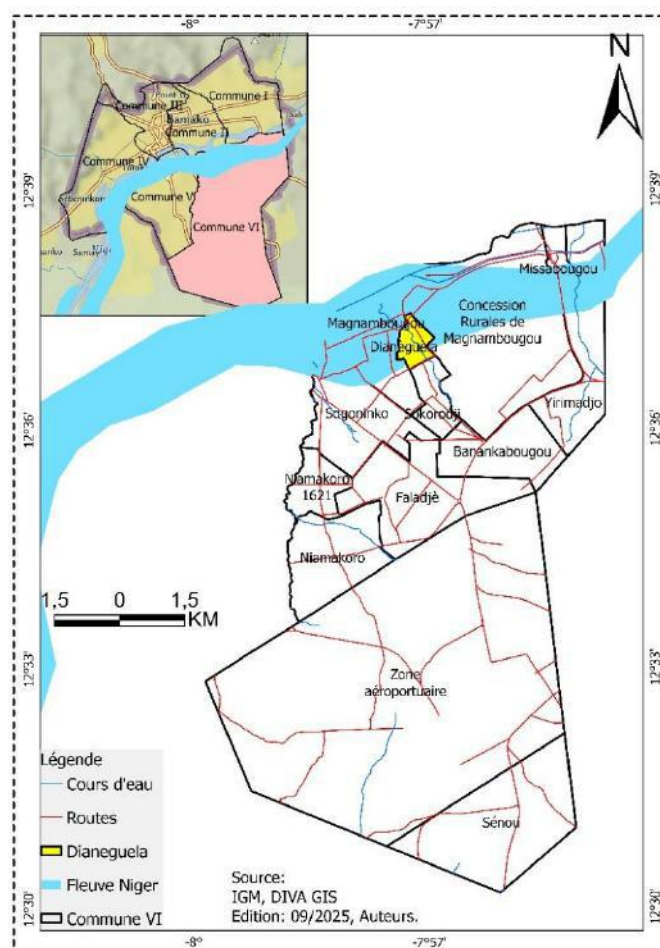


Fig.1 : Carte de la commune VI du district de Bamako

2.2. Matériel

Les matériels utilisés sont le GPS de marque compass pour la géolocalisation des points de prélèvement. Un conductimètre LF 197 de marque WTW nous a permis de déterminer la conductivité électrique de l'eau. Un chromatographe ionique de marque « Metrohm » nous a permis de déterminer les anions majeurs. Un titreur automatique muni d'électrodes spécifiques de marque « Metrohm nous a permis de mesurer le pH, le TA, le TAC. Le photomètre à flamme de marque jenway a permis de déterminer le sodium et le potassium. Le DR 3900 de marque HACH a permis de déterminer la couleur et la teneur en fer et enfin le TL2310 de marque HACH nous a permis de déterminer la turbidité des eaux. Le GPS a permis le relever les coordonnées géographiques des points de prélèvement.

2.3. Méthode

La méthodologie adoptée combine : **1)** une recherche documentaire qui a consisté en une revue des études

antérieures sur la pollution des eaux souterraines à Bamako et dans d'autres villes africaines ; **2)** la collecte des données de terrain à travers l'identification et géoréférencement (GpS) des points d'eau souterraines utilisés par la population de Dianéguela. Un total de 26 points dont 15 puits et 11 forages a été retenu pour l'étude. Les prélèvements ont été réalisés en novembre 2024. Les coordonnées géographiques des points de prélèvement ont été enregistrées via GPS et cartographiés (figure 2). Une liste des points de prélèvement a ensuite été établie (tableau 1) ; **3)** analyses physicochimiques en laboratoire : Elles ont concerné le pH et la conductivité électrique mesurés in situ avec un multiparamètre portable, nitrates (NO₃⁻) et nitrites (NO₂⁻) analysés par chromatographie ionique dosé par titrage automatique. Les résultats ont été comparés aux normes de potabilité de l'organisation mondiale de la santé (OMS, 2017) et aux recommandations nationales maliennes.

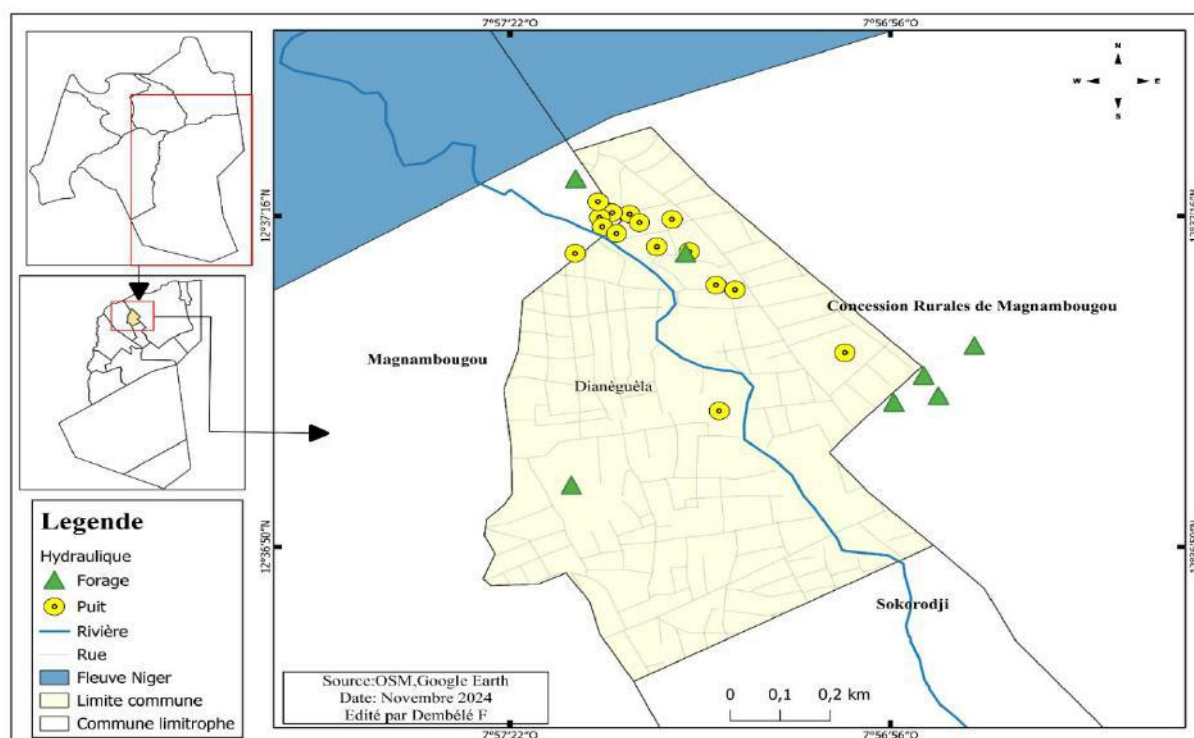


Fig. 2. Figure de présentation de Dianéguela avec les points de prélèvement

Suivant les points de prélèvement, les échantillons d’eaux ont été prélevés dans des flacons de 1 litre, préalablement rincés trois fois avec de l’eau destinée à l’analyse. Par la

suite, les flacons été remplis jusqu’au bord sans laisser d’air. Les eaux prélevées ont été conservées dans une glacière et transportées au Laboratoire National des Eaux pour analyse.

Tableau 1 : Points de prélèvement des eaux souterraines

Points de prélèvement	Longitude	Latitude
Puits		
P1	7.9538	12.62104
P2	7.95412	12.62107
P3	7.95435	12.62097
P4	7.95438	12.6213
P5	7.9543	12.62077
P6	7.95404	12.62063
P7	7.95362	12.62086
P8	7.9533	12.62035
P9	7.95302	12.62093
P10	7.9527	12.62025
P11	7.95221	12.61954
P12	7.95186	12.61944
P13	7.94984	12.61811
P14	7.95215	12.61688
P15	7.9548	12.62021

Forages		
F1	7.95277	12.62021
F2	7.94746	12.61826
F3	7.94839	12.61763
F4	7.94812	12.6172
F5	7.94894	12.61706
F6	7.95487	12.61531
F7	7.95479	12.62179
F8	7.95479	12.62179
F9	7.95411	12.62309
F10	7.95441	12.6245
F11	7.95366	12.62404

Source : enquêtes de terrain, novembre 2024

III. RESULTATS

A l'issue de l'analyse des données, les anions et cations majeurs des forages et puits ont pu être déterminés.

L'analyse des anions au niveau des eaux de puits ont porté sur le pH, la conductivité électrique, la turbidité, la couleur, le bicarbonate, les chlorures, les nitrates, les nitrites, les sulfates et le fluor (tableau 1).

3.1. RESULTATS D'ANALYSE DES ANIONS MAJEURS DES EAUX DE PUITS

Tableau 2. Résultats d'analyses des anions majeurs des eaux puits de Dianéguela

Points de prélèvement	pH	Cond $\mu\text{S}/\text{cm}$	Turb NTU	Coul UCV	HCO_3^- mg/L	Cl^- mg/L	NO_3^- mg/L	NO_2^- mg/L	SO_4^{2-} mg/L	F^- mg/L
P1	6,54	1866	10	56	434	208,36	289,50	21,385	58,77	0,177
P2	5,62	2160	2	13	410	255,32	362,51	21,806	78,57	0,215
P3	6,49	1900	1	0	335	252,56	331,80	27,051	51,00	0,001
P4	6,59	2130	9	41	441	255,70	336,30	24,593	70,22	0,234
P5	6,64	2190	2	5	335	311,68	391,01	33,490	64,03	0,214
P6	6,79	2350	6	31	458	300,68	384,42	29,598	62,69	0,251
P7	6,34	1802	2	12	466	193,44	239,89	16,143	51,42	0,164
P8	6,62	2450	2	11	420	338,58	416,21	21,393	68,11	0,001
P9	6,79	1310	4	35	324	142,97	199,59	0,165	25,70	0,193
P10	6,77	2250	1	0	440	280,21	336,40	0,107	99,04	0,485
P11	6,74	1021	3	15	158	1,96	0,001	0,001	5,44	0,105
P12	7,19	2660	20	143	1230	120,90	158,75	4,995	25,93	0,001
P13	5,49	297	1	0	13	252,98	299,09	0,400	172,54	0,316
P14	7,18	2860	2	5	801	27,94	58,05	0,071	0,76	0,207
P15	6,52	142	0	0	184	346,52	350,44	10,976	128,86	0,279
Norme Malienne	5,5-9,5	≤ 1500	<10	<25	PVG	≤ 600	≤ 50	$\leq 0,02$	≤ 500	$\leq 0,5$

Source : Laboratoire National des Eaux, novembre 2024

Les résultats d'analyses du tableau 1 montrent que sur les 15 eaux de puits analysés, 14 puits sont de mauvaise qualité physicochimique due à la teneur en bicarbonate, en nitrate et en nitrite. Les eaux de tous les puits sont non conformes à l'exception de la teneur en bicarbonate, en nitrate et en nitrite de P11 qui est conforme.

3.2. RESULTATS D'ANALYSE DES CATIONS MAJEURS DES EAUX DE PUIITS

Les analyses des cations au niveau des eaux de puits ont porté sur l'ammoniac, le calcium, le magnésium, le sodium, le potassium, le fer²⁺ et le titre hydrotimétrique (tableau 2).

Tableau 3. Résultats d'analyses des cations des eaux puits de Dianéguela

Points de prélèvement	NH ₄ ⁺ mg/L	Ca ²⁺ mg/L	Mg ²⁺ mg/L	Na ²⁺ mg/L	K ⁺ mg/L	Fe ²⁺ mg/L	TH mg/L
P1	0,14	46,45	15,12	315,20	61,78	0,02	178
P2	0,04	40,52	16,12	315,20	54,78	0,00	168
P3	0,00	49,26	27,50	298,80	49,89	0,00	236
P4	0,04	56,56	31,12	334,20	53,81	0,01	269
P5	0,01	88,15	37,45	306,40	42,80	0,00	374
P6	0,04	108,42	42,34	298,40	62,27	0,00	445
P7	0,03	20,55	11,30	333,40	60,78	0,00	98
P8	0,02	105,65	62,25	293,30	52,82	0,01	520
P9	0,04	17,26	8,72	238	44,85	0,03	79
P10	0,29	66,43	35,62	352,25	36,35	0,00	313
P11	0,03	17,26	8,75	184,60	22,41	0,00	79
P12	0,19	105,35	42,37	390,30	34,35	0,15	438
P13	0,00	20,27	4,83	32,22	6,37	0,00	71
P14	0,02	110,65	52,32	379,75	87,66	0,00	491
P15	0,09	102,15	48,59	203,37	55,80	0,01	455
Norme Malienne	≤0,5	≤400	≤100	≤400	≤100	≤0,3	≤500

Source : Laboratoire National des Eaux, novembre 2024

Les résultats d'analyses du tableau 2 montrent que sur les 15 eaux de puits analysées, les cations sont conformes dans l'ensemble excepté le titre hydrotimétrique de P8 qui est non conforme.

L'analyse des anions au niveau des eaux de forage ont porté sur le pH, la conductivité électrique, la turbidité, la couleur, le bicarbonate, les chlorures, les nitrates, les nitrites, les sulfates et le fluor (tableau 3).

3.3. RESULTATS D'ANALYSE DES ANIONS MAJEURS DES EAUX DES FORAGES

Tableau 3 : Résultats d'analyse des anions majeurs des eaux de forage de Dianéguela

Points de prélèvement	pH	Cond μS/cm	Turb NTU	Coul UCV	HCO ₃ ⁻ mg/L	Cl ⁻ mg/L	NO ₃ ⁻ mg/L	NO ₂ ⁻ mg/L	SO ₄ ²⁻ mg/L	F ⁻ mg/L
F1	7,25	1682	0	3	816	69,66	84,95	3,385	5,66	0,042
F2	5,74	110	1	4	35	173,09	243,51	8,546	60,79	0,180
F3	6,45	244	272	1195	103	10,03	28,41	0,356	0,09	0,071
F4	6,07	210	10	3	39	20,21	53,75	2,387	1,26	0,083
F5	5,93	32	1	0	7	14,83	63,13	1,557	2,22	0,154

F6	7,31	1080	0	0	336	96,11	125,62	8,196	26,86	0,118
F7	7,43	668	1	3333	319	32,31	14,37	4,257	15,05	0,335
F8	7,16	1576	572	0	363	175,35	200,64	18,536	77,52	0,241
F9	7,29	917	1	0	259	92,27	66,99	9,788	59,93	0,280
F10	7,11	987	1	6	304	81,69	110,05	13,686	38,50	0,195
F11	7,48	562	1	0	251	26,15	20,86	0,001	11,76	0,067
Norme Malienne	5,5- 9,5	≤1500	<10	<25	PVG	≤600	≤50	≤0,02	≤500	≤0,5

Source : Laboratoire National des Eaux, 2024

Les résultats d'analyses du tableau 3 montrent que sur les 11 points d'eaux de forages analysés, 8 points d'eaux sont de mauvaise qualité physicochimique due à des teneurs en bicarbonate, en nitrate et en nitrite non conformes.

La teneur en bicarbonate des points F2, F4, F5 sont conformes. Aussi, celle en nitrate des points F3, F7, F11

sont conformes. Par ailleurs, les résultats révèlent que la teneur en nitrite du point F11 est conforme.

3.4. Résultats d'analyse des cations majeurs des eaux de forage

L'analyse des cations au niveau des eaux de forage ont porté sur l'ammoniac, le calcium, le magnésium, le sodium, le potassium, le fer2 et le titre hydrotimétrique (tableau 4).

Tableau 4. Résultats d'analyses des cations majeurs des eaux de forage de Dianéguela

Point de prélèvement	NH ₄ ⁺ mg/L	Ca ²⁺ mg/L	Mg ²⁺ mg/L	Na ²⁺ mg/L	K ⁺ mg/L	Fe ²⁺ mg/L	TH mg/L
F1	0,04	92,75	42,56	181,20	30,9	0,00	407
F2	0,00	5,62	1,72	13,89	2,89	0,00	21
F3	0,07	15,32	6,54	13,32	20,91	0,57	65
F4	0,08	11,22	3,75	26,94	1,89	0,07	43
F5	0,00	1,22	0,34	3,44	3,29	0,00	6
F6	0,08	84,36	38,57	65,45	21,90	0,00	369
F7	0,02	50,96	22,62	31,80	34,35	0,00	220
F8	0,14	75,25	40,86	181,20	28,85	0,31	356
F9	0,00	60,26	32,78	72,60	11,94	0,00	285
F10	0,05	45,57	17,12	127,42	24,68	0,21	184
F11	0,02	46,11	22,23	27,38	11,36	0,00	207
Norme Malienne	≤0,5	≤400	≤100	≤400	≤100	≤0,3	≤500

Source : Laboratoire National des Eaux, novembre 2024

Les résultats d'analyse du tableau 4 montrent que sur les 11 eaux de forages, les cations analysés sont conformes dans l'ensemble des points de prélèvement.

3.5. SYNTHESE GENERALE EN FONCTION DES PARAMETRES

La synthèse effectuée nous a permis d'avoir une vue d'ensemble des paramètres au niveau des eaux de puits et des forages. La paramètres d'analyse ont porté sur les bicarbonates, les nitrates et les nitrites (tableau 5).

Tableau 5. Indication de la conformité des paramètres des points d'eau par rapport à la norme malienne

Paramètres	HCO ₃ ⁻ mg/L	%	NO ₃ ⁻ mg/L	%	NO ₂ ⁻ mg/L	%
Points d'eau conformes	4	15,38	3	11,53	1	3,84
Points d'eau non conformes	22	84,65	23	88,46	25	96,15
Total	26	100	26	100	26	100

Source : Laboratoire National des Eaux, novembre 2024

En récapitulatif, l'analyse des résultats du tableau 5 indique que 84,65 % des eaux analysées ont des teneurs en bicarbonate non conformes contre 15,68 % conformes. Par rapport aux nitrates, 88,46 % des eaux ont des teneurs non conformes contre 11,53 % conformes. Par ailleurs, 96,15 % des eaux ont des teneurs en nitrites non conformes contre 3,84 % conformes.

IV. DISCUSSION

L'étude réalisée sur les impacts des rejets domestiques sur la qualité des eaux souterraines de Dianéguela du district de Bamako a permis d'analyser la qualité physicochimique des eaux de 11 forages et de 15 puits prélevés en 2024. Les résultats obtenus indiquent que 84,65 % des eaux analysées ont des teneurs en bicarbonate non conformes ; 88,46 % des eaux ont des teneurs en nitrates non conformes ; 96,15 % des eaux ont des teneurs en nitrites non conformes.

Ces eaux de puits ou de forages de Dianéguela présentant des non conformités (89,75 % en moyenne) sont déconseillées à la consommation humaine ou animale sans un traitement au préalable. Les résultats d'analyse indiquent aussi que la qualité des eaux de forage et de puits sont impactées négativement par les rejets d'eaux usées dans les rues du quartier de Dianéguela. En effet, le quartier dispose peu de canalisation d'évacuation des eaux, ce qui laisse plus de temps aux eaux usées domestiques de s'infiltrer dans la nappe phréatique.

4.1 Impacts des eaux chargées de bicarbonates sur la consommation humaine

Il n'y a pas de norme OMS, ni norme malienne pour cet élément, mais une concentration élevée en bicarbonates donne une saveur salée et un goût amer à l'eau. Les teneurs en bicarbonate de l'étude varient entre 7 mg/L et 1230 mg/L, convergent d'avec les résultats d'une étude menée au Burkina Faso [12] sur la caractérisation physico-chimique des eaux souterraines de la localité de Yamtenga (Burkina Faso). Cette étude a relevé des teneurs en bicarbonate variant entre 39,04 mg/L et 268,4 mg/L. Nos résultats sont similaires à ceux de l'étude menée par [13] sur l'origine et le processus de minéralisation des eaux souterraines dans la partie sud du Marais Poitevin (Poitou-Charentes-France) et de son substratum carbonaté de l'oxfordien supérieur. Les résultats de cette étude révèlent des teneurs de bicarbonates

variant entre 497,8 mg/L (valeur minimale) dans les eaux du site expérimental de Saint Hilaire la Palud à 1007,7 mg/L (valeur maximale) dans celles du site expérimental de Marans. Nos résultats sont similaires à ceux de [14] au Maroc sur l'évaluation de la qualité physico-chimique des eaux souterraines des nappes du Jurassique du haut bassin de Ziz dont les teneurs en bicarbonate oscillaient entre 50 et 400 mg/l. Selon [15], qui ont mené une étude sur la caractérisation physico-chimique des eaux des aquifères du continental intercalaire / hamadien et du continentalsiems terminal de la région de Zinder (Zinder), les ions bicarbonates proviennent de l'altération de certains minéraux (feldspaths, calcite...). Donc les teneurs enregistrées en bicarbonate dans les eaux souterraines de ces aquifères renseignent sur le temps de séjour de l'eau dans l'aquifère. Les teneurs en bicarbonate oscillaient entre 4,880 mg/L et 351,360 mg/L sont inférieurs aux résultats de cette étude. En somme, la forte teneur en bicarbonate des eaux de notre zone d'étude n'a pas de risques sanitaires sur les populations. Cependant, elle donne un goût amer aux eaux, ce qui pousse certains ménages à ne pas utiliser les eaux pour des besoins de consommation humaine.

4.2. Impacts des Nitrates par rapport à la consommation humaine

Les résultats de notre étude indiquent des teneurs en nitrates qui varient entre 0,001 mg/L et 416, 21 mg/L donc supérieures à la norme OMS et celle malienne. Les résultats de l'étude sont largement supérieurs à ceux de [16] sur la pollution des eaux souterraines par les nitrates du sous bassin du Massili au Burkina Faso dont les teneurs variaient entre 0,00 mg/L et 98,88 mg/L. Les résultats de l'étude sont très proches des résultats de l'étude menée par [17] sur l'impact des teneurs en nitrates sur le taux de desserte en eau potable des populations en zone du socle de Damagaram Mounio (Est du Niger) avec des teneurs variant entre 0,22 mg/L et 479 mg/L. Nos résultats concordent avec ceux de l'étude menée en Côte d'Ivoire par [18] sur l'évaluation de la contamination chimique des eaux souterraines par les activités anthropiques dans la Zone d'Ity-Floleu (Sous-Préfecture de Zouan-Hounien). Cette étude révèle des teneurs en nitrates au niveau de toutes les stations ne respectaient pas les normes OMS. Au-delà de 100 mg/L de nitrate, l'eau ne doit ni, être bu, ni être utilisée pour préparer les aliments. La dégradation de la qualité de

l'eau souterraine de la zone d'étude (Côte d'Ivoire) est liée essentiellement aux altérations par les nitrates et les phosphates. Les nitrates sont des sels très solubles qui sont facilement entraînés en profondeur par les eaux d'infiltration. Les apports de cet élément dans les eaux souterraines sont causés par des impacts liés à des problèmes d'assainissement, plus qu'à des pratiques agricoles. Ce qui pourrait confirmer nos fortes teneurs en nitrate dues à l'insuffisance de réseau de drainage des eaux usées et aux rejets d'eaux usées dans les rues qui finissent par s'infiltrer dans la nappe phréatique.

4.3 Impacts des Nitrites par rapport à la consommation humaine

Nos résultats indiquent des teneurs obtenues en nitrite oscillent entre 0,001 mg/L à 33,490 mg/L. Ces résultats sont supérieurs aux résultats d'une étude menée par [19] sur les risques sanitaires liés aux composés chimiques contenus dans l'eau de boisson dans la ville de Fès, ces teneurs de nitrite oscillaient entre 0,001 mg/l à 0,09 mg/l.

Ces auteurs affirment qu'en effet, les nitrates et les nitrites sont toxiques pour les êtres vivants et transforment l'hémoglobine du sang en une substance proche, la méthémoglobine qui ne joue plus son rôle dans l'oxygénation des cellules et des tissus. L'excès de nitrites et de nitrates peut donc entraîner une anémie grave, surtout chez les nourrissons. Les nitrites peuvent aussi se combiner au cours de la digestion avec des dérivés de protides et former des substances soupçonnées d'être cancérogènes : les nitrosamines. Les effets potentiels de ceux-ci sur la santé sont nombreux : la méthémoglobinémie chez les enfants, le goitre, la cancérogénicité, un effet sur la zone glomérulaire adrénaire.

Les résultats de l'étude vont en droite ligne avec les constats de [20] qui a mené une étude en Côte d'Ivoire sur l'évolution spatio-temporelle des teneurs en nitrates des eaux souterraines de la ville d'Abidjan dont les teneurs en nitrites des eaux variaient de 0 à 11 mg/L. Ces auteurs affirment que les causes des fortes teneurs en nitrites rencontrées dans les eaux du quaternaire traduisent une contamination superficielle issue des eaux usées des égouts, des fosses septiques et des puits perdus de la ville.

Les résultats d'une étude menée au Maroc par [21] sur l'impact des facteurs de pollution sur la qualité des eaux de la zone aval de la vallée de l'oued Nekor (Al-Hoceima, Maroc) confirment que l'activité humaine accélère le processus d'enrichissement des composés azotés (nitrate, nitrite, phosphate, ammoniac) sur les sols subissant l'érosion, ce qui provoque l'infiltration des eaux usées, par les rejets des industries minérales, d'engrais azotés dans le sous-sol contribuant à la pollution des eaux souterraines, ces

constats convergent dans le même sens que les résultats de notre étude.

V. CONCLUSION

L'étude menée à Dianéguela en commune VI du district de Bamako a mis en évidence une dégradation progressive de la qualité des eaux souterraines, principalement liées aux rejets domestiques non maîtrisés. Les résultats obtenus confirment la présence de polluants chimiques tels que le bicarbonate à des teneurs variant entre ($7 \text{ mg/L} < \text{HCO}_3^- < 1230 \text{ mg/L}$), le nitrate à des teneurs variant entre ($0,001 \text{ mg/L} < \text{NO}_3^- < 416,21$) et le nitrite à des teneurs variant entre ($0,12 < \text{NO}_2^- < 33,490 \text{ mg/L}$) dépassant les normes de potabilité, ce qui soulève des inquiétudes sanitaires pour les populations utilisant ces ressources sans traitement préalable.

En somme, quatre-vingt-dix pour cent (90 %) des paramètres physico-chimiques analysés sont non conformes à la norme malienne d'une eau de consommation humaine et à celle de l'OMS

L'un des avantages majeurs de cette recherche est qu'elle apporte des données locales actualisées sur la contamination des eaux souterraines, permettant ainsi une meilleure compréhension des impacts environnementaux des rejets domestiques. Elle met également en évidence la vulnérabilité des nappes phréatiques dans les zones urbaines à forte densité et souligne l'urgence des stratégies d'assainissement adaptées au contexte local.

Cependant cette étude présente certaines limites. Elle se concentre sur une période et une zone géographique restreinte, ce qui, peut limiter la généralisation des résultats, de plus l'analyse s'est principalement focalisée sur les paramètres physicochimiques sans inclure d'autres contaminants potentiels comme les métaux lourds ou les résidus pharmaceutiques.

Malgré ces limites, les résultats de cette recherche peuvent servir de base pour plusieurs applications concrètes :

- la mise en place de politiques locales de gestion des eaux usées ;
- l'orientation des campagnes de sensibilisations sur les bonnes pratiques d'hygiène et d'assainissement ;
- la planification de la réalisation des forages ou puits dans les lieux surs, éloignés des zones de rejets à haut risque de pollution ;
- le développement de système de traitement à petite échelle pour améliorer la qualité de l'eau destinée à la consommation ;

Enfin, cette étude met en lumière la nécessité d'une approche intégrée et participative pour préserver

durablement les ressources en eau souterraine dans les zones urbaines en expansion comme Dianéguela.

REMERCIEMENT

Nous adressons nos vifs remerciements au décanat de la Faculté d'Histoire et de Géographie (FHG et la Direction Générale du Laboratoire National des eaux (LNE) qui ont facilité la mise en œuvre de la présente étude ainsi que toutes les bonnes volontés.

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The Study on Soil Erosion and Carbon Sequestration in Zhanjiang City Using RUSLE-InVEST Model

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Received: 21 Aug 2025; Received in revised form: 23 Sep 2025; Accepted: 27 Sep 2025; Available online: 06 Oct 2025

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Abstract— This study aims to reveal the spatiotemporal differentiation characteristics of soil erosion and carbon sequestration in Zhanjiang City and their underlying causes, providing a scientific basis for ecosystem monitoring in the region. Based on the RUSLE-InVEST model and geographic detectors, data from four years (2013, 2017, 2020, and 2023) were selected to analyze the main driving factors and mechanisms of soil erosion, as well as the spatiotemporal variations in carbon sequestration in the study area. The results indicate that soil erosion in Zhanjiang City exhibits significant spatial heterogeneity, with an overall increasing trend in erosion severity. In single-factor analysis, precipitation and land use type factors showed higher explanatory power for soil erosion. In interaction factor analysis, all factor interactions demonstrated either two-factor enhancement or nonlinear enhancement. Overall, the intensity of soil erosion in Zhanjiang City fluctuated over time, and carbon sequestration showed a significant correlation with changes in soil erosion intensity. High-risk erosion areas require prioritized management, and region-specific differentiated governance strategies are recommended based on local conditions.



Keywords— Soil erosion, RUSLE model, InVEST model, Carbon sequestration, Geographic detector

I. INTRODUCTION

Ecosystems can generate a range of goods and services essential for human well-being, collectively referred to as Ecosystem Services (ES) (Nelson et al., 2009). Since the Millennium Ecosystem Assessment (2005), ecosystem services have become one of the key indicators for measuring ecosystem quality and sustainability. Since then, the assessment of ecosystem services has emerged as a major research topic, with significant progress being made.

Water conservation, soil retention, and carbon sequestration, as key components of ecosystem services, play a crucial role in the synergistic development of

ecosystems (Zuo et al., 2024). However, in the context of economic development and urbanization, climate change and land-use alterations are severely impacting global ecosystem services, threatening the sustainable development of human society (Babbar et al., 2021). Consequently, the increase in the greenhouse effect and climate change has become a global concern, particularly as carbon sequestration significantly influences global carbon reduction and mitigates global warming (Deng et al., 2021). Carbon sequestration, as an ecosystem service, may play a vital role in offsetting anthropogenic carbon sources and regulating climate. Moreover, soil erosion severely damages soil resources, affects soil carbon

sequestration, harms ecosystems, and simultaneously hinders crop growth, impeding regional ecological-economic development (Ma et al., 2021). Understanding the impacts and mechanisms of these ecological chains can help address the related scientific issues mentioned above.

Soil erosion is the process by which soil and its parent materials are destroyed, eroded, transported, and deposited under the action of hydraulic forces, gravity, and other factors (Dotterweich, 2013). Soil erosion can lead to a decline in soil quality, deterioration of physical and chemical properties, land desertification, reduced agricultural productivity, sedimentation in rivers and lakes, and many other environmental issues, making it one of the largest global ecological problems (Wang, 2011). Due to China's large population, complex geological conditions, and topographic structures, soil erosion has severely threatened survival and socio-economic development, becoming one of the major challenges for sustainable development in the 21st century (Qi et al., 2011). The multi-scale characteristics of soil erosion and the complexity of its influencing factors impose higher demands on soil erosion prevention and control. Therefore, studying the spatiotemporal distribution characteristics of soil erosion holds special significance and necessity (Yan, 2022).

The Revised Universal Soil Loss Equation (RUSLE) model was developed by the U.S. Department of Agriculture as an improvement upon the Universal Soil Loss Equation (USLE) (Chen, 2011). Due to its straightforward calculation formula and relatively low data requirements, it has become a widely used quantitative estimation model for soil erosion globally (Wu et al., 2012). For example, Hu Xianpei et al. analyzed the spatiotemporal characteristics of soil erosion in Tongren from 1987 to 2015 using the RUSLE model (Hu et al., 2019). Li Yanmeng et al. explored the spatiotemporal evolution patterns of karst soil erosion based on GIS and the RUSLE model (Li et al., 2023). These studies collectively demonstrate that the RUSLE model has a certain applicability in estimating soil erosion in most regions of China (Liang et al., 2019). Additionally, Chen Jinxing et al. (Chen et al., 2016) found that RUSLE is more suitable for areas with significant forest and farmland

coverage, while the Chinese Soil Loss Equation (CSLE) is preferable for regions dominated by other land use types. Larger regions are generally more suited for RUSLE (Huang et al., 2025). Given that Zhanjiang covers a relatively large area with substantial forest and farmland coverage, this study adopts the RUSLE model combined with remote sensing and geographic information technology. By extracting factors such as the rainfall erosivity factor (R), soil erodibility factor (K), land use data, cover management factor (C), and support practice factor (P) for Zhanjiang, the study aims to investigate soil erosion in the region.

In addition, regarding the analysis and exploration of carbon sequestration, there is a close relationship between carbon sequestration, soil, and ecological service systems. Soil serves as the largest terrestrial carbon sink for organic carbon. Through organic matter management, soil carbon sequestration can be increased, thereby enhancing its carbon sequestration capacity. This also brings multiple benefits, including improved fertility, soil and water conservation, and environmental protection. Forest management can also increase carbon sequestration by boosting productivity and extending rotation periods, complementing the soil carbon pool to jointly support the health and functionality of ecosystems. In recent years, using the carbon sequestration module of the InVEST model to study the spatiotemporal characteristics of ecosystem carbon sequestration and its relationship with land use patterns has become a hot topic (Wu and Wang, 2023; Zhou, 2025). Compared to other research methods, the InVEST model requires less data and operates faster, enabling spatial mapping of carbon sequestration distribution and dynamic changes. It reflects the relationship between land use changes and carbon sequestration, facilitating the dynamic quantification of ecological service function value (Liu et al., 2021).

Most previous studies have employed single-variable analysis and rarely combined soil erosion with carbon sequestration in research on the same region. Therefore, this study utilizes the RUSLE model for soil erosion analysis, employs geographic detectors to examine its driving factors, and explores the spatiotemporal characteristics of ecosystem carbon sequestration using the carbon module of the InVEST model. By adopting a

coupled approach, it aims to provide an in-depth understanding of the ecological service system in the study area, thereby offering a scientific reference.

II. STUDY AREA AND DATA SOURCES

2.1 Study Area

Zhanjiang City ($20^{\circ}13'N$ - $21^{\circ}57'N$, $109^{\circ}31'E$ - $110^{\circ}58'E$) is located in the southwest of Guangdong Province. It serves as the central city of the western Guangdong and Beibu Gulf urban agglomeration and is one of China's first batch of coastal open cities (Figure 1). The total area under its jurisdiction is 13,263 km², with a coastline stretching 2,023.6 km, including 1,243.7 km of mainland coastline and 779.9 km of island coastline. Situated south of the Tropic of Cancer, it enjoys a tropical northern marginal monsoon climate, with an average annual temperature ranging between 23°C and

24°C. The region experiences distinct monsoons, diverse climate types, abundant heat, plentiful rainfall, and clearly defined wet and dry seasons. Frequent and severe meteorological disasters such as typhoons and torrential rain-induced floods make the area prone to soil erosion.

Zhanjiang is located in the southern red soil region of China, characterized by uneven spatial and temporal distribution of precipitation, acidic, barren, and heavy-textured soil properties. Predominantly composed of red soil, which is relatively heavy and not prone to forming granular structures, these soil characteristics are prone to causing soil erosion. Coupled with unreasonable land development and utilization, it is highly susceptible to ecological and environmental issues such as soil desertification, salinization, water and soil loss, and soil pollution.

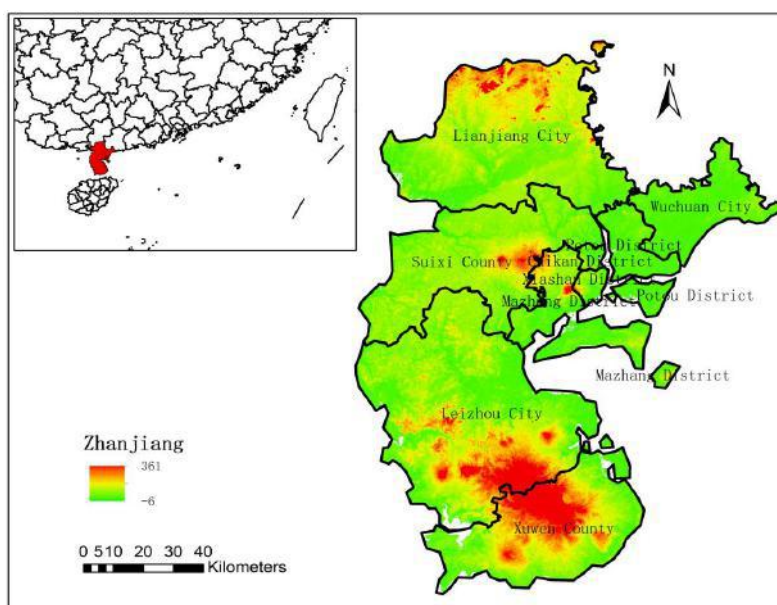


Fig.1 Geographical Location and Elevation Distribution Map of Zhanjiang City

2.2 Data Sources

The elevation data with a spatial resolution of 30 m was obtained from the Geospatial Data Cloud (<http://www.gscloud.cn>) for creating terrain generalization maps and LS factors; Landsat 8 data was downloaded and processed in ENVI to derive NDVI (Normalized Difference Vegetation Index), which was then used to calculate FVC (Fractional Vegetation Cover) through confidence extraction and formula computation, ultimately applied to determine the vegetation cover factor.

The world soil database (HWSD) was downloaded from the National Earth System Science Data Center (<https://www.geodata.cn>), and after mask extraction and raster calculation, the K factor (soil erodibility factor) was obtained. Rainfall data for the study area was also acquired, and the R (Rainfall) factor was calculated using ArcGIS raster computation.

Land use/land cover data was downloaded from the Resource and Environment Science Data Center (<http://www.resdc.cn>). Additionally, GDP (Gross Domestic

Product) data was sourced from the Resource and Environment Science Data Registration and Publishing System (<http://www.resdc.cn/DOI>, 2017. DOI: 10.12078/2017121102), while population density data was retrieved from the Zenodo database

(<https://zenodo.org/records/11179644>) for investigating the influencing factors of soil erosion using geographic detectors. The data required for model operation and their sources are listed in Table 1.

Table 1 Description of Research Data Sources and Uses

Data category	Data source	Purpose
Annual precipitation	Downloaded from the National Earth System Science Data Center (https://www.geodata.cn),	Generate R-factor layer
HWSD (Harmonized World Soil Database)		Generate K-factor layer
30m DEM	Geospatial Data Cloud (http://www.gscloud.cn)	Calculate LS factor
NDVI		Generate C-factor layer
Global Land Use/Land Cover Map (LUCC)	Resource and Environmental Science Data Center (https://www.resdc.cn/)	Generate land use layers
GDP data	Resource and Environmental Science Data Registration and Publishing System (http://www.resdc.cn/DOI)	Geographic Detector Parameters
Population density data	Zenodo Database (https://zenodo.org/records/11179644) ,	Geographic Detector Parameters

III. METHODOLOGY

This study employs the RUSLE model to assess soil erosion, utilizes a geographic detector to analyze its driving factors, and investigates the spatiotemporal variations in ecosystem carbon sequestration using the carbon module of the InVEST model. The RUSLE model incorporates data such as annual precipitation, HWSD,

NDVI, and DEM for analysis, while the InVEST model primarily relies on the Global Land Use/Land Cover Map (LUCC) as its analytical material. The geographic detector analysis involves 11 factors, encompassing both natural and anthropogenic elements. Finally, the analytical results are integrated to derive scientific conclusions for the study area (Figure 2).

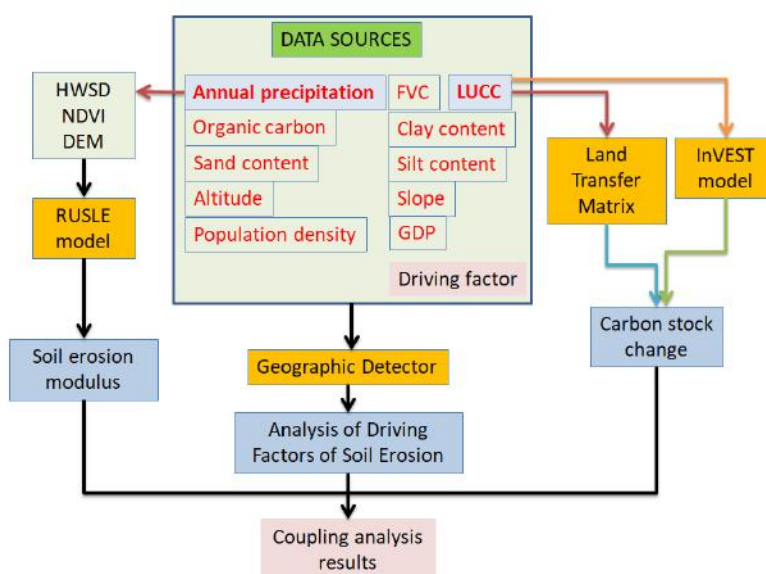


Fig.2 Technical Framework of This Study

3.1 Soil Erosion Model Parameter Construction

This study employs the RUSLE model, which features standardized and clear definitions for each factor, along with a straightforward calculation process. The calculation formula is as follows:

$$A=R \times K \times LS \times C \times P \quad (1)$$

In the formula: A is the soil erosion modulus, Unit: $t/(hm^2 \cdot a)$; R is the rainfall erosivity factor, in $MJ \cdot mm/(hm^2 \cdot h \cdot a)$; K is the soil erodibility factor, in $t \cdot h/(MJ \cdot mm)$; LS is the slope length and steepness factor, dimensionless; C is the cover and management factor, dimensionless, ranging from 0 to 1.

3.1.1 R Factor

Precipitation is one of the important causes of soil erosion, mainly triggered by the kinetic energy carried by raindrops impacting soil particles and causing erosion. The R value is a function of rainfall energy and intensity, generally used to reflect the impact of rainfall on soil erosion (Wang et al., 2005). This study adopts the "Guidelines for Calculating Soil Loss in Production and Construction Projects SL773-2018." After obtaining annual precipitation data from 2012 to 2022, the

$$K_{epic} = \left\{ 0.2 + 0.3 \exp \left[-0.0256 SAN \left(1 - \frac{SIL}{100} \right) \right] \right\} \times \left(\frac{SIL}{CLA + SIL} \right)^{0.3} \times \left[1 - \frac{0.25C}{C + \exp(3.72 - 2.95C)} \right] \times \left[1 - \frac{0.7SN}{SN + \exp(-5.51 + 22.9SN)} \right] \quad (4)$$

$$K = -0.01383 + 0.51575 \times K_{epic} \quad (5)$$

3.1.3 L、S Factor

Slope length and gradient are fundamental topographic factors. Slope length refers to the distance from the starting point of surface runoff generation on a slope to the point where runoff converges into a gully (Wang et al., 2020). A longer slope length indicates greater runoff accumulation and stronger erosive power. Gradient refers to the degree of inclination at a specific point on the land surface. By influencing the velocity of water flow, gradient affects infiltration rate, runoff volume, and soil scouring, making it the best micro-scale indicator for soil erosion assessment (Wang et al., 2020). The LS factor effectively reflects the influence of topography on rainfall erosion. In this study, DEM was used to extract the LS factor.

$$L = \left(\frac{\lambda}{22.13} \right)^m \quad (6)$$

precipitation erosivity factor was calculated using Formula (2).

$$R = 0.067 P_d^{1.627} \quad (2)$$

In Equation (2), R represents the multi-year average rainfall erosivity factor, with units of $MJ \cdot mm/(hm^2 \cdot h)$; P_d denotes the multi-year average rainfall, measured in mm, and the spatial distribution of the precipitation erosivity factor is calculated accordingly.

3.1.2 K Factor

Soil erodibility refers to the spatial variation in the ability of soil's inherent physicochemical structure to resist erosion at different locations, serving as a crucial quantitative parameter for assessing soil sensitivity to erosion. This study employs the widely used calculation methods from the Erosion Productivity Impact Calculator (EPIC) model proposed by Sharpley et al. (1990) and Williams et al. (1983). Additionally, based on the modified algorithm for soil erodibility factor (K) in Chinese regions introduced by Zhang et al. (2007), the soil erodibility factor (K) for the study area was calculated using the following formula:

$$SN = 1 - SAN/100 \quad (3)$$

$$\lambda = L \times \cos \alpha \quad (7)$$

In the formula: L is the slope length factor (dimensionless); λ is the horizontal projection slope length (m), which is the length of surface water flow along the direction; α is the slope value of the water flow area; m is the variable slope exponent (Jin, 2019).

When $\theta < 0.57^\circ$, $m = 0.2$.

When $0.57^\circ \leq \theta < 1.72^\circ$, $m = 0.3$.

When $1.72^\circ \leq \theta < 2.86^\circ$, $m = 0.4$.

When $2.86^\circ \leq \theta$, $m = 0.5$.

The slope calculation is based on grading. For slopes below 10° , the McCoolDK formula (McCool et al., 1987) is used, while for slopes above 10° , the formula calibrated by Liu (2001) is applied:

$$S = \begin{cases} 10.80 \times \sin \theta + 0.03 & \theta < 5^\circ \\ 16.80 \times \sin \theta - 0.50 & 5^\circ < \theta \leq 10^\circ \end{cases} \quad (8)$$

$$21.91 \times \sin \theta - 0.96 \quad \theta > 10^\circ$$

Where: S is the slope factor (dimensionless); θ is the slope ($^\circ$)

3.1.4 C Factor

Vegetation coverage (C) refers to the percentage of vegetation in the vertical projection area relative to the total area. In this study, the pixel dichotomy model was used to calculate the vegetation coverage of the study area as a measure of vegetation factors, with the calculation formula as follows:

$$Fg = (NDVI - NDVI_{soil}) / (NDVI_{veg} - NDVI_{soil}) \quad (9)$$

In the equation, Fg represents vegetation coverage, NDVI_{soil} is the NDVI value of pure soil pixels, which corresponds to the NDVI value closest to the 0.5% position in the minimum NDVI probability distribution table; NDVI_{veg} is the NDVI value of pure vegetation pixels, corresponding to the NDVI value closest to the 0.5% position in the maximum NDVI probability distribution table. By downloading Landsat8 OIL_TIRS product data and performing radiometric calibration, atmospheric correction, and NDVI calculation, the vegetation coverage of Zhanjiang City in 2016 was estimated.

3.1.5 P Factor

The P-value of the soil conservation factor refers to the ratio of soil erosion on sloping farmland with conservation measures to that without such measures under otherwise identical conditions, reflecting the efficacy of these measures in reducing soil erosion. The value ranges from 0 to 1, with P=1 indicating no soil conservation measures are implemented. Based on previous research (Zheng et al., 2023), the assigned values are as follows: Cropland is assigned 1, forestland 0.3, grassland 0.6, impervious surfaces 0.2, water bodies 0.7, and bare land 0.8.

3.2 Soil Erosion Characterization Index

3.2.1 Comprehensive Index Assessment of Soil Erosion

To compare and analyze soil erosion conditions across different units, a comprehensive soil erosion index (Yang et al., 2000) can be used. The grading values for hydraulic erosion—slight, mild, moderate, strong, extremely strong, and severe—are assigned as 0, 2, 4, 6, 8, and 10, respectively.

3.2.2 Soil Erosion Intensity Dynamic Degree

The dynamic degree of soil erosion intensity is an

indicator that depicts the rate and magnitude of regional differences in a certain soil erosion intensity over a period, aiming to reflect the severity and overall trend of its changes (He et al., 2024). When J is positive, it means that the area of a certain soil erosion intensity has increased during the study period, indicating an intensification of soil erosion at that intensity; when J is negative, it indicates a mitigation of soil erosion at that intensity. The calculation formula is as follows:

$$J = \frac{U_b - U_a}{U_a} \times \frac{1}{T} \times 100\% \quad (10)$$

In the formula: J represents the dynamic degree of soil erosion at a certain intensity; U_a and U_b are the areas of a certain erosion intensity at the beginning and end of the study period, respectively; T is the duration of the study period.

3.2.3 Entropy of Soil Erosion Intensity

The lower the information entropy of soil erosion intensity, the greater the proportion of slight erosion, and the smaller the proportion of other intensities of soil erosion, indicating an improvement in soil erosion conditions. The calculation formula is as follows:

$$H = -\sum_{i=1}^n Q_i \times \ln Q_i \quad (11)$$

In the formula: H represents the information entropy of soil erosion intensity; Q_i is the proportion of the area of soil erosion at the i-th intensity level to the total area of soil erosion in the watershed; n is the number of soil erosion intensity levels (Huang et al., 2025).

3.3 Geographical Detector

Based on the geographic detector, by combining different spatial data discretization methods with discontinuous parameters, the q value is used to quantitatively measure the quality of the discretization parameters. The larger the q value, the stronger the explanatory power of X on Y. The calculation formula is as follows:

$$q = 1 - \sum_{h=1}^L N_h \times \sigma_h^2 / N \sigma^2 \quad (12)$$

In the formula: q is the detection value of the driving factor; it refers to the explanatory power of the independent variable X on the dependent variable Y, with a range of [0, 1]. The larger the value, the stronger the explanatory power of X on the spatial differentiation of Y. N is the total number of samples; L is the number of strata for variable X or Y; h is the number of strata for variable X

or Y ; N_h is the number of samples in the h -th stratum; σ^2 is the total variance of the dependent variable Y for the entire population; σ^2 is the variance of Y values in the h -th stratum.

This study established random sampling points for fishing net extraction within Zhanjiang City, extracting various classification values to their corresponding sample points. Based on the GD package in R language, equal interval, natural breaks, and quantile methods were selected as discretization approaches, with classification numbers ranging from 3 to 7 categories. Subsequently, the geographical detector was employed to analyze the influence of various factors in the RUSLE model on soil erosion. Using the factor detector and interaction detector modules within the model, the explanatory power of 11 influencing factors (land use type, annual rainfall, vegetation coverage, elevation, slope, sand content, silt content, clay content, organic carbon content, GDP, and population density) on the spatial heterogeneity of soil erosion was quantified.

3.4 Carbon Sequestration Estimation

Carbon sequestration services, as a core regulatory function in addressing climate change, directly influence

atmospheric carbon cycles and global climate patterns through ecosystem carbon sequestration and storage processes. They serve as a vital natural solution for achieving the "dual carbon" goals, with their quantitative research providing a scientific basis for carbon sink accounting and climate policy formulation (Zhou, 2025). This study utilizes the Carbon module within the InVEST model to estimate total carbon sequestration based on the sum of carbon pools corresponding to land use types, calculated using the formula (13):

$$Ci_tot = Ci_above + Ci_below + Ci_soil + Ci_dead \quad (13)$$

i represents the i th land use type; Ci_tot denotes the total carbon sequestration, Ci_above represents the aboveground biomass carbon sequestration, Ci_below indicates the underground biomass carbon sequestration, Ci_soil stands for the soil carbon sequestration, and Ci_dead refers to the dead organic matter carbon sequestration. The units of the aforementioned carbon sequestration are all in tons. Based on previous studies, the carbon density values c for various land use types in Guangdong Province were compiled, as shown in Table 2 (Dai et al., 2025).

Table 2 Carbon Density c (t/hm²) of Land Use Types in Guangdong Province

Land use type	ci_above	ci_below	ci_soil	ci_dead
Cropland	15.74	3.15	10.84	0.00
Forestland	19.24	5.77	19.28	2.82
Grassland	16.06	83.50	9.99	0.24
Water bodies	0.28	1.37	3.03	1.24
Construction Land	11.29	2.26	17.97	0.00
Bare land	13.92	2.78	5.33	0.00

IV. RESULTS AND ANALYSIS

4.1 Spatiotemporal Analysis of Soil Erosion Models

Zhanjiang is in the southern red soil hilly region within the water erosion zone, classified under the southern red soil hilly area. According to the "Classification and Grading Standards for Soil Erosion" (SL190 - 2007), its permissible soil erosion modulus (allowable soil loss) is 500 t/(km²·a), equivalent to 5 t/(hm²·a). Analysis reveals that the average erosion modulus in Zhanjiang City is 138.7 t/(hm²·a), exceeding the permissible soil erosion modulus for the study area. Additionally, the analysis indicates that the average soil

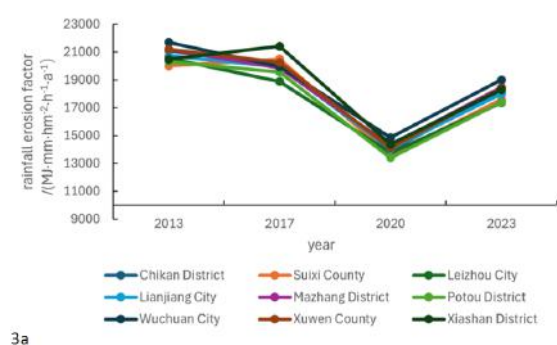
erosion volume in Zhanjiang City reaches 1.3064 million tons.

Analysis of various factors in the erosion model reveals that the R-factor exhibits fluctuating changes, with an overall "first decline then rise" trend from 2013 to 2023 (reaching its lowest point in 2020). Significant peaks occurred in 2013 and 2017, largely aligning with changes in soil erosion modulus, making it the core driving factor of erosion (Figure 3a). The C and P factors remained relatively stable. The C-factor (vegetation factor) mostly maintained within the range of 0.8-1.0 across most districts and counties over the long term, with only minor

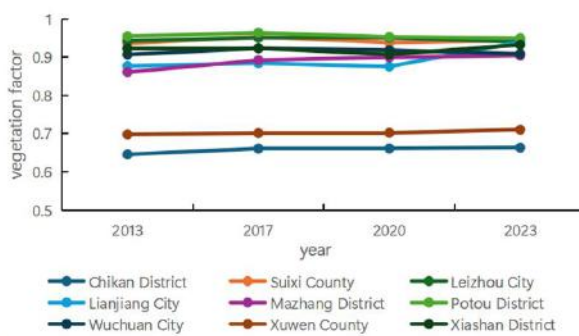
fluctuations in a few years/regions (Figure 3b).

The P-factor (soil and water conservation measures factor) also remained relatively stable, though some districts and counties (e.g., Chikan District and Mazhang District) showed a slow decline from 2013 to 2023 (Figure 3c). Over time, the soil erosion modulus (K-factor) in most districts and counties displayed a "first decrease then increase" trend (Figure 3d): from 2013 to 2020, the modulus decreased in sync with the decline in the R-factor, while from 2020 to 2023, it rose again as the R-factor

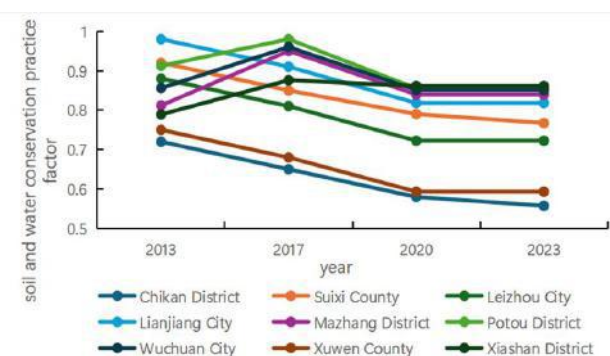
rebounded. Regional differences were significant: agricultural districts and counties such as Leizhou City and Suixi County, influenced by both natural and human factors, had high erosion modulus values strongly linked to the R-factor. In contrast, areas like Chikan District and Mazhang District, characterized by gentle terrain and enhanced conservation measures, exhibited lower modulus values with suppressed growth, confirming the buffering effect of vegetation and measures on erosion.



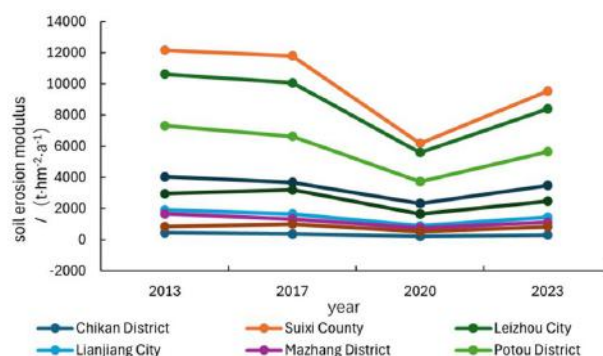
3a



3b



3c



3d

Fig.3. Factor Variation and Soil Erosion Modules from 2013 to 2023

4.2 Comprehensive Assessment of Soil Erosion Levels

This study refers to the "Classification and Grading Standards for Soil Erosion" (SL190-2007) issued by the Ministry of Water Resources of the People's Republic of China, dividing soil erosion intensity into six levels: slight, mild, moderate, strong, extremely strong, and severe erosion. Areas with mild erosion and above are considered as regions affected by soil erosion.

As shown in Figure 4, the overall trend of soil erosion in Zhanjiang City is dominated by slight erosion, accounting for approximately 98.13% of the total erosion

area. From 2013 to 2023, the area of slight erosion exhibited a trend of first decreasing and then increasing, with an average rate of 0.061 km²/year. The area of mild erosion showed a similar trend of first decreasing and then rising, with an average rate of -0.064 km²/year. Meanwhile, the areas of moderate, strong, extremely strong erosion all displayed a pattern of initial decline followed by an increase, with average rates of 0.053 km²/year, 0.033 km²/year, and 0.001 km²/year, respectively.

In terms of spatial distribution, Leizhou City had the highest proportion of slight erosion area, followed by

Lianjiang City, while Wuchuan City had the lowest proportion. Additionally, in 2020, the proportion of slight erosion area experienced a slight decline, while the proportions of other erosion categories increased, indicating a worsening trend of erosion in some regions.

As shown in Figure 5, soil erosion in the study area peaked in 2020, reaching 927,500 tons. The erosion intensity in Xiashan District was relatively low, indicating better ecological conditions and more stable vegetation coverage in the area. In contrast, Leizhou City experienced higher erosion levels, reflecting poorer ecological

conditions, lower vegetation coverage, and weaker soil and water conservation measures. According to Figure 6, slight erosion was the dominant intensity level in the study area. Overall, the distribution of erosion intensity grades showed a pattern of lower levels in the northwest and higher levels in the southeast. The northern regions had relatively lower soil erosion modulus, while the eastern and southern regions exhibited higher values. In Leizhou City, located in the south, the areas affected by slight and moderate erosion increased significantly.

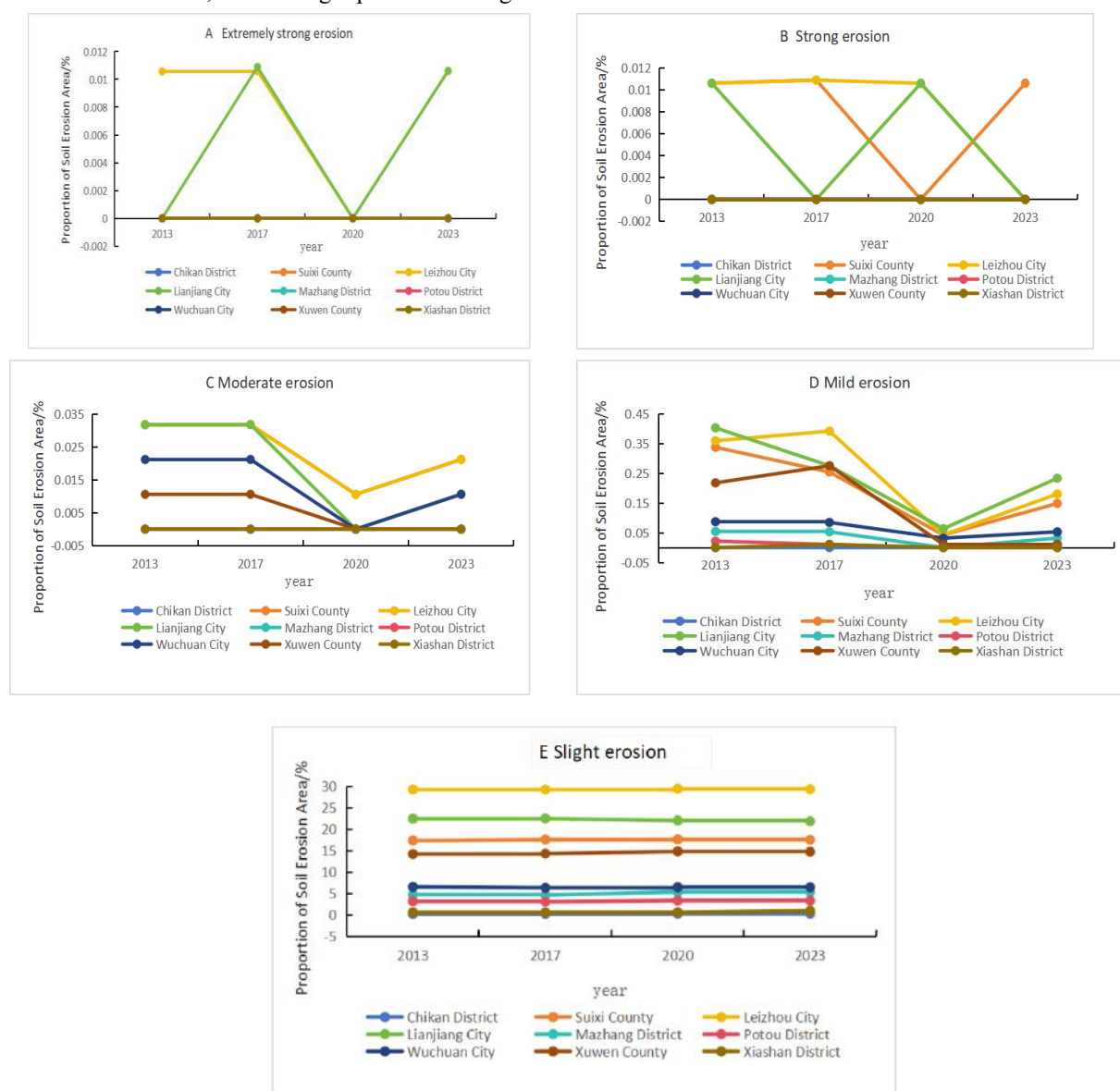


Fig.4 Proportion of Soil Erosion Area

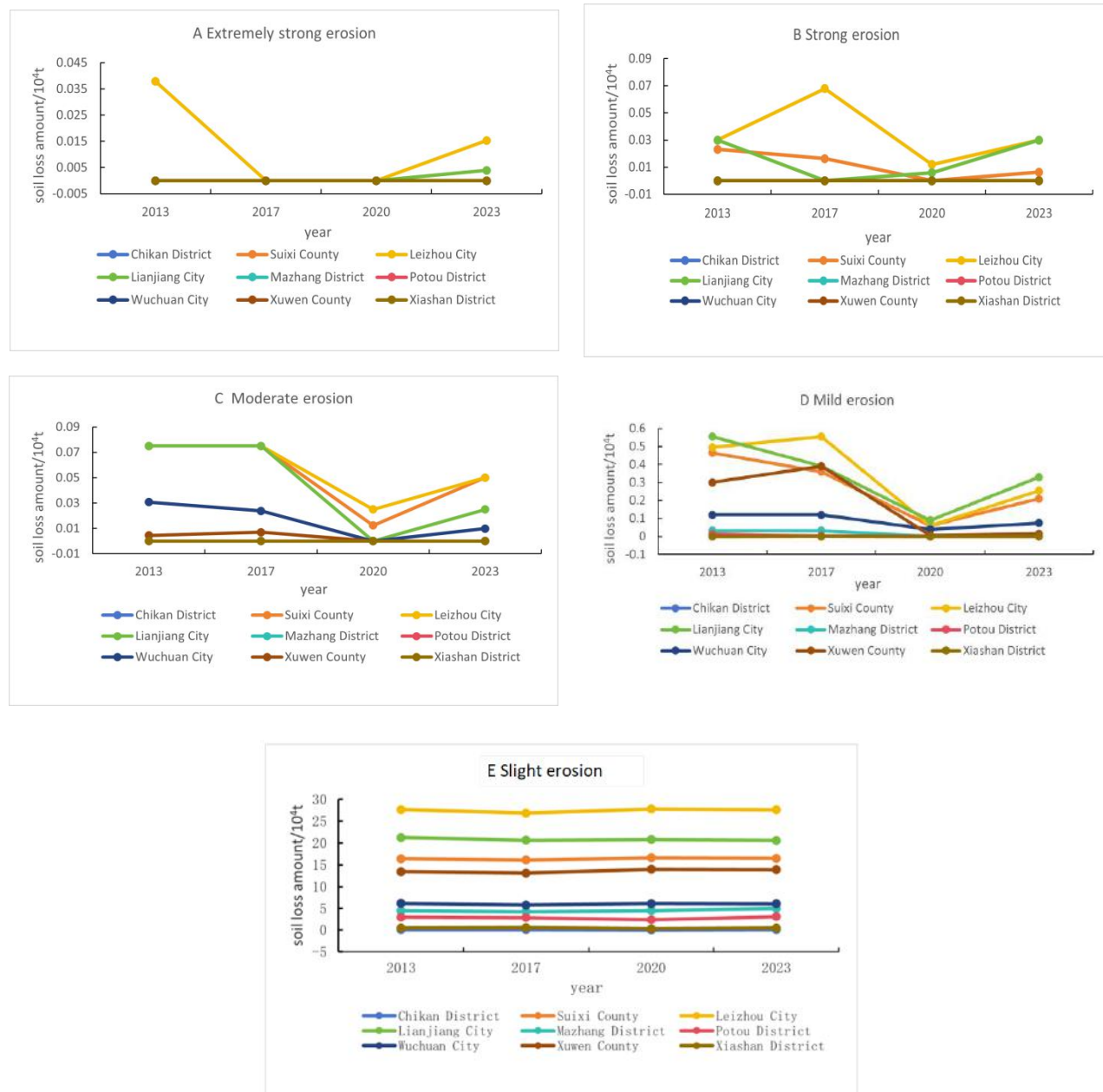


Fig.5 Spatiotemporal Statistics of Soil Erosion

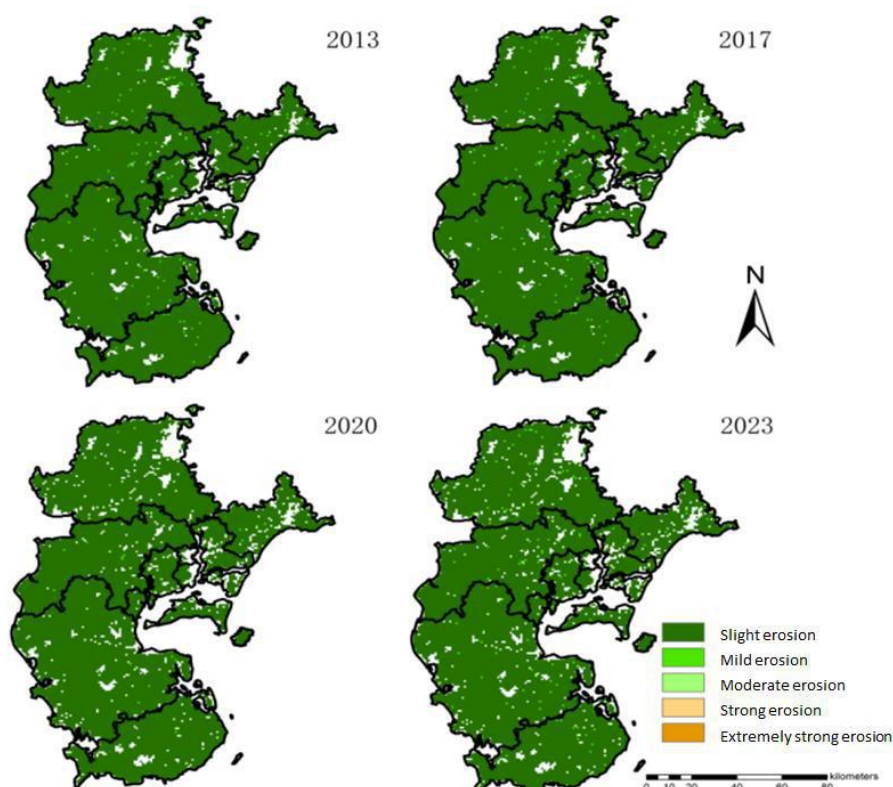


Fig.6 Distribution Map of Soil Erosion Intensity from 2013 to 2023

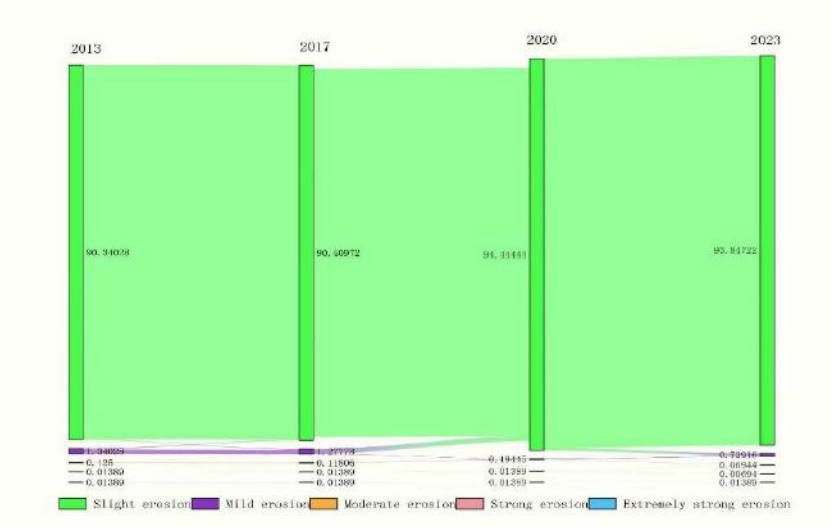
4.3 Dynamic Changes in Soil Erosion Levels

From 2013 to 2023, the trend of area transfer changes in different erosion intensity levels in the study area was not significant (Figure 7). During the periods 2013–2017, 2017–2020, and 2020–2023, 96.44%, 88.70%, and 99.16% of the areas, respectively, showed no change in erosion intensity levels. Over the study period, the newly added and transferred areas of slight erosion were 3.51 km² and 1.49 km², accounting for 3.70% and 1.57% of the total area, respectively. The reduced and transferred area of mild erosion was 0.61 km², accounting for 0.63% of the total area. Moderate and strong erosion trends decreased by 0.059% and 0.0073%, respectively, indicating minimal changes in soil erosion intensity in the study area.

As shown in Figure 8, the dynamic degree of slight erosion was predominantly negative from 2013–2017 and 2020–2023, indicating a gradual reduction around such regions, with more areas transitioning to mild erosion and higher grades. Meanwhile, the dynamic degrees of mild, moderate, strong, and extremely strong erosion were mainly positive, suggesting an increasing area of erosion

and a worsening trend in erosion conditions. Particularly during 2020–2023, the dynamic degree of strong erosion reached its peak at 412.5%. Although the dynamic degrees of most erosion intensities were negative during 2017–2020, indicating some mitigation of erosion, the dynamic degrees of moderate and strong erosion rose again to 131.8%, 63.40%, and 412.5% in 2020–2023, demonstrating that the trend of intensifying erosion has not been effectively curbed.

Comparing the comprehensive soil erosion index with the changes in information entropy of soil erosion intensity in the study area (Table 3), the trends show similar patterns. Between 2013 and 2017, the comprehensive index and information entropy values were comparable, indicating that the overall intensity of soil erosion in the study area did not change significantly. From 2017 to 2020, the comprehensive index showed a slight decline, reaching 0.010 in 2020, while the information entropy also decreased from 0.091 in 2017 to 0.027 in 2020. This suggests that during this period, the extent of soil erosion narrowed, and the intensity of erosion weakened.



Note A: Slight erosion B: Mild erosion C: Moderate erosion D: Strong erosion E: Extremely strong erosion

Fig.7 Soil Erosion Intensity Grade Transfer Map

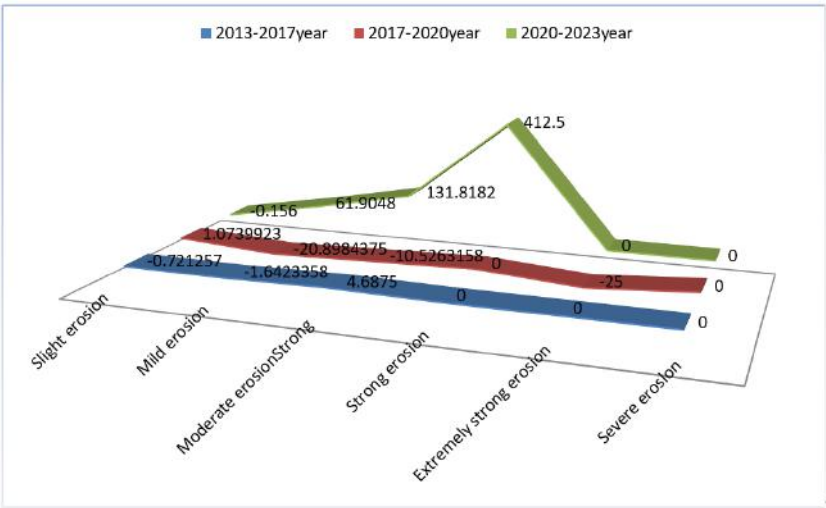


Fig.8 Dynamic Degree of Soil Erosion Intensity

Table 3 Soil Erosion Characterization Indexes in Zhanjiang City

Year		2013Year	2017Year	2020Year	2023 Year
Characterization Index	Composite Index	0.0378	0.038	0.010	0.0678
	Information entropy	0.0911	0.091	0.027	0.114

4.4 Analysis of Factors Influencing Soil Erosion

The significance analysis of soil erosion influencing factors (Table 4) reveals that the order of factors affecting the soil erosion modulus from greatest to least is annual precipitation ($q=0.4648$) > land use type ($q=0.2855$) > organic carbon content ($q=0.1959$) > slope ($q=0.1922$) > sand content ($q=0.1917$) > elevation ($q=0.1879$) > GDP

($q=0.1506$) > clay content ($q=0.1453$) > silt content ($q=0.1304$) > population density ($q=0.1281$) > vegetation coverage ($q=0.1281$). The results indicate that the primary factors contributing to the spatial variation of soil erosion modulus are natural environmental factors, with annual precipitation having the highest explanatory power, followed by land use type and organic carbon content.

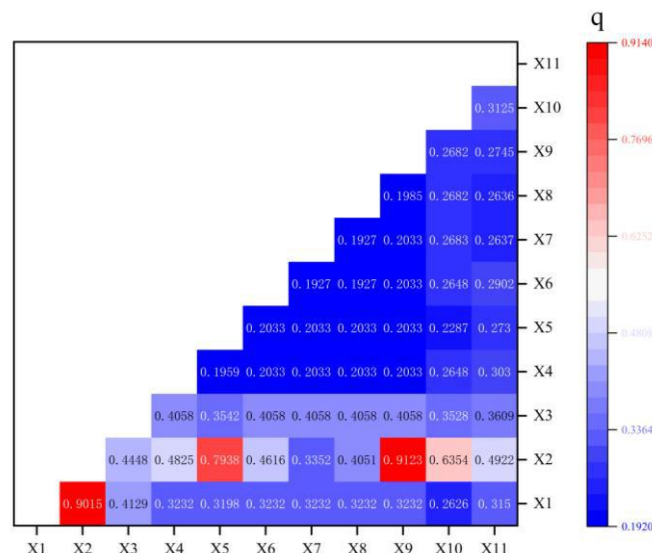
Among anthropogenic factors, GDP exhibits the greatest explanatory influence.

Table 4 q-values of Driving Factors

Category	Factors	q value	p value
Natural Factors	LUCC	0.2855	0.2340
	Annual precipitation	0.4648	0.0080
	FVC	0.1281	0.7911
	Organic carbon content	0.1959	0.5168
	Clay content	0.1453	0.2406
	Sand content	0.1917	0.5275
	Silt content	0.1304	0.7131
	Altitude	0.1879	0.5415
	Slope	0.1922	0.5025
Humanistic Factors	Population density	0.1281	0.7157
	GDP	0.1506	0.6459

Additionally, the interaction analysis of driving factors (Figure 9) revealed that precipitation (X2) $\cap$ slope (X9) ($q=0.9123$) exhibited nonlinear enhancement. After the synergistic effect of the two factors, the explanatory power surged from 0.46 (single factor X2) to 0.91, strongly driving soil erosion synergistically. The combination of concentrated precipitation and steep slopes significantly enhanced runoff scouring force. Precipitation (X2) $\cap$ land use type (X1) ($q=0.9015$) also showed nonlinear enhancement, with a synergistic explanatory

power close to 0.90. Bare land and farmland under heavy precipitation represent a "typical pattern" of high erosion risk: low vegetation coverage combined with concentrated runoff leads to a sharp increase in erosion risk. Precipitation (X2) $\cap$ vegetation coverage (X3) ($q=0.4938$) demonstrated dual-factor enhancement, with improved synergistic explanatory power ($>$ single factor X2). The combination of low vegetation coverage and intense precipitation exacerbates erosion.



NOTE: X1: land use type X2: annual precipitation X3: vegetation coverage X4: soil organic carbon content X5: clay content X6: sand content X7: silt content X8: elevation X9: slope gradient X10: population density X11:GDP

Fig.9 Interaction Diagram of Driving Factors for Soil Erosion

4.5 Analysis of Spatiotemporal Distribution of Carbon Sequestration

4.5.1 Characteristics of Temporal Changes in Carbon Sequestration

According to the model calculations, the carbon sequestration in Zhanjiang City was 32.561 t/ha in 2013, 32.588 t/ha in 2018, 32.597 t/ha in 2020, and 32.652 t/ha in 2023, showing an overall upward trend. When analyzed by land use type (Figure 10), the carbon sequestration from largest to smallest is forest land > cropland > construction land > grassland > water bodies > unused land. By 2023, forest land accounted for 50.10% of the total carbon sequestration in Zhanjiang City, followed by cropland (38.33%) and construction land (2.411%). Overall, from 2013 to 2023, the soil erosion modulus decreased, and carbon sequestration capacity improved. Both grassland carbon sequestration and construction land carbon sequestration showed fluctuating increases, rising by 329.37 tons and 2,427.04 tons, respectively.

Using carbon sequestration dynamics from land use type conversions between 2020 and 2023, the relationship between soil erosion and carbon sequestration was explored. The conversion of cropland to forestland (an area of 6.00 km²) resulted in a net increase in carbon sequestration (104.28 t), with simultaneous accumulation in vegetation carbon (36.72 t) and soil carbon (67.56 t). This may be attributed to the restoration of forest vegetation, where increased vegetation cover intercepts rainfall and stabilizes soil, reducing hydraulic erosion's

stripping of topsoil. Additionally, the input of vegetation litter enhances soil organic carbon, establishing a positive feedback loop of "erosion reduction-carbon sequestration." Conversely, the conversion of forestland to cropland (an area of 7.00 km²) led to a significant decrease in carbon sequestration (-121.66 t), with synchronous losses in vegetation carbon (-42.84 t) and soil carbon (-78.82 t) (Table 5). This is directly related to the decline in vegetation coverage after forestland reclamation. The destruction of forest ecosystems reduces soil erosion resistance (e.g., aggregate stability and root soil-binding capacity), making topsoil more susceptible to stripping by rainfall and runoff. This results in the loss of organic-rich topsoil and weakens the ecosystem's carbon sequestration capacity, reflecting the logical chain of "vegetation destruction → intensified erosion → carbon loss."

In addition, the conversion of water bodies to grassland (covering an area of 1.00 km²) resulted in a significant increase in carbon sequestration (103.87t), with both vegetation carbon (97.91t) and soil carbon (5.96t) rising simultaneously (Table 5). When water bodies are transformed into grassland, the aquatic carbon pool is disrupted, but the development of grassland vegetation promotes the sedimentation of silt (carrying organic carbon). If hydraulic erosion occurs in the area, the silt eroded and transported from around the water bodies can accumulate during grassland restoration, serving as a potential source of soil carbon replenishment.

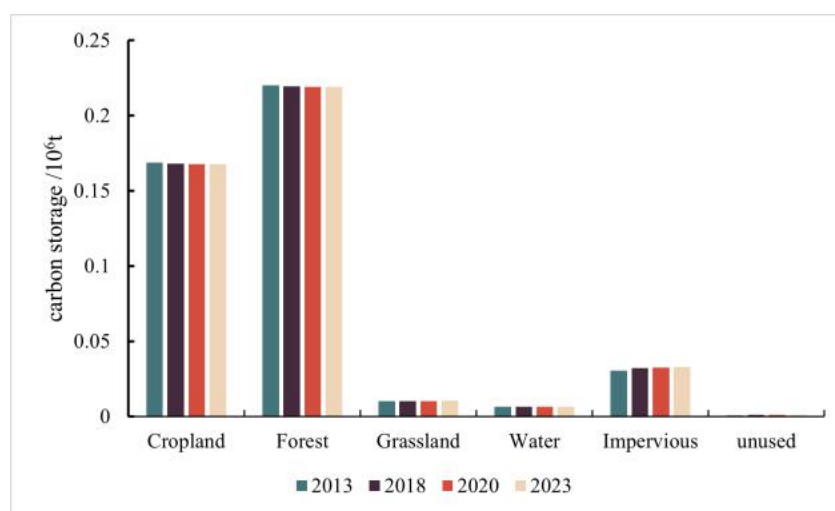


Fig.10 Carbon Sequestration by Land Use Type for Each Year

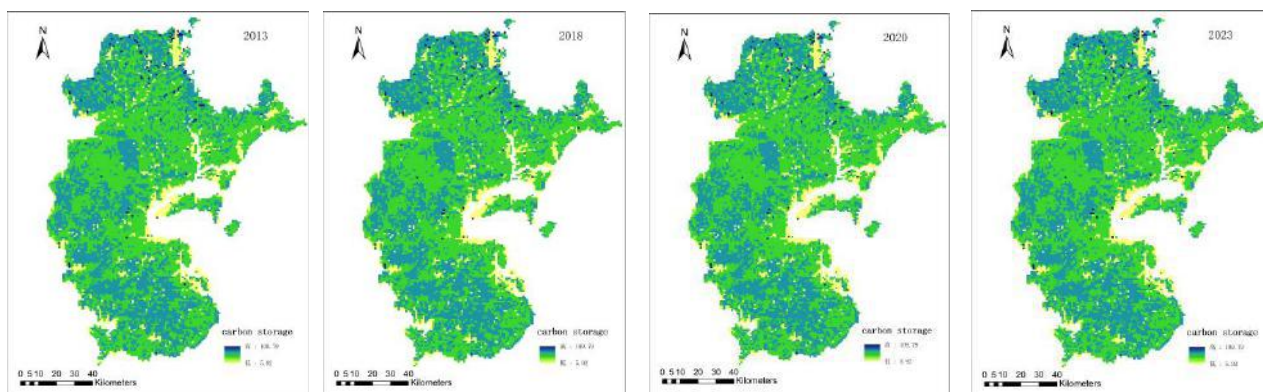
Table 5 Land Use Type Conversion and Corresponding Carbon Stock Changes from 2020 to 2023

Land use type change	Area/km ²	Changes in carbon sequestration/t	Changes in vegetation carbon sequestration/t	Changes in soil carbon sequestration/t
Cropland-Cropland	5624.00	0.00	0.00	0.00
Cropland -Forestland	6.00	104.28	36.72	67.56
Cropland - Grassland	1.00	80.06	80.67	-0.61
Cropland - Water bodies	1.00	-23.81	-17.24	-6.57
Cropland - Construction	6.00	10.74	-32.04	42.78
Forestland - Cropland	7.00	-121.66	-42.84	-78.82
Forestland - Forestland	4640.00	0.00	0.00	0.00
Forestland - Grassland	1.00	62.68	74.55	-11.87
Forestland - Water bodies	1.00	-41.19	-23.36	-17.83
Forestland - Construction	4.00	-62.36	-45.84	-16.52
Water bodies - Forestland	1.00	-62.68	-74.55	11.87
Grassland - Grassland	93.00	0.00	0.00	0.00
Water bodies - Cropland	1.00	23.81	17.24	6.57
Water bodies - Grassland	1.00	103.87	97.91	5.96
Water bodies - Water bodies	1082.00	0.00	0.00	0.00
Water bodies - Construction	3.00	76.80	35.70	41.10

4.5.2 Spatial Variation Characteristics of Carbon Sequestration

In terms of spatial distribution, the carbon sequestration patterns in Zhanjiang City across different periods are relatively consistent, exhibiting an overall "higher in the north and lower in the south, with localized variations" characteristic (Figure 11). By comparing it with the spatial distribution map of land use types in Zhanjiang City (Figure 12), it can be observed that areas with high carbon sequestration per unit area are primarily located in forested and natural ecological regions in the northern and western parts of the city. Conversely, areas with low carbon sequestration per unit area are concentrated in

water bodies and croplands with intensive human activities in the central, eastern, and surrounding regions. The increase in carbon sequestration is mainly attributed to ecological restoration and improved vegetation coverage, showing scattered distribution in localized areas. The decrease in carbon sequestration is largely associated with the expansion of construction land and intensified land development, primarily concentrated in urban core development zones and their peripheries. Overall, while the spatial distribution characteristics of the city's average carbon sequestration show minor localized adjustments over time, the general pattern remains closely linked to ecological changes and human activities.



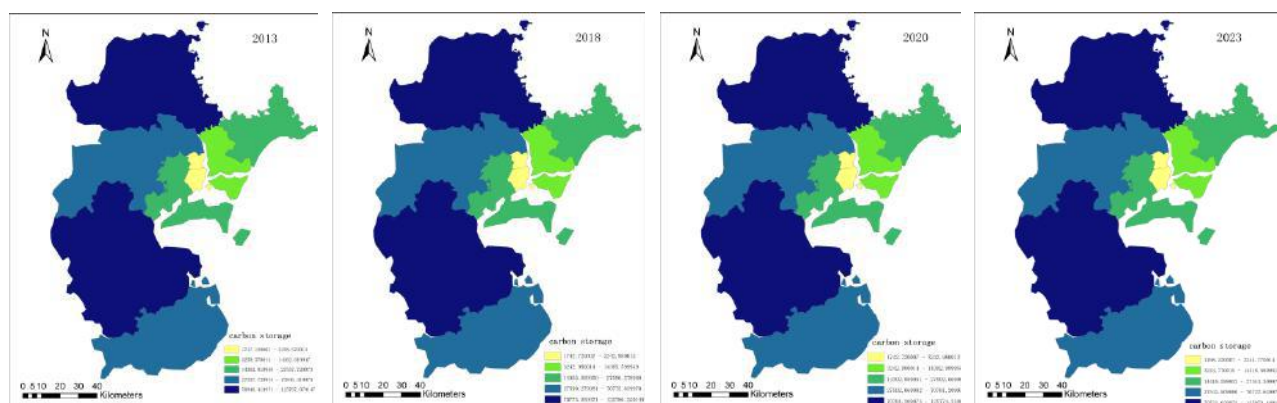


Fig.11 Spatial Distribution of Carbon Sequestration in Zhanjiang

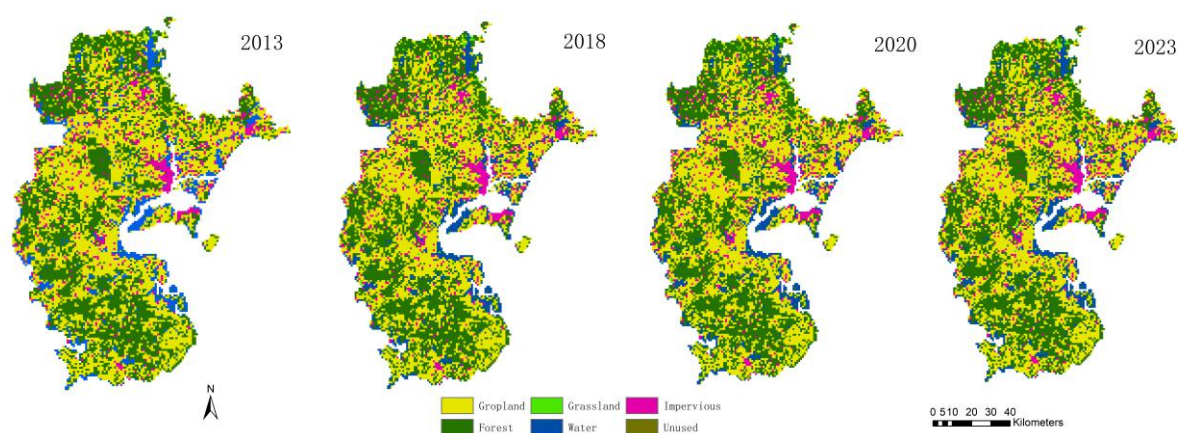


Fig.12 Spatial Distribution of Land Use Types in Zhanjiang City

V. CONCLUSION

(1) The intensity of soil erosion in Zhanjiang City exhibits spatial heterogeneity. Areas with moderate and moderate erosion are primarily distributed in the southern and eastern regions. The overall trend of soil erosion in Zhanjiang City is dominated by slight erosion, accounting for over 98.13% of the total eroded area. From 2013 to 2023, the area of slight erosion showed a trend of first decreasing and then increasing, with an average rate of 0.061 km²/year. The area of mild erosion displayed a similar trend of first decreasing and then increasing, with an average rate of -0.064 km²/year. The areas of moderate, strong, and extremely strong erosion all exhibited a trend of first decreasing and then increasing, with average rates of 0.053 km²/year, 0.033 km²/year, and 0.001 km²/year, respectively.

(2) From 2013 to 2023, the soil erosion intensity in Zhanjiang City generally showed a fluctuating trend.

During the periods of 2013–2017, 2017–2020, and 2020–2023, 96.44%, 88.70%, and 99.16% of the areas, respectively, experienced no change in erosion intensity levels. The dynamic degree of slight erosion was predominantly negative in 2013–2017 and 2020–2023, indicating a gradual reduction in such areas, with more transitioning to mild erosion or higher levels. Meanwhile, the dynamic degrees of mild, moderate, strong, and extremely strong erosion were mainly positive, reflecting an ongoing increase in erosion area and worsening conditions. Notably, during 2020–2023, the dynamic degree of strong erosion peaked at 412.5%. Although most erosion intensities showed negative dynamic degrees from 2017 to 2020, suggesting some mitigation, the dynamic degrees of moderate and strong erosion surged again to 131.8%, 63.40%, and 412.5% in 2020–2023, indicating that the trend of intensifying erosion remains unchecked and urgent governance reinforcement

is needed.

(3) Based on the factor analysis of the geographic detector model, this study selected 11 factors for quantitative analysis with the soil erosion modulus in Zhanjiang City. The single-factor analysis revealed that among natural factors, annual precipitation and land use type are the primary factors influencing soil erosion, with annual precipitation exhibiting the strongest explanatory power. In 2020, the precipitation erosivity factor significantly decreased, corresponding to a notable decline in the soil erosion modulus for the same year, highlighting the dominant role of precipitation. Among human activity factors, GDP demonstrated relatively high explanatory power. The interaction factor analysis results indicated that all interactions between factors exhibited either two-factor enhancement or nonlinear enhancement characteristics. When the factors influencing soil erosion changes interacted, their synergistic driving effects were significantly stronger than those of individual factors. Interactions such as precipitation $\cap$ slope and precipitation $\cap$ land use notably enhanced the explanatory power for the spatial differentiation of the erosion modulus.

(4) The conversion of land use profoundly influences the carbon sequestration "source-sink" process by altering vegetation cover, soil erosion resistance, and erosion environment. The "carbon accumulation" from cropland to forestland and the "carbon loss" from forestland to cropland are essentially the result of the combined effects of soil erosion risk and changes in vegetation ecological functions. This provides a typical scenario for quantifying the "erosion-carbon sequestration" relationship and also offers a basis for optimizing regional carbon sequestration and soil erosion processes through land use regulations.

(5) From 2020 to 2023, as the dominant influencing factor of soil erosion in Zhanjiang City (precipitation factor) intensified, coupled with changes in land use types (conversion of forest land to cropland), the intensity of soil erosion increased. The enhanced erosion stripped away the topsoil rich in organic matter, exposing previously stable carbon to an oxidizing environment and accelerating its decomposition (CO_2 release). Consequently, carbon sequestration declined, the climate regulation function weakened, and the performance of the

ecological service system was affected.

(6) Based on the above analysis, in order to effectively manage and protect the soil resources in this region, this study proposes strengthening the management and monitoring of areas with complex terrain, implementing soil and water conservation measures such as vegetation restoration, gully control, and shelterbelt construction to reduce the risks of soil erosion and water resource loss. Additionally, continuous monitoring of soil erosion conditions and timely adjustment of management strategies are crucial for restoring the ecological balance of the area and achieving sustainable development.

ACKNOWLEDGEMENTS

The author is grateful for the research grants given to Ruei-Yuan Wang from GDUP T Talents Recruitment (No.2019rc098), Peoples R China under Grant No.702-519208, and Academic Affairs in GDUP T for Goal Problem-Oriented Teaching Innovation and Practice Project Grant No.701-234660.

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Exploring Agriculture Students' Insights towards Sustainable Livelihood

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Received: 29 Aug 2025; Received in revised form: 28 Sep 2025; Accepted: 03 Oct 2025; Available online: 11 Oct 2025

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Abstract— This research investigates the socio-demographic and institutional factors affecting students' perceptions toward sustainable livelihood. Within the framework of the Sustainable Livelihood Approach, education becomes vital for developing the human capital needed in agriculture. Purposive sampling was carried out with 202 B.Sc. and M.Sc. agriculture students from a university in Lucknow District, using a quantitative, descriptive survey design. The researcher himself developed the instrument for collecting responses through the "Perception Scale of Agriculture Students Towards Sustainable Livelihood" (PSASSL). To conduct the statistical analysis, the Mann-Whitney U-test was used. Perception differences were significant with respect to gender, locale, parental occupation, and type of institutions, with no perception differences on the basis of socioeconomic status. The perceptions of the students on sustainable livelihood were influenced highly through their own exposure, agricultural tradition, and the standard of the institution's infrastructure. The results provide greater emphasis on the need for a more equitable curriculum process as well as an entry into experiential learning, most especially in government institutions. In this regard, the results are consistent with the thrust of the National Education Policy (NEP) 2020 objectives and promoting integrated skill-based as well as sustainability-oriented education. This research strengthens the argument regarding sustainability in agricultural education, showing how different cohorts of students conceptualize sustainability, thus making a case for recommendations that should be made for educators, agencies, and policymakers wishing to promote sustainable development and livelihood preparation among prospective agricultural professionals.

Keywords— Sustainable Livelihood, Agricultural Education, Student Perceptions, Livelihood Strategies, Agricultural Curriculum, Higher Education.



I. INTRODUCTION

The "Sustainable livelihood" has been defined as the ability of individuals to maintain, preserve and oppose outside tremors in a long time. Its fundamental objective is to enhance life-supporting capacity, resources, and activities without weakening the environmental, social, or economic structures from which human beings derive (Krantz, 2001).

According to the Sustainable Livelihood Framework of 1999 developed by the Department for International Development, the five assets that a livelihood should comprise of would be human, natural, physical, social, and financial capital. Efficient asset management enables an individual or a community to combat stresses from economic crises, environmental degradation, and political disturbances (Scoones, 1998). An important aspect of

livelihood sustainability is human capital-often referred to as education. From this, a person acquires knowledge, self-confidence, and some usable skills. It is, therefore, linked to increased productivity, reduction of risks, and adoption of new techniques (Chambers & Conway, 1992). According to Mavhungu (2023), rural communities that have undergone agricultural instruction have a higher tendency of adopting conservation farming techniques and making improvements in their productivity. Rural populations that have received agricultural education are more likely to embrace conservation farming methods and productivity enhancement.

Another fundamental value of sustainable living is concern for the environment. Although more than 60% of Indians are working in agriculture, most of the industry is still dependent on the monsoon rains (Patel & Sharma, 2021). Integrated pest management, organic farming, and soil conservation are examples of sustainable practices that help to preserve the ecosystem and reduce the need for outside inputs (Singh, 2020).

Inclusive development is also guaranteed by access to training and education. For instance, when agricultural training covers the general range of learning capabilities and is gender-responsive, the marginalized and the women benefit (UNESCO, 2017). Educated women manage the family finance more effectively, improve the well-being of the family, and participate in family community decisions (World Bank, 2012). Agricultural education provides the sustainable livelihood best by bridging academic learning with the practical training for the conservation of the environment, production of crops, testing the soil, and irrigation of waters (FAO, 2017). As per Rivera and Qamar (2003) Comprehension of the local and the scientific systems of knowledge prepares the students best for the real-life agronomical challenges.

Agricultural usability of technology, such as GIS, drones, and sensors, boosts livelihood activities. As Mavhungu (2023) noted, the technology makes resources more efficient and cheaper due to the facilitation of more information-based as well as sustainable agriculture. Owing to this connection, innovation as well as productivity correlates highly with sustainability. The sustainable living must be social as well as adaptable, and it must continually change. Market forces, migration, as well as change induced by the climate, dictate how the approach evolves (Scoones, 1998). Enterprise, innovation, as well as environmental protection plans, ensure the today's livelihood does not risk the tomorrow's livelihood (Morse & McNamara, 2013).

II. REVIEW OF RELATED LITERATURE

This evaluation of global studies provides an observation of the relationship between agricultural education and sustainable lifestyle. By highlighting the students' approaches, institutional responsibilities, course intervals and livelihood approaches, it emphasizes how the stability of education and local development is integrated into different situations. Hussein and Nelson (1998) have investigated on permanent livelihoods and diversification strategies between the African and Asian population in the countryside. The purpose is to find out how institutional structures affect enteritis and livelihood, the most important strategy for livelihood is the most important strategy. Brocklesby and Fisher (2003) present sustainable livelihoods approaches, including the increase and uptake within organizations. These authors argue that community development thinking has been largely absent from such approaches because of the local nature of the former and the technocratic impetus of sustainable livelihoods intervention, conflicting with its principles. The sustainable livelihoods strategy was suggested by Adato and Meinzen-Dick (2002) as the approach by which the effect of agricultural research on poverty may be measured. Their transdisciplinary method addresses how technologies are aligned with various household strategies, including social and cultural considerations in addition to economic ones. Apine et al. (2019) comments on the sustainable livelihoods approach as applied to small-scale mud crab fisheries in Southwest India. This emphasizes how frameworks establish societal characteristics, risk, impressive institutions and appropriate management strategies, given how framework uncertainty and socio-economic factors, in view of uncertainty and socio-economic factors. Serrat, O. (2017) It structures drivers of livelihood opportunity and helps plan and evaluate development action. The article also emphasizes grasping institutions for policy appraisal on a contextual basis. McDowell and de Haan (1997) analyze critically sustainable livelihoods and migration. Migration is then more of a rule than an exception, and according to them, it is usually household livelihood strategy often practiced together with others and two-way processes keeping ties with origins stressing the complicated institutional factors. Allison and Horemans (2006) show how the SLA can be utilized in developing fisheries in West Africa. The SLA thus showed the way to align fisheries policy and poverty reduction objectives, as well as approaches to lower poverty without increasing pressure on already over-exploited fish stocks. Researching how sustainable development is understood in Ghana by agricultural students, Quansah et al. (2024) reported that their subjects were predominantly rural dwellers who would like to practice farming but do not have access to starting funds. The study recommends establishing

government incentives that encourage students to pursue agriculture after graduation. Alam (2023) study was on how universities handle the issue of sustainability as a fallout of globalization and commercialization. It uses qualitative measures such as case studies and interviews across the nations to reveal that there is a policy-practice gap where institutions are more profit-oriented than that actual sustainable development. Schulz et al. (2019) An analysis of 374 students in 21 Latin American countries found a significant relationship between knowledge and approach to permanent agriculture. Most placed a very positive attitude, but their knowledge was more moderate and weaker correlated. Education should be practical and sustainability-oriented. Of students in agriculture, Agumagu et al. (2018) from Nigeria's Rivers State found that 73% would engage in farming for income, but fewer wanted government or private sector employment. Major issues included high equipment costs, limited access to land, and insufficient resources. Government and NGO incentives and interventions were suggested. Sitienei and Morrish (2014) It was found that students at American College registered in agricultural programs did not have a high level of knowledge related to permanent agriculture, which reveals large knowledge intervals in important principles and practice. He suggested changing the course to include extensive content addressing of stability. Fröhlich et al. (2013) described how students at the age of 24 in Germany see agricultural responsibilities differently. While older students attached importance to plants and animals, younger students tended to point out agriculture and processing. In general, agricultural education did not focus much on contemporary practice and the environment. Entrapped in student farms, experiential learning was researched on sustainable US agriculture by Parr and Trexler (2011). Using qualitative focus groups, they were able to conclude that all which involved effective community of practice in complementing classroom teaching with fieldwork did transfer knowledge by actual experience and collective work. Borsari and Vidrine (2005) surveyed the degree of sustainability in the undergraduate curricula of institutions involved in agriculture. The surveys of faculty indicated that courses continued to be traditional with very limited emphasis on sustainability. The study recommended changes in the curriculum to include sustainable agriculture towards effective agricultural education. Agbaje et al. (2001) assessed the impact of sustainable agriculture on secondary agricultural education programs and teachers in the U.S North Central region. Findings indicated a fairly moderate use of sustainable agriculture; terms were mostly neutral on such usage, suggesting space for more incorporated teaching materials. Alonge and Martin (1995) analyzed farmer perceptions on sustainable agriculture

practices in the U.S. Farmers find the practices fit and profitable, with certain negative elements. Such findings recommend research alterations to improve capability and profitability for wider use of sustainable techniques.

RESEARCH QUESTIONS

What factors influence the insights of Agriculture Students towards Sustainable Livelihood (e.g. Gender, Locale, Parental Occupation, Social Category and Type of institutions (Central and State)?

RESEARCH OBJECTIVES

To identify the factors that influence the insights of Agriculture Students towards Sustainable Livelihood (e.g. Gender, Locale, Parental Occupation, Social Category and Type of institutions).

RESEARCH HYPOTHESIS

H₁: Gender significantly influences agriculture students' insights towards sustainable livelihood.

H₂: Locale significantly influences agriculture students' insights towards sustainable livelihood.

H₃: Parental occupation significantly influences agriculture students' insights towards sustainable livelihood.

H₄: Social category significantly influences agriculture students' insights towards sustainable livelihood.

H₅: Type of institution significantly influences agriculture students' insights towards sustainable livelihood.

III. METHODOLOGY

Research Method

The researcher used a quantitative method to explore the insights of agriculture students towards Sustainable Livelihood.

Research Design

The Descriptive Research Design has been used in this study. This design helped in systematically studying the trends and influencing factors such as gender, locale, parental occupation, social category, and type of institutions.

Study of the Population

The study population comprises B.Sc. and M.Sc. Agriculture students' who are enrolled in different universities in Lucknow District.

Sample size

The present study comprised 202 agriculture students (B.Sc. and M.Sc.) from 2 different universities in Lucknow District.

Sampling

The present study used purposive sampling to select agriculture students actively enrolled in accredited B.Sc. and M.Sc. agricultural programs. This method ensured that the participants had direct experience and knowledge relevant to Sustainable Livelihood.

Process of Data Collection

The Perception Scale of Agriculture Students towards Sustainable Livelihood (PSASSL) have been self-constructed by the investigators to gather data on students' insights and attitudes towards Sustainable Livelihood.

IV. ANALYSIS AND DISCUSSION

This shall illuminate the factors affecting agricultural students' perception of sustainable livelihood through exhaustive statistical data analysis and interpretation. Five main factors-Gender, Locale, Parental Occupation, Social Category, and Type of Institutions will direct this study in relation to its research aim and hypotheses. Student perceptions of different institutional and demographic

variables will be tested using the Mann-Whitney U-test. Results will then be discussed in relation to relevant empirical research and theoretical frameworks identified in the literature review. There are some conclusions that arise out of the mixing of both contextual and numerical data where the exact nature and direction of these discrepancies are afforded insight. By means of this methodology, the study would make an effort to connect the theoretical concepts informing sustainable livelihoods with the actual learning experience of agriculture education students. This could, ultimately, amount to a more holistic, practical, and policy-relevant understanding of sustainability in agricultural education.

Analysis 1:

Objective-1: To identify the influence of Gender on insights of Agricultural Students towards Sustainable Livelihood

Ho 1: There is no significant difference in the insights of Agriculture Students towards Sustainable Livelihood with reference to Gender.

Table 1: Ranks

Ranks				
	Gender	N	Mean Rank	Sum of Ranks
Student Sustainable Livelihood	Male	105	109.48	11495.00
	Female	97	92.87	9008.00
	Total	202		

Table 2: Test Statistics

	Student Sustainable Livelihood
Mann-Whitney U	4255.000
Wilcoxon W	9008.000
Z	-2.020
Asymp. Sig. (2-tailed)	0.043

Table 3: Gender-wise Mean Ranks, N and Mann-Whitney U-values of Student Sustainable Livelihood

Variable	Gender	Mean Ranks	N	Mann-Whitney U-values	Remark
Student Sustainable Livelihood	Male	109.48	105	4255.000	P<0.05
	Female	92.87	97		

Table-3 shows that statistically, utilizing the Mann-Whitney U-test, it is possible to determine whether gender influences the agricultural students' perspectives towards sustainable

livelihood by analyzing 105 male and 97 female students. The results indicate that males had a higher mean rank of 109.48 compared to females with 92.87. $U = 4255.000$, $Z =$

-2.020, and $p = 0.043$ (two-tailed). The result is significant since $p < 0.05$; therefore, it can be concluded that male and female students have significantly different views on sustainable livelihood, rejecting the null hypothesis. This reinforces the need to support gender-sensitive program designs with equal opportunities and practical exposure to all students, thus ensuring a balanced understanding of sustainability in agriculture. The findings suggest, possibly due to differential exposure to practical aspects of sustainable livelihood, that male students appear to have more favorable or informed views, possibly owing to differential exposure to field practices, livelihood planning, or sociocultural roles that favor engagement within agriculture.

Discussion

The perceptions of agricultural students regarding sustainable livelihood were found to be statistically significantly different; male students held more positive

opinions ($U = 4255.000$, $p = 0.043$). Such results echo with previous. The present research lends credence to the argument that sociocultural roles and differences in experiences shape sustainability perceptions and aligns with Alam's (2023) call for inclusive and indispensable sustainability education, especially among higher education. Such findings emphasize building gender-sensitive pedagogies and equitable learning atmospheres for offering equal opportunities for male and female students to contemplate theoretical and practical pursuits of sustainable living.

Analysis 2:

Objective-2: To identify the influence of locale on insights of Agriculture Students towards Sustainable Livelihood

Ho 2: There is no significant difference in the insights of Agriculture Students towards Sustainable Livelihood with reference to Locale.

Table 4: Ranks

Ranks				
	Locale	N	Mean Rank	Sum of Ranks
Student Sustainable Livelihood	Rural	116	117.35	13612.50
	Urban	86	80.12	6890.50
	Total	202		

Table 5: Test Statistics

	Student Sustainable Livelihood
Mann-Whitney U	3149.500
Wilcoxon W	6890.500
Z	-4.480
Asymp. Sig. (2-tailed)	<.001

Table 6: Locale-wise Mean Ranks, N and Mann-Whitney U--values of Student Sustainable Livelihood

Variable	Locale	Mean Ranks	N	Mann – Whitney U-values	Remark
Student Sustainable Livelihood	Rural	117.35	116	3149.500	P<0.05
	Urban	80.12	86		

Table-6 shows that The Mann-Whitney U-test assessed whether students' location is influential to the perception of sustainable livelihood. The sample included 86 students from urban areas and 116 students from rural areas. The analysis shows that mean rank of urban students was 80.12 while mean rank of rural students was 117.35. Asymptotic significance (two-tailed) computed at less than 0.001 value of U was 3149.500, Z was -4.480. Since $p < 0.05$, the

outcome is statistically significant. The null hypothesis is therefore rejected, and this confirms students think about sustainable livelihood differently depending on their location. Rural students generally had a more favorable and knowledgeable attitude toward sustainable livelihoods since this attitude is grounded in their practical knowledge of rural lifestyles, family involvement in farming, and direct exposure to agricultural practices. The findings highlight the

importance of experiential and contextual learning but equally highlight the need to narrow the perception gap by implementing targeted programs to enhance urban students' understanding of sustainable rural livelihoods.

Discussion

There is a statistically significant difference in rural students' perceptions of sustainable livelihoods categorized by location ($U = 3149.500$, $Z = -4.480$, $p < 0.001$). McDowell and de Haan (1997) did recognize as important that local experience contributes to the making of livelihood strategies, inexcusably for rural areas; the more familiar rural students would be with agricultural challenges and sustainability practices, then the more favorable perception would be; however, Apine et al. (2019) showed local knowledge and community assets to be essential for planning sustainable livelihoods while urban students would

not be equally exposed, reinforcing the case for contextualized education. Therefore, these findings entail the necessity of an experiential learning approach such as field trips and rural immersion programs to close the perception gap between the two areas, wherein urban students would be able to relate the academic concepts to the practical realities of agriculture, thus contributing to more equitable and balanced educational outcomes.

Analysis 3:

Objective 3-: To identify the influence of Parental Occupation on insights of Agriculture Students towards Sustainable Livelihood.

Ho 3: There is no significant difference in the insights of Agriculture Students towards Sustainable Livelihood with reference to Parental Occupation.

Table 7: Ranks

Ranks				
	Parental Occupation	N	Mean Rank	Sum of Ranks
Student Sustainable Livelihood	Farming	114	109.61	12496.00
	Non-farming	88	90.99	8007.00
	Total	201		

Table 8: Test Statistics

	Student Sustainable Livelihood
Mann-Whitney U	4091.000
Wilcoxon W	8007.000
Z	-2.248
Asymp. Sig. (2-tailed)	0.025

Table 9: Parental Occupation-wise Mean Ranks, N and Mann-Whitney U--values of Student Sustainable Livelihood

Variable	Parental Occupation	Mean Ranks	N	Mann-Whitney U-values	Remark
Student Sustainable Livelihood	Farming	109.61	114	4091.000	P<0.05
	Non-farming	90.99	88		

Table-9 shows that The Mann-Whitney test was used to analyze if the occupation of the parents would bring about differences on agricultural students' perceptions about sustainable livelihoods. The two groups of respondents consisted of 114 students from farmer families and 88 students coming from non-farming households. The mean

ranks for the former group were 109.61, while the latter carried a mean rank of 90.99. The Mann-Whitney U-value obtained was 4091.000, Z-score (-2.248), and a two-tailed significance value of 0.025. Since the p-value less than 0.05, it implies that the difference is significant which means that the perceptions can be significant between both groups or is

rejected to assume with the null hypothesis. Results show that students who were brought up by farmers had a more positive and deeper understanding of sustainable livelihood than their counterparts, which could be attributed to the exposure they received while growing up, as well as experiencing practical agricultural practices and the challenges that accompany them. The results demonstrated that parental occupation strongly influenced the attitude and comprehension of students, thus indicating the need for more work-based learning in agricultural education to sensitize students to non-farming households.

Discussion

There was very significant statistical evidence of difference in students' perception towards sustainable livelihood according to parental occupation ($U = 4091.000$, $Z = -2.248$, $p = 0.025$), with students from farming households having an evident higher mean rank (109.61) compared to those from non-farming homes (mean rank of 90.99). Direct engagement with farming sometimes enhances understanding and perception of sustainability. According

to Adato and Meinzen-Dick (2002), coupled to the fact that sustainability education is important in household strategies, it also needs to be placed within diverse social and cultural contexts. Quansah et al. (2024) discovered that students from rural farming Ghana were better disposed to adopt sustainable farming but encountered financial barriers that prevented them from adopting such farming practices. The inferences from these findings imply experiential gaps among non-farming students, and thereby, introducing hands-on fieldwork learning in agricultural education can help close the gap in understanding sustainability. This will thus make agricultural education more far-reaching and equitable in experience.

Analysis 4:

Objective-4: To identify the influence of Social Categories on insights of Agriculture Students towards Sustainable Livelihood.

Ho 4: There is no significant difference in the insights of agriculture students towards Sustainable Livelihood with reference to Social Categories.

Table 10: Ranks

Ranks				
	Social Category	N	Mean Rank	Sum of Ranks
Student Sustainable Livelihood	Reserved	116	108.26	12558.00
	Unreserved	86	92.38	7945.00
	Total	202		

Table 11: Test Statistics

	Student Sustainable Livelihood
Mann-Whitney U	4204.000
Wilcoxon W	7945.000
Z	-1.910
Asymp. Sig. (2-tailed)	0.056

Table 12: Social Category-wise Mean Ranks, N and Mann-Whitney U--values of Student Sustainable Livelihood

Variable	Social Category	Mean Ranks	N	Mann-Whitney U-values	Remark
Student Sustainable Livelihood	Reserved	108.26	116	4204.000	P>0.05
	Unreserved	92.38	86		

Table-12 shows that there were 86 unreserved students and 116 reserved students in the sample. Mean rank for unreserved students stands at 92.38, while, for reserved

category students, it stands at 108.26. The Mann-Whitney U-value is 4204.000, with a two-tailed p-value of 0.056 and z-equivalent score of -1.910. Given that p is more than 0.05,

the result can therefore not be considered significant. Therefore, we cannot reject the null hypothesis. That social category had a non-significant effect on the perceptions of students on sustainable livelihood. Such results imply that students share approximately similar attitudes and understandings towards sustainable livelihood irrespective of social category. Such similarities may have arisen from the positive results from inclusive practices of effective curricula and equal exposure through agricultural education programs.

Discussion

Results indicate that there is no significant difference statistically between the students' perceptions of sustainable livelihood based on social category ($U = 4204.000$, $Z = -1.910$, $p = 0.056$). The reservation category students do have a slightly higher mean rank (108.26) compared to unreserved students (92.38); however, this difference does not provide enough evidence to reject the null hypothesis. This implies that most of the students regard the sustainable livelihood scenario quite similarly, irrespective of their

social category. The findings may be reflective of a more inclusive approach being adopted in agricultural education programs while providing equal exposure for different sections. Alam (2023) Told that while educational policy emphasizes stability, practice varies. However, institutions making an effort to embed sustainability fairly across populations of students can help bridge social divides. Likewise, Adato and Meinzen-Dick (2002) advocated for social context to be introduced into educational and livelihood strategies. In that light, the data suggest that equal access to sustainability education, curriculum content, and field experiences may be leveling the playing field for students across social categories.

Analysis 5:

Objective-5: To identify the influence of Type of Institutions on insights of Agriculture Students towards Sustainable Livelihood.

Null Hypothesis 5: There is no significant difference in the insights of Agriculture Students towards Sustainable Livelihood with reference to Type of Institutions.

Table 13: Ranks

Ranks				
	Type of Institutions	N	Mean Rank	Sum of Ranks
Student Sustainable Livelihood	Central	69	137.11	460.50
	State	133	83.03	11042.50
	Total	202		

Table 14: Test Statistics

	Student Sustainable Livelihood
Mann-Whitney U	2131.500
Wilcoxon W	11042.500
Z	-6.242
Asymp. Sig. (2-tailed)	<.001

Table 15: Type of Institutions-wise Mean Ranks, N and Mann-Whitney U--values of Student Sustainable Livelihood

Variable	Type of Institutions	Mean Ranks	N	Mann Whitney U-values	Remark
Student Sustainable Livelihood	Central	137.11	69	2131.500	$P < 0.05$
	State	83.03	133		

Table-15 shows that the Mann-Whitney U-test was utilized to identify whether pupils' perceptions of sustainable livelihood differed with regard to the type of institutions. A sample of 69 students from central institutions and 133 from

state institutions was taken. A statistical difference was indicated with the mean ranks for central institution students at 137.11 and state institution students at 83.03. The p-value of the two-tailed test was found to be < 0.001 , $Z = -6.242$, and

$U=2131.500$; thus, considering $p<0.05$, the difference now considered statistically significant with rejection of the null hypothesis, suggesting that the type of institution considerably influences students' conceptualizations of sustainable living. Central institutions appear to endorse their students to acknowledge or be knowledgeable from exposure to academia with access to modern facilities and hands-on learning experiences. Hence state institutions must keep on with the improvements on the human resources and quality of education delivered in order to promote more equitable learning outcomes.

Discussion

The results reveal a statistically significant association between institutions type and students' conception of a sustainable lifestyle ($U=2131.500$, $Z=-6.242$, $p<0.001$). Undoubtedly, the results indicate that mean ranks for Central institution students (137.11) far exceed those of state students (83.03), demonstrating that they are indeed determined by a different kind of institution. Alam (2023) further brought to the fore the contradiction between institutional policy and practice where market-driven paths inhibit real sustainability education. Such could hinder access to the state universities as far as modern pedagogical materials and experiential learning are concerned. Meaning that, based on the above findings, immediate improvement of facilities and quality of teaching will have to be undertaken by the state institutions so as to enhance fairer and effective sustainability education across all types of institutions.

V. FINDING

The research conducted, socio-demographic factors and institutional influences have effects on agricultural students' views on sustainable livelihood. As stated in the findings, one of the significant aspects that shaped perceptions among students was the gender of the students, as the male students were found to show more positive inclination towards it. This may be because they have experienced field work and planning for a livelihood more than their female counterparts. Locale was important as well, where rural students cut across the understanding of sustainable livelihoods better than urban students, indicating that exposure to actual world situations in rural areas enhanced attitudes. Another independent variable was the parental occupation; children from farming backgrounds had a better understanding of sustainability because of their experience in farming operations and related issues. The variable socioeconomic category bore no statistically significant difference, implying that equal exposure to curriculum and policies might fill perception gaps across reserved and unreserved categories. The difference was, however, mostly

explained by the type of institutions: the students from central universities understood principles of sustainable lifestyles much better than their state-university counterparts.

VI. CONCLUSION

Sustainable livelihood was perceived differently by agricultural students on the basis of gender, Locale, parental occupation, and institutional type; socioeconomic classes seem to have very little bearing on these perceptions. Male students from rural and farming backgrounds seemed to develop somewhat enhanced perceptions of sustainable development due to their exposure through large-scale and practically oriented experiences. Central University was evaluated as being superior on the understanding aspect, thereby inferring that within the system there were differences of access or otherwise to learning opportunities and resources. These perceptual gaps could then be filled by providing greater visibility of field-based learning, reforming the curriculum, and improving the facilities among the institutions, especially public ones. This project strives to empower the students with theoretical knowledge and practical skills towards advancing sustainable rural development, environmental sustainability, and livelihoods security by educational institutions under the Sustainable Livelihoods and NEP 2020 framework.

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Half diallel analysis through griffing's approach in sesame (*Sesamum indicum* L.)

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Received: 03 Sep 2025; Received in revised form: 30 Sep 2025; Accepted: 05 Oct 2025; Available online: 12 Oct 2025

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Abstract— Ten sesame (*Sesamum indicum* L.) genotypes were crossed in a half diallel mating fashion to produce 45 hybrids. The analysis of variance for combining ability revealed that mean sum of squares due to GCA and SCA were found highly significant for all the traits. The lower estimates of potence ratio indicated that non-additive gene action was predominant for the inheritance of all the traits except oil content. The estimates of general combining ability effects revealed that the parents, Keriya 10, U-6-3 and ST-12-25 were good general combiners for seed yield per plant. The genotype Keriya 10 was also good general combiner for plant height (cm), number of braches per plant, number of capsules per plant, number of seeds per capsule, biological yield per plant (g) and 1000-seed weight (g). The cross combinations viz., Keriya 10 × BS-6-1, U-6-3 × Khadkala-1 and U-6-3 × EI-233 were found to be good specific cross combination for seed yield per plant and its attributes. Crosses with high sca effect in seed yield per plant were in combinations of good × poor general combiners.



Keywords— Combining ability, Gene action, Griffing's approach, Half-diallel, Sesame

I. INTRODUCTION

Sesame is a very ancient oilseed crop grown next to groundnut, mustard and soybean in India. Sesame (*Sesamum indicum* L.) belongs to the order *Tubiflorae*, family *Pedaliaceae* with chromosome number $2n=26$. It is basically considered a crop of tropical and sub-tropical regions but, it has also spread to the temperate parts of the world. Africa has been considered to be the primary centre of origin of sesame and it spread early through West Asia to India, China and Japan which they became secondary distribution centers (Weiss, 1983). Sesame is called as the "Queen of Oilseeds" because of its excellent qualities of the seed, oil and meal. Sesame oil contains a high unsaturated essential fatty acids content [linoleic acid (37–47%), oleic acid (35–43%)] and low saturated fatty acid content [palmitic acid (8–11%) and stearic acid (5–10%)], the seeds also contain 14.1–29.5% proteins, 4.3–20.5%

carbohydrates, 4.2–6.9% ash and 2.7–6.7% fiber content, along with vitamin E, minerals, lignans (sesamolin and sesamin), and tocopherols. In addition, sesame seed mineral composition includes K (349–851 mg/100g), P (50–890 mg/100g), Mg (305–79 mg/100g), Ca (80–1263 mg/100g) and Na (123 mg/100g) (Couch *et al.*, 2017). In India, sesame is cultivated in an area of 19.01 lakh ha with a production of 8.10 lakh tones annually and productivity of 426 kg/ha (Anon., 2022). In Gujarat, sesame is cultivated in an area of 2.46 lakh ha with a production of 1.16 lakh tones and productivity of 471 kg/ha (Anon., 2022). The choice of parents to be incorporated in hybridization programme is a crucial step for plant breeders, particularly if the aim is improvement of complex quantitative characters, such as yield and its components. The use of parents of known superior genetic worth ensures much better success. It requires extensive

and detailed genetic assessment of existing germplasm as well as newly developed promising lines, which could be used in future breeding programme or could be directly released as a cultivar after thorough testing. Combining ability analysis gives useful information regarding the selection of parents in terms of the performance of their hybrids. On the basis of this information, decisions on the selection of the parents can be taken most effectively. Estimates of general combining ability (gca) and specific combining ability (sca) effects are widely used in planning of breeding programs.

II. MATERIALS AND METHODS

The experimental material comprised of 10 parents, one standard check (G.Til 7) and 45 F₁'s derived by crossing 10 different genotypes of sesame in a half-diallel fashion at Main Oilseeds Research Station, Junagadh Agricultural University, Junagadh, during summer-2023. A total 56 genotypes, including 10 parents, 45 F₁'s and one standard check (G.Til 7) were grown in a randomized complete block design with three replications during *kharij*-2023 at Instructional Farm, Department of Agronomy, College of Agriculture, Junagadh Agricultural University, Junagadh. Each entry was accommodated in a single row plot of 3 meter length with row to row and plant to plant distances of 45 cm and 10 cm, respectively. All the recommended cultural practices and plant protection measures were followed uniformly to grow healthy crop. The observations were recorded both as plot basis (days to 50% flowering, days to maturity, 1000-seed weight (g) and oil content (%)) and measurement on five randomly selected plants (plant height (cm), number of branches per plant, number of capsules per plant, length of capsule (cm), number of seeds per capsule, seed yield per

plant (g), biological yield per plant (g) and harvest index (%)). The replication wise mean values of each entry for the twelve traits were analyzed using randomized block design (RBD) as suggested by Panse and Sukhatme (1995). The combining ability analysis was carried out according to Model-I (Fixed effect), Method-2 (Parents and one set of F₁'s without reciprocals) of Griffing (1956). The replicated mean data were analyzed statistically using the software INDOSTAT version 8.1.

III. RESULTS AND DISCUSSION

The analysis of variance for combining ability for different characters has been presented in Table 1. The results revealed that mean squares due to general combining ability (GCA) and specific combining ability (SCA) were highly significant for all the characters. This indicated that both additive and non-additive type of gene effects imparting a vital role in the inheritance of all these traits. The results, in general, are in accordance with the findings of Reddy *et al.* (2015), Rajput *et al.* (2017), Ram *et al.* (2018), Jeeva *et al.* (2020), Kumar *et al.* (2021), Sikarwar *et al.* (2021), Rathod *et al.* (2022) and Gadhiya *et al.* (2023). The magnitude of GCA and SCA variances revealed that the SCA variances were higher than their respective GCA variances for all the characters except oil content where GCA variance is equal to SCA. The $\sigma^2_{GCA}/\sigma^2_{SCA}$ ratio less than unity confirmed the preponderance of non-additive gene action for all the traits except oil content where $\sigma^2_{GCA}/\sigma^2_{SCA}$ ratio equal to unity confirmed the preponderance of both additive and non-additive gene action. The predominance of non-additive gene action for seed yield and its component traits were also reported by Reddy *et al.* (2015), Ram *et al.* (2018), Kumar *et al.* (2021), Sapara *et al.* (2022) and Gadhiya *et al.* (2023).

Table 1: Analysis of variance (mean squares) for combining ability of different characters in sesame

Source of variation	Df	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of branches per plant	Number of capsules per plant	Length of capsule (cm)
GCA	9	4.99**	29.95**	271.69**	1.25**	109.86**	0.10**
SCA	45	1.67**	14.45**	102.38**	0.37**	96.31**	0.03**
Error	108	0.46	1.23	17.36	0.01	10.82	0.01
σ^2_{GCA}		0.28	1.29	14.11	0.07	1.13	0.01
σ^2_{SCA}		1.23	13.22	85.02	0.36	85.49	0.02
$\sigma^2_{GCA}/\sigma^2_{SCA}$		0.22	0.10	0.16	0.21	0.01	0.33
Source of variation	Df	Number of seeds per capsule	Seed yield per plant (g)	Biological yield per plant (g)	1000-seed weight (g)	Harvest index (%)	Oil content (%)

GCA	9	22.78**	6.91**	213.91**	0.10**	5.81**	2.87**
SCA	45	20.76**	3.69**	130.54**	0.05**	2.47**	0.35**
Error	108	3.54	0.36	12.00	0.01	1.27	0.14
σ^2_{GCA}		0.17	0.27	6.95	0.004	0.28	0.21
σ^2_{SCA}		17.22	3.34	118.54	0.04	1.20	0.21
$\sigma^2_{GCA/}$		0.01	0.08	0.06	0.11	0.23	1.01
σ^2_{SCA}							

*,** Significant at 5% and 1% levels, respectively

The general combining ability effects of the parents (Table 2) revealed that none of the parents was found to be good general combiner for all the characters. An overall appraisal of gca effect revealed that parents, Keriya 10, U-6-3 and ST-12-25 was good general combiners for seed yield per plant. The parent Keriya 10 was also found good gca effect for plant height, number of branches per plant, number of capsules per plant, number of seeds per capsule, biological yield per plant and 1000-seed weight. The parent G. Til 6 had good gca effect for days to 50% flowering, days to maturity, length of capsule

and harvest index. For oil content B-14-1, ST-12-25 and EI-233 were found good general combiner. It is suggested that population involving these parents in a multiple crossing programme may be developed for isolating desirable recombinants. Further, the varieties or lines showing good general combining ability for particular component may also be utilized in component breeding programme for effective improvement in particular components, ultimately seeking improvement in seed yield itself.

Table 2: Estimates of general combining ability (gca) effects of parents for different characters in sesame

Parents	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of branches per plant	Number of capsules per plant	Length of capsule (cm)
Keriya 10	0.16	2.03**	4.89**	0.38**	4.18**	0.01
KMR 102	-0.68**	1.64**	6.98**	0.34**	-0.38	0.04
BS-6-1	-0.23	1.17**	-4.53**	-0.13**	-3.35**	0.04
U-6-3	0.93**	0.95**	-0.34	0.46**	4.49**	-0.08**
B-14-1	-0.09	-0.74*	3.02**	0.03	-0.51	-0.04
ST-12-25	0.91**	-0.58	3.30**	0.09**	3.72**	0.005
EI-233	-0.32	-2.58**	-5.28**	-0.44**	-2.47**	-0.14**
Khadkala-1	-0.18	-1.41**	-7.81**	-0.08*	-3.06**	-0.10**
AT 482	0.54**	1.12**	0.77	-0.21**	-1.74	0.10**
G.Til 6	-1.04**	-1.61**	-0.99	-0.44**	-0.88	0.16**
SE(gi)	0.18	0.30	1.14	0.03	0.90	0.02
SE(gi-gj)	0.28	0.43	1.70	0.04	1.34	0.03

Parents	Number of seeds per capsule	Seed yield per plant (g)	Biological yield per plant (g)	1000-seed weight (g)	Harvest index (%)	Oil content (%)
Keriya 10	2.11**	1.21**	5.72**	0.09**	0.25	-1.08**
KMR 102	0.52	0.07	-0.93	0.04*	0.46	0.15
BS-6-1	1.09*	-0.58**	-3.35**	-0.09**	0.08	-0.61**
U-6-3	1.67**	0.89**	4.24**	-0.001	0.18	0.04

B-14-1	-1.69**	-0.43*	-1.41	-0.11**	-0.48	0.50**
ST-12-25	0.48	0.76**	5.50**	-0.05*	-0.56	0.44**
EI-233	-1.72**	-0.56**	-1.80	0.08**	-0.48	0.36**
Khadkala-1	-1.27*	-1.21**	-6.37**	-0.12**	-0.10	0.04
AT 482	-0.69	0.02	2.40*	0.15**	-0.90**	0.14
G.Til 6	-0.49	-0.16	-3.99**	-0.01	1.56**	-0.01
SE(gi)	0.52	0.16	0.95	0.02	0.31	0.10
SE(gi-gi)	0.77	0.24	1.41	0.03	0.46	0.15

*,** Significant at 5% and 1% levels, respectively

For days to 50% flowering out of 45 hybrids significant sca effects in the desirable (negative) direction were exhibited by five hybrids. The range of sca effects varied from -2.67 (Keriya 10 × KMR 102) to 2.52 (KMR 102 × G.Til 6 (Table 3). The hybrid Keriya 10 × KMR 102 (-2.67) depicted highest significant sca effects in desirable direction followed by B-14-1 × AT 482 (-2.31), Keriya 10 × ST-12-25 (-2.26), BS-6-1 × G.Til 6 (-1.92) and KMR 102 × BS-6-1 (-1.62) indicating that they may be promising hybrids for exploiting earliness in flowering.

The ranged of sca effects for days to maturity in hybrids varied from -6.77 (BS-6-1 × AT 482) to 6.18 (B-14-1 × ST-12-25) (Table 3). Out of 45 crosses, six crosses exhibited significant and negative sca effects for early maturity. The highest significant and negative sca effect was observed in cross BS-6-1 × AT 482 (-6.77) followed by EI-233 × AT 482 (-3.68), Khadkala-1 × AT 482 (-3.18), BS-6-1 × EI-233 (-3.07), ST-12-25 × G.Til 6 (-2.96) and ST-12-25 × Khadkala-1 (-2.82) indicating that they may be promising hybrids for exploiting earliness.

The magnitude of sca effects in hybrids varied from -12.79 (EI-233 × Khadkala-1) to 17.96 (BS-6-1 × U-6-3) for plant height (Table 3). Out of 45 hybrids, 12 hybrids exhibited significant and desirable (positive) sca effects for this trait. The highest significant and negative sca effect was observed in cross BS-6-1 × U-6-3 (17.96) followed by Keriya 10 × G.Til 6 (17.29) and B-14-1 × AT 482 (16.56), seven hybrids showed significant and negative sca effects, thus they were poor combinations.

The results of sca effect revealed that 11 hybrids recorded significant positive sca effects for number of capsules per plant. The significant positive sca effects ranged from -11.47 (B-14-1 × EI-233) to 29.28 (Keriya 10 × BS-6-1) (Table 3). The crosses, Keriya 10 × BS-6-1 (29.28) followed by U-6-3 × Khadkala-1 (22.56) and U-6-3 × EI-233 (18.87) had maximum sca effects for number of capsules per plant. These superior crosses involved one

good and one poor combiner parents. Eleven crosses exhibited sca effects in negative direction.

Out of 45 crosses, eight crosses exhibited significant and positive sca effects which was ranged from -0.26 (Keriya 10 × BS-6-1) to 0.28 (Khadkala-1 × AT 482) for length of capsule (Table 3). The highest significant and positive sca effect was reported in cross Khadkala-1 × AT 482 (0.28) followed by B-14-1 × G.Til 6 (0.27), U-6-3 × G.Til 6 (0.26), Keriya 10 × AT 482 (0.21) and EI-233 × G.Til 6 (0.20). Six crosses exert significant and negative sca effects for this trait, thus they were poor combinations.

The spectrum of variation for sca effects in hybrids ranged from -8.27 (Keriya 10 × BS-6-1) to 9.79 (B-14-1 × G.Til 6) for number of seeds per capsule (Table 4). Out of 45 crosses, 10 hybrids exhibited significant and positive sca effects, therefore, they were considered as good specific combinations for number of seeds per capsule. Some of the good specific combinations were B-14-1 × G.Til 6 (9.79) followed by KMR 102 × B-14-1 (8.14), KMR 102 × EI-233 (7.20), Khadkala-1 × AT 482 (6.96) and BS-6-1 × U-6-3 (4.70). The seven crosses noted as poor specific cross combinations as they noted significant and negative sca effects. Some of the poor specific combinations were Keriya 10 × BS-6-1 (-8.27), B-14-1 × Khadkala-1 (-7.60) and BS-6-1 × B-14-1 (-5.20).

Significant sca effects for seed yield per plant was exhibited by 20 hybrids, of which, 12 hybrids possessed positive sca effects, hence they were good specific combinations for higher seed yield per plant (Table 4). Eight crosses showing negative sca effects were registered as poor specific combinations. The hybrids U-6-3 × Khadkala-1 (4.20) followed by Keriya 10 × BS-6-1 (4.17), U-6-3 × EI-233 (3.16) and B-14-1 × AT 482 (3.07) for seed yield per plant. The range of sca effect for seed yield per plant varied from 4.20 (U-6-3 × Khadkala-1) to -2.08 (B-14-1 × EI-233).

The magnitude of sca effects ranged from -14.47 (Keriya 10 × U-6-3) to 24.33 (U-6-3 × Khadkala-1) for

biological yield per plant (Table 4). The results revealed that 18 hybrids recorded significant positive sca effects in desirable direction. The highest significant and positive sca effect was observed in cross U-6-3 × Khadkala-1 (24.33) followed by U-6-3 × EI-233 (20.54), Keriya 10 × BS-6-1 (18.09), ST-12-25 × EI-233 (15.93) and BS-6-1 ×

EI-233 (15.27). Eleven crosses had found significant and negative sca effects for this trait. Some of the poor specific combinations for biological yield per plant were Keriya 10 × U-6-3 (-14.47), EI-233 × Khadkala-1 (-12.46) and EI-233 × AT 482 (-12.31).

Table 3: Estimates of specific combining ability effects of hybrids for days to 50% flowering, days to maturity, plant height, number of branches per plant in sesame

Sr. No.	Crosses	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of branches per plant	Number of capsules per plant	Length of capsule (cm)
1.	Keriya 10 × KMR 102	-2.67**	2.51*	-10.79**	0.69**	9.67**	-0.05
2.	Keriya 10 × BS-6-1	-0.12	2.65*	1.86	0.75**	29.28**	-0.26**
3.	Keriya 10 × U-6-3	0.05	0.54	-9.03*	0.05	-1.19	-0.03
4.	Keriya 10 × B-14-1	0.41	1.23	-3.06	0.90**	4.84	-0.09
5.	Keriya 10 × ST-12-25	-2.26**	-0.60	2.19	0.02	-4.75	-0.17*
6.	Keriya 10 × EI-233	-0.37	1.07	0.41	-0.51**	-9.36**	-0.10
7.	Keriya 10 × Khadkala-1	2.49**	2.57*	0.57	0.59**	1.83	0.003
8.	Keriya 10 × AT 482	1.11	2.37*	12.42**	-0.23*	-0.69	0.21**
9.	Keriya 10 × G.Til 6	0.36	3.43**	17.29**	-0.86**	-8.85**	-0.15*
10.	KMR 102 × BS-6-1	-1.62*	-0.30	-2.44	-0.36**	-3.03	0.05
11.	KMR 102 × U-6-3	0.22	-0.07	1.41	0.74**	-7.33*	-0.10
12.	KMR 102 × B-14-1	-0.42	-0.05	6.35	0.39**	2.90	0.16*
13.	KMR 102 × ST-12-25	-0.42	0.79	-6.37	0.29**	-2.13	0.14
14.	KMR 102 × EI-233	-0.53	-1.55	2.88	0.66**	2.73	0.14
15.	KMR 102 × Khadkala-1	0.33	1.29	10.17**	0.28**	-0.78	-0.11
16.	KMR 102 × AT 482	0.94	0.09	-5.48	0.26*	-5.67	-0.07
17.	KMR 102 × G.Til 6	2.52**	5.48**	8.76*	0.19	5.50	-0.05
18.	BS-6-1 × U-6-3	-0.23	1.40	17.96**	-0.37**	3.97	0.01
19.	BS-6-1 × B-14-1	1.80**	0.43	-3.87	0.10	-7.76*	-0.10
20.	BS-6-1 × ST-12-25	1.13	5.26**	8.81*	0.93**	-0.59	-0.003
21.	BS-6-1 × EI-233	0.69	-3.07**	14.26**	-0.21*	8.94**	-0.002
22.	BS-6-1 × Khadkala-1	0.22	6.09**	-8.45*	-0.11	-4.57	0.08
23.	BS-6-1 × AT 482	-1.17	-6.77**	-9.23*	-0.49**	-7.10*	-0.18*
24.	BS-6-1 × G.Til 6	-1.92**	3.29**	-10.53**	-0.13	-2.16	-0.08
25.	U-6-3 × B-14-1	0.97	-0.35	0.64	-0.40**	-7.30*	0.12

Table 3: contd.....

Sr. No.	Crosses	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of branches per plant	Number of capsules per plant	Length of capsule (cm)
26.	U-6-3 × ST-12-25	-0.03	2.82**	4.96	0.01	7.24*	0.14
27.	U-6-3 × EI-233	1.19	1.48	4.94	0.24*	18.87**	0.14
28.	U-6-3 × Khadkala-1	0.72	-1.68	6.90	-0.09	22.56**	-0.02
29.	U-6-3 × AT 482	1.33*	4.12**	11.11**	0.91**	15.20**	-0.09
30.	U-6-3 × G.Til 6	-0.09	4.51**	5.19	0.83**	-7.09*	0.26**
31.	B-14-1 × ST-12-25	0.66	6.18**	-0.97	0.21*	3.14	0.04
32.	B-14-1 × EI-233	-1.12	0.84	10.12**	-0.43**	-11.47**	-0.11
33.	B-14-1 × Khadkala-1	0.08	2.01	-5.43	-0.01	10.22**	-0.25**
34.	B-14-1 × AT 482	-2.31**	-0.52	16.56**	0.29**	8.66**	0.16*
35.	B-14-1 × G.Til 6	-0.06	-1.13	8.16*	1.12**	0.67	0.27**
36.	ST-12-25 × EI-233	-0.12	3.01**	16.30**	-0.16	10.97**	0.16*
37.	ST-12-25 × Khadkala-1	0.08	-2.82**	5.52	0.09	-8.44**	0.01
38.	ST-12-25 × AT 482	1.02	2.32*	10.61**	-0.77**	-4.16	-0.10
39.	ST-12-25 × G.Til 6	1.94**	-2.96**	-3.56	0.30**	-5.62	0.02
40.	EI-233 × Khadkala-1	0.30	3.84**	-12.79**	-0.57**	-9.65**	-0.02
41.	EI-233 × AT 482	-0.76	-3.68**	0.99	0.48**	-6.37*	-0.14
42.	EI-233 × G.Til 6	1.16	0.70	2.06	0.09	1.17	0.20**
43.	Khadkala-1 × AT 482	-0.23	-3.18**	3.18	0.21*	2.02	0.28**
44.	Khadkala-1 × G.Til 6	-0.98	-1.46	-4.28	-0.36**	-1.14	0.09
45.	AT 482 × G.Til 6	1.30*	2.01	2.87	-0.25*	6.84*	-0.21**
SE (S _{ij})		0.62	1.02	3.84	0.10	3.03	0.07
SE (S _{ij} – S _{ik})		0.91	1.50	5.64	0.15	4.45	0.11
SE (S _{ij} – S _{kl})		0.87	1.43	5.38	0.14	2.43	0.11
Range		-2.67 to	-6.77 to	-12.79 to	-0.86 to	-11.47 to	-0.26 to
		2.52	6.18	17.96	1.12	29.28	0.28
Number of significant and desirable crosses		5	6	12	20	11	8

*,** Significant at 5% and 1% levels, respectively

The estimates of sca effects in hybrids ranged from -0.77 (B-14-1 × G.Til 6) to 0.34 (KMR 102 × ST-12-25) (Table 4). Among 45 crosses, 14 crosses were

identified as good specific combinations by exhibiting significant and positive sca effects for 1000-seed weight. The most superior cross combinations with respect to sca

effect were KMR 102 $\times$ ST-12-25 (0.34), KMR 102 $\times$ G.Til 6 (0.31) and AT 482 $\times$ G.Til 6 (0.26). The superiority of these three crosses was confirmed by their highly significant positive sca effects. While seven hybrids were poor specific combinations as they possessed significant and negative sca effects.

Only one hybrids Keriya 10 $\times$ U-6-3 exhibited significant positive sca effects for harvest index (Table 4). The magnitude of sca effects among hybrids varied -3.19 (BS-6-1 $\times$ B-14-1) to 4.04 (Keriya 10 $\times$ U-6-3). Three hybrids BS-6-1 $\times$ B-14-1 (-3.19), U-6-3 $\times$ G.Til 6 (-2.87) and KMR 102 $\times$ G.Til 6 (-2.50) showed significant and negative sca effects, thus they were poor combinations.

The range of sca effects for oil content among hybrids varied from -1.81 (Keriya 10 $\times$ G.Til 6) to 1.04 (Keriya 10 $\times$ Khadkala-1) (Table 4). The results revealed that three hybrids recorded significant positive sca effects in desirable direction. The highest significant and positive sca effect was observed in cross Keriya 10 $\times$ Khadkala-1 (1.04) followed by KMR 102 $\times$ U-6-3 (1.01) and ST-12-25

$\times$ EI-233 (0.85). Five crosses had found significant and negative sca effects for this trait. Some of the poor specific combinations for oil content were Keriya 10 $\times$ G.Til 6 (-1.81), KMR 102 $\times$ Khadkala-1 (-1.23) and Keriya 10 $\times$ AT 482 (-1.14).

IV. CONCLUSION

From this study, it was concluded that both additive and non-additive gene actions were observed in expression of the traits studied. Among the parents Keriya 10, U-6-3 and ST-12-25 was found to be good general combiner for seed yield per plant. The best combiners Keriya 10, G.Til 6 and ST-12-25 could be utilized in future breeding programmes. The crosses Keriya 10 $\times$ BS-6-1, U-6-3 $\times$ Khadkala-1 and U-6-3 $\times$ EI-233 showed highly significant sca effect with high *per se* for seed yield. Since heterosis breeding is not feasible in sesame on commercial scale at present, above three crosses could be exploited to isolate transgressive segregants in segregating generation to develop high yielding pureline in sesame.

Table 4: Estimates of specific combining ability effects of hybrids for biological yield per plant, 1000-seed weight, harvest index and oil content in sesame

Sr. No.	Crosses	Number of seeds per capsule	Seed yield per plant (g)	Biological yield per plant (g)	1000-seed weight (g)	Harvest index (%)	Oil content (%)
1.	Keriya 10 $\times$ KMR 102	-3.76*	0.78	10.07**	0.22**	-1.94	0.47
2.	Keriya 10 $\times$ BS-6-1	-8.27**	4.17**	18.09**	0.07	0.96	0.53
3.	Keriya 10 $\times$ U-6-3	-4.04*	-0.74	-14.47**	-0.21**	4.04**	-0.16
4.	Keriya 10 $\times$ B-14-1	1.32	1.06	6.43*	0.07	-0.27	-0.23
5.	Keriya 10 $\times$ ST-12-25	-2.32	-1.92**	-10.67**	0.04	0.15	0.05
6.	Keriya 10 $\times$ EI-233	-2.65	-0.96	-2.59	0.09	-0.22	0.45
7.	Keriya 10 $\times$ Khadkala-1	3.16	0.98	2.78	0.15*	0.88	1.04**
8.	Keriya 10 $\times$ AT 482	-2.21	-0.60	-0.11	-0.09	-1.09	-1.14**
9.	Keriya 10 $\times$ G.Til 6	3.32	-0.27	-2.06	0.13*	-0.34	-1.81**
10.	KMR 102 $\times$ BS-6-1	1.45	-0.34	-5.65	-0.28**	1.94	0.67
11.	KMR 102 $\times$ U-6-3	-0.39	-0.59	-7.26*	0.01	1.62	1.01**
12.	KMR 102 $\times$ B-14-1	8.14**	1.64**	6.45*	0.13*	0.79	0.31
13.	KMR 102 $\times$ ST-12-25	-1.00	-0.03	-4.15	0.34**	1.32	0.39
14.	KMR 102 $\times$ EI-233	7.20**	0.06	-1.08	-0.51**	0.48	-0.54
15.	KMR 102 $\times$ Khadkala-1	0.15	-0.96	-6.62*	-0.41**	0.93	-1.23**
16.	KMR 102 $\times$ AT 482	-0.22	-0.87	-6.49*	0.03	0.63	-1.02**

17.	KMR 102 × G.Til 6	-2.56	1.56**	13.61**	0.31**	-2.50*	0.33
18.	BS-6-1 × U-6-3	4.70**	2.16**	13.67**	0.06	-0.80	-0.06
19.	BS-6-1 × B-14-1	-5.20**	-1.42*	0.03	0.16*	-3.19**	-0.12
20.	BS-6-1 × ST-12-25	0.43	-0.01	-0.35	0.01	0.24	0.04
21.	BS-6-1 × EI-233	1.79	2.64**	15.27**	0.10	-0.55	-0.73*
22.	BS-6-1 × Khadkala-1	1.08	-0.75	-5.50	0.08	0.77	-0.26
23.	BS-6-1 × AT 482	0.53	-1.90**	-11.95**	-0.06	0.72	0.08
24.	BS-6-1 × G.Til 6	-0.27	-0.73	-3.71	-0.01	-0.17	0.02
25.	U-6-3 × B-14-1	2.16	-0.36	-5.07	0.04	1.24	0.05

Table 4: contd.....

Sr. No.	Crosses	Number of seeds per capsule	Seed yield per plant (g)	Biological yield per plant (g)	1000-seed weight (g)	Harvest index (%)	Oil content (%)
26.	U-6-3 × ST-12-25	-1.62	0.99	7.59*	-0.08	-0.85	-0.17
27.	U-6-3 × EI-233	4.45*	3.16**	20.54**	0.19**	-1.29	0.50
28.	U-6-3 × Khadkala-1	3.53*	4.20**	24.33**	0.13*	-0.73	0.22
29.	U-6-3 × AT 482	-1.24	2.19**	13.15**	0.21**	-0.36	-0.05
30.	U-6-3 × G.Til 6	3.75*	-1.13*	-0.17	-0.05	-2.87**	0.13
31.	B-14-1 × ST-12-25	3.82*	1.54**	7.47*	0.06	0.32	-0.10
32.	B-14-1 × EI-233	-1.99	-2.08**	-11.34**	0.11	-0.09	-0.27
33.	B-14-1 × Khadkala-1	-7.60**	0.64	6.73*	0.08	-1.34	0.23
34.	B-14-1 × AT 482	4.06*	3.07**	15.03**	0.10	0.73	0.34
35.	B-14-1 × G.Til 6	9.79**	-0.87	-3.94	-0.77**	-0.52	0.44
36.	ST-12-25 × EI-233	2.04	2.61**	15.93**	0.14*	-0.44	0.85*
37.	ST-12-25 × Khadkala-1	2.53	-1.88**	-11.14**	-0.17*	0.19	0.32
38.	ST-12-25 × AT 482	1.28	-0.70	-8.66**	-0.16*	1.67	-0.10
39.	ST-12-25 × G.Til 6	-0.92	-0.93	-3.88	0.12	-0.95	-0.22
40.	EI-233 × Khadkala-1	-4.01*	-2.07**	-12.46**	0.04	0.44	-0.35
41.	EI-233 × AT 482	-0.55	-1.39*	-12.31**	0.09	1.83	0.27
42.	EI-233 × G.Til 6	2.71	1.08	8.63**	0.14*	-1.66	0.25
43.	Khadkala-1 × AT 482	6.96**	1.07	10.40**	-0.04	-1.62	-0.21
44.	Khadkala-1 × G.Til 6	2.26	0.90	0.70	0.13*	1.78	-0.03
45.	AT 482 × G.Til 6	-4.18*	1.81**	11.88**	0.26**	-1.38	0.63
SE (S _{ij})		1.73	0.55	3.19	0.06	1.04	0.34
SE (S _{ij} – S _{ik})		2.55	0.80	4.69	0.09	1.53	0.50
SE (S _{ij} – S _{kl})		5.38	0.76	4.47	0.09	1.46	0.48
Range		-8.27 to 9.79	-2.08 to 4.20	-14.47 to 24.33	-0.77 to 0.34	-3.19 to 4.04	-1.81 to 1.04
Number of significant and desirable crosses		10	12	18	14	1	3

*,** Significant at 5% and 1% levels, respectively

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The Impact of *Aethina tumida* Infestation on *Apis mellifera* Colonies: A Review of Control Measures and Future Prospects

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Received: 02 Sep 2025; Received in revised form: 02 Oct 2025; Accepted: 05 Oct 2025; Available online: 13 Oct 2025

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Abstract— The small hive beetle (*Aethina tumida* Murray) is a significant invasive pest affecting *Apis mellifera* colonies worldwide, leading to severe consequences for beekeeping and agricultural pollination. This review examines *A. tumida* biology, its economic impact, and current control measures. While chemical pesticides such as Coumaphos and Permethrin have been widely used, concerns over toxicity, environmental contamination, and resistance development highlight the need for sustainable alternatives. Emerging strategies provide eco-friendly solutions, including biological control agents (*Beauveria bassiana*, *Steinernema* spp.), botanical pesticides (*Jatropha curcas*, neem oil), and mechanical trapping. Integrated Pest Management (IPM) combines multiple approaches, is increasingly seen as an effective strategy for long-term *A. tumida* management. However, research gaps persist in optimizing field applicability, standardizing botanical pesticide formulations, and assessing economic feasibility. This review underscores the need for regulatory frameworks, beekeeper adoption of sustainable practices, and large-scale studies to validate these emerging approaches. It also explores prospects, including biotechnological innovations, genetic engineering, artificial intelligence-driven monitoring, and regulatory improvements, to ensure sustainable beekeeping practices.



Keywords— *Aethina tumida*, *Apis mellifera*, , biological control, botanical pesticides, Integrated Pest Management

I. INTRODUCTION

1.1 Importance of Honeybees in Agriculture

Honeybees (*Apis mellifera*) play an essential role in global agriculture, primarily as pollinators supporting biodiversity and food security [1]. According to Hristov *et al.* (2020) and Malav *et al.* (2022), approximately 75% of the world's flowering plants and 35% of crops depend on animal pollination, with honeybees being among the most efficient pollinators. The ability of honeybees to forage over long distances, adapt to diverse environments, and maintain large colony sizes makes them indispensable to both natural ecosystems and managed agricultural systems [2,3,4].

The economic value of honeybee pollination services is estimated between \$235 billion and \$577 billion annually, demonstrating their critical role in sustaining food production [5,6,7]. Crops such as almonds, apples, blueberries, cucumbers, and coffee are highly dependent on honeybee pollination for improved yields and quality [8,9,10].

Beyond pollination, honeybees also support the global beekeeping industry, generating billions of dollars in revenue through honey, beeswax, propolis, and royal jelly production [11,12,13]. The global honey market is valued at over \$8 billion, with China, the United States, Turkey, and Argentina being the leading producers [14,15,16]. Beeswax

is widely used in cosmetics, pharmaceuticals, and candle-making, while propolis and royal jelly have gained attention for their antimicrobial and immune-boosting properties [17]. Beekeeping also serves as a primary livelihood for millions of people, particularly in rural areas, where honey production supports economic stability [18,19].

Despite their importance, honeybee populations are in decline due to multiple stressors that threaten both wild and managed colonies [20,21]. Habitat destruction caused by urbanization and deforestation has reduced floral resources, leading to nutritional deficiencies and weakened immune responses [22,23,24,25]. Climate change further exacerbates these challenges by altering flowering periods, disrupting foraging behavior, and increasing the frequency of extreme weather events [26,27,28]. Pesticide exposure, particularly from neonicotinoids, has been linked to navigation impairment, reduced foraging efficiency, and decreased reproductive success, contributing to large-scale colony losses [29,30,31]. Additionally, emerging pathogens and parasites, such as *Varroa destructor*, *Nosema* spp., and viral infections, have increased honeybee mortality rates globally [32,33,34]. Among these threats, the small hive beetle [35,36].

II. OVERVIEW

2.1 Overview of *Aethina tumida* Lifecycle, Behavior, and Economic Impact

The small hive beetle [37]. The lifecycle begins when female beetles lay clusters of eggs inside beehives, typically within cracks or near pollen and brood cells [38]. The eggs hatch within 2-4 days, giving rise to larvae that immediately begin feeding on hive resources, including honey, pollen, and bee brood [38,39]. The larval stage lasts approximately 10-14 days, during which larvae grow and create extensive tunneling damage in honeycombs, causing honey to ferment and become unpalatable [40]. Once mature, the larvae exit the hive and burrow into the soil surrounding the hive to pupate, undergoing metamorphosis over a period of 3-6 weeks before emerging as adults [41]. Adult beetles then seek out new hives to invade, perpetuating the infestation cycle [42].

Behaviorally, *A. tumida* exhibits highly opportunistic behavior, often targeting weakened or stressed bee colonies for invasion [43]. Adult beetles possess a keen ability to detect and locate beehives from considerable distances by responding to olfactory signals emitted by bee pheromones and volatile compounds produced within the hive [44]. Once inside, they employ adaptive survival strategies, including mimicry, which enables them to avoid detection and aggression from worker bees [45]. Additionally, these beetles demonstrate exceptional mobility and resilience,

allowing them to persist without food for extended periods, particularly in cooler environments where their metabolic rates slow down [46]. This adaptability contributes to their success as persistent hive pests, making them a significant challenge for beekeepers [43].

A. tumida is highly opportunistic, exploiting weak or stressed colonies. Adult beetles have the ability to detect and invade hives from significant distances, using olfactory cues from bee pheromones and hive volatiles [38,47]. Within the hive, they exhibit defensive strategies such as mimicry, allowing them to evade worker bee aggression. [48] Furthermore, beetles are highly mobile and resilient, making them capable of surviving for extended periods without food, particularly in cooler environments [43].

The economic impact of *A. tumida* infestations on beekeeping operations is severe [49]. The destruction of honeycombs results in significant losses in honey production, while the contamination of honey with beetle larvae excrement renders it unsellable [50]. Infected colonies may abscond due to stress, leading to further economic damage for beekeepers [51,52]. Additionally, the need for pest control measures, including chemical treatments, hive replacements, and increased labor for monitoring and management contributes to rising production costs [53]. In regions where *A. tumida* infestations have become widespread, such as North America and Australia, regulatory agencies have imposed quarantine measures, further affecting trade and hive movement [47]. These cumulative losses underscore the urgent need for sustainable and effective control strategies.

2.2 Current Control Strategies for *Aethina tumida*

The management of *Aethina tumida* infestations requires an integrated approach that incorporates chemical, biological, mechanical, and cultural strategies [36]. While synthetic pesticides have traditionally been the most commonly used control method, their long-term viability is increasingly being questioned due to pesticide resistance, environmental contamination, and harmful effects on honeybees. As a result, researchers and beekeepers are exploring sustainable alternatives such as biological control agents, botanical insecticides, and mechanical trapping techniques [54]. This section examines the strengths and limitations of these control strategies.

The effective management of *Aethina tumida* infestations requires a multifaceted approach that integrates chemical, biological, mechanical, and cultural control strategies [36]. This is because *A. tumida* is a highly adaptable pest capable of rapid reproduction and dispersal, making single-method control strategies insufficient [55,56]. The spread of *A. tumida* from its native sub-Saharan Africa to regions such as the United States, Australia, and Europe has necessitated

regional adaptations in management techniques, as environmental conditions and honeybee behaviors differ globally [57,58].

While synthetic pesticides have historically been the primary method of control, growing concerns over pesticide resistance, environmental toxicity, and adverse effects on honeybee health have led researchers and beekeepers to seek more sustainable alternatives [59,60]. Emerging control strategies, such as biological control agents [61,62]

2.2.1 Chemical Control Methods

Synthetic pesticides have historically been the primary method for controlling *Aethina tumida*. [63,36]. These chemicals primarily target adult beetles inside the hive or larvae pupating in soil [64]. One of the most commonly used insecticides is Coumaphos, a potent organophosphate sold under the trade name CheckMite+™ [65]. This compound is applied as strips inside beehives to eliminate adult beetles. However, studies have shown that Coumaphos residues can accumulate in wax and honey, posing potential risks to honeybee health and human consumers [66,67,68]. Another widely used insecticide is Permethrin, a synthetic pyrethroid that is typically applied to soil near infested hives to kill *Aethina tumida* larvae before they pupate. Although Permethrin has proven effective, its long-term application can harm non-target soil organisms and contribute to pesticide resistance among *A. tumida* populations [69,70,71].

Several newer insecticides, including Fipronil and Imidacloprid, have demonstrated high efficacy against *A. tumida*. However, these chemicals have been banned in multiple countries due to their detrimental effects on pollinators and environmental persistence [72,73]. The overuse of synthetic pesticides has led to growing concerns about honey contamination, colony stress, environmental degradation, and resistance development, prompting researchers to explore more sustainable solutions [74,75,76,77].

2.2.2 Biological Control Strategies

Biological control offers an alternative to chemical pesticides by using natural enemies to suppress *Aethina tumida* populations [49,78]. Entomopathogenic fungi have emerged as one of the most effective biological agents [79,80,81]. Species such as *Beauveria bassiana* and *Metarhizium anisopliae* are capable of infecting *A. tumida* adults and larvae by penetrating their exoskeleton and disrupting their physiological functions [82,83,36]. Laboratory studies have demonstrated that *B. bassiana* can reduce *A. tumida* populations by over 70%, while *M. anisopliae* is particularly effective when applied to soil around hives to prevent larval pupation [84,85]. However, the efficacy of these fungi is highly dependent on

temperature and humidity, which can limit their field applicability, particularly in hot or dry climates [86,87,88].

In addition to fungal pathogens, entomopathogenic nematodes have been explored as a potential biological control strategy [89,90,91]. *Steinernema* and *Heterorhabditis* species are known to actively seek out and infect *A. tumida* pupae in soil, significantly reducing adult emergence rates [85]. Field trials have reported up to 80% suppression of SHB populations when nematodes were applied to hive surroundings [92,47]. However, nematodes are highly sensitive to environmental conditions, requiring adequate moisture levels for survival. This sensitivity presents challenges for their large-scale deployment in regions with dry or variable climates [93,94].

Several studies have also investigated the role of predatory insects in *A. tumida* suppression [95,84,96]. Certain ant species, including *Pheidole megacephala* and *Solenopsis invicta*, have been observed preying on *A. tumida* larvae in natural settings [97,98]. While these predators can contribute to population control, introducing them intentionally into apiaries raises concerns regarding ecological disruption and unintended consequences for other beneficial insect species [99,100,101,102].

2.2.3 Botanical Pesticides

Botanical pesticides are being increasingly explored as an alternative to chemical insecticides due to their biodegradability, low toxicity to honeybees, and reduced risk of environmental contamination [103,104,105]. One of the most promising plant-based insecticides is *Jatropha curcas* oil, which contains phorbol esters that interfere with insect development and physiological functions [106,107]. Experimental trials have shown that *Jatropha curcas* oil is highly effective in repelling and killing *A. tumida* larvae, making it a potential candidate for sustainable SHB control [108].

Neem oil [109,110]. Neem-based treatments act as feeding deterrents and growth inhibitors, preventing larvae from reaching maturity [59]. Essential oils derived from plants such as thyme, clove, and peppermint have been tested for their repellent activity against *A. tumida*, though their efficacy in field conditions remains inconsistent [111]. While botanical pesticides hold significant potential, further research is needed to optimize their formulations and standardize application techniques for large-scale use [112,113].

2.2.4 Mechanical and Physical Controls

Mechanical and physical control strategies offer non-toxic methods for reducing *A. tumida* populations [114,84]. One of the most widely used mechanical control methods is the bottom board trap, which is placed at hive entrances to

physically capture beetles before they gain access to the colony [53,115]. Other approaches include beetle barns, which contain pesticide-laced bait to lure and eliminate beetles, and oil traps, which suffocate adult beetles that enter the traps [116]. While mechanical interventions are effective in reducing beetle numbers, they require regular monitoring and maintenance [117]. These strategies are most successful when integrated with biological or chemical methods as part of a broader pest management program [118,119].

III. FUTURE PROSPECTS IN *AETHINA TUMIDA* MANAGEMENT

The future of *A. tumida* management lies in technological advancements, innovative biological strategies, and strengthened policy frameworks [75,120]. Selective breeding programs are being developed to produce *A. mellifera* strains with enhanced hygienic behavior, making them more capable of detecting and removing beetles [121]. Genetic engineering, particularly CRISPR-based modifications, presents an opportunity to enhance resistance traits in honeybee populations, reducing susceptibility to *A. tumida* infestations [122].

Biotechnological innovations such as RNA interference [123,96]. Additionally, microbial symbiont manipulation is being explored to alter the gut microbiota of *A. tumida*, disrupting digestion and survival [124]. These methods offer environmentally friendly and sustainable pest control strategies.

Artificial intelligence [125]. Smart hives incorporating automated pest management mechanisms, such as robotic trapping and real-time environmental adjustments, could significantly improve pest control efficiency while minimizing human intervention. [126]

Integrated Pest Management [127]. Standardizing best practices through large-scale field trials will improve the economic feasibility and practical adoption of these approaches among beekeepers worldwide [128].

Policy and regulatory frameworks will also play a crucial role in sustainable *A. tumida* management. Implementing safer pesticide alternatives, monitoring residue levels in honey products, and enhancing international cooperation on biosecurity measures will be necessary to prevent further spread [129]. Collaborative efforts between researchers, beekeepers, and regulatory agencies will be key to ensuring sustainable and effective control measures.

IV. DISCUSSION

While chemical insecticides remain the dominant method for *Aethina tumida* control, their drawbacks—including pesticide resistance, honey contamination, and non-target toxicity—have driven the search for alternative solutions. Biological control strategies, such as fungal pathogens and nematodes, have shown significant potential but require further optimization to overcome environmental limitations. Botanical pesticides, particularly *Jatropha curcas* and neem oil, provide a natural and eco-friendly approach but still lack standardized field application protocols. Mechanical trapping methods, while effective in reducing beetle populations, require frequent monitoring and integration with other control measures for long-term efficacy. Given these challenges, Integrated Pest Management (IPM) is increasingly viewed as the most sustainable approach, as it combines multiple control strategies to achieve effective, long-term suppression of *A. tumida* populations.

V. CONCLUSION

The threat posed by the small hive beetle on honey bee colonies cannot be over emphasized. Although chemical insecticides remain the most widely used control method, their associated risks highlight the need for more sustainable alternatives. Biological control agents, including fungi and nematodes, offer promising eco-friendly solutions but require further research to optimize their field effectiveness. Botanical pesticides, particularly *Jatropha curcas* and neem oil, present a natural alternative, though issues regarding dosage optimization and application consistency remain. Mechanical control methods provide immediate population reduction, but their success is enhanced when used in combination with other strategies.

Future research should focus on improving biological control formulations, field trials for botanical pesticides, and regulatory frameworks to support sustainable beekeeping practices. By adopting Integrated Pest Management (IPM) approaches and increasing beekeeper education, *Aethina tumida* infestations can be effectively mitigated while ensuring honeybee health, agricultural productivity, and biodiversity conservation.

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Impact of Soil Salinity on Growth and Yield of Different Millet Crops

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Received: 30 Jun 2025; Received in revised form: 01 Aug 2025; Accepted: 06 Sep 2025; Available online: 13 Oct 2025

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Abstract— Millets are a diverse group of small-seeded cereal crops that have been cultivated for thousands of years. Millets are rich in essential nutrients such as dietary fiber, proteins, vitamins and minerals. With the rising challenges of climate change and food security, millets are being promoted globally as nutri-cereals for their adaptability, nutritional value, and role in sustainable agriculture. Excessive accumulation of soluble salts in the root zone adversely affects plant growth and yield. Crops grown under saline conditions often exhibit reduced germination, stunted growth and poor yield. Millets can also tolerate salinity to some extent but are sensitive to high levels of soil salinity. High salt levels can reduce the grain yield. The experiment was laid out in factorial RBD consists of three main treatments (pearl millet, finger millet and foxtail millet) and five sub treatments (2,3,4,5 dS m⁻¹ and control). The salinity of respective pots was developed with addition of salt water prepared by additions of NaCl, NaSO₄ and CaSO₄ in 2:2:1 ratio. Data on yield attribute (panicle/ear head length) and grain yield were recorded the results revealed that panicle length decreased with increasing salinity. Pearl millet recorded the highest panicle length (21 cm), followed by foxtail millet (18 cm), while finger millet exhibited the lowest mean panicle length (9 cm). Among the salinity levels, the highest panicle length (19 cm) was observed under control while the lowest panicle length (13 cm) occurred at the highest salinity level 5 dS m⁻¹. A significant reduction in grain yield with increasing salinity. Among the millets, finger millet recorded the highest average grain yield (288 g pot⁻¹) across salinity levels, followed by pearl millet with 213 g pot⁻¹, and foxtail millet with the lowest at 194 g pot⁻¹. Among the salinity levels, the highest yield was observed under control with 257 g pot⁻¹, while the lowest yield occurred at the highest salinity level 5 dS m⁻¹ with of 200 g pot⁻¹. Grain yield decreased progressively with increasing salinity across all three millets, indicating the negative impact of salt stress on reproductive performance.



Keywords— Millets, nutrients, salinity, panicle length, grain and straw yield.

I. INTRODUCTION

The changes foreseen under climate change scenarios are the changes in the pattern of rainfall, rather than the quantum, leading to long spells of drought and spells of water-logging of the soils as well as salinity. Salinity is the main abiotic factor influencing crop development, productivity and germination (Sairam *et al.*, 2002). Salinity is the process of accumulation of soluble salts, it affects plant productivity in arid and semi-arid regions of the world where evapo-transpiration is high and

the rain is not enough for leaching the salt out of the root zone (Al-Hilal, 1999).

Millets have been cultivated for thousands of years and because of their substantial advantages in terms of nutrition and easy adaptable to environment, they are currently receiving more attention. Pearl millet (*Pennisetum glaucum* L) is well acclaimed for its great tolerance to various forms of adversity, especially its capacity to endure high concentrations of salinity and one of the most important economic crops to face climate change and maintain food security. It has an advantage due to its

promising dual purpose (grain and fodder), short duration and quick growing nature. Foxtail millet (*Setaria italica* L.), a member of the Paniceae family is extensively grown for grain and forage throughout Asia, Europe, North America, Australia and North Africa. It's an environment-friendly crop with high drought-stress resistance, short life cycle, dual grain and forage use, wide adaptability, and low water consumption, making it the prospective model crop for investigating the effects of salinity plants.

Finger millet (*Eleusine coracana*) is a staple crop grown in different agronomical regions of the world. It's renowned for its exceptional nutritional profile, containing minerals such as calcium and phosphorus, essential amino acids like methionine, tryptophan and cysteine, dietary fibers and high-quality protein, anti-diabetic, antioxidant and antimicrobial effects. Despite its resilience to multiple stresses, salinity poses major impacts on finger millet growth and development by reducing water content, leaf expansion, plant height, grain weight and delaying flowering. Keeping this in view, the present study was taken up to know the effect of variable salinity levels on yield attributes and yield of millet crops.

II. MATERIAL AND METHODS

The experiment was conducted during Kharif 2024 at Agricultural Research Station, Perumallapalle, Tirupati, Acharya N.G. Ranga Agricultural University, which is geographically situated at 13° 36' 761" N latitude and 79° 20' 704" E longitude with an altitude of 182.9 m above the mean sea level, which falls under Southern Agro Climatic Zone of Andhra Pradesh. Three Millets (pearl millet, finger millet and foxtail millet) using five salinity levels (EC of 2, 3, 4, 5 dS m⁻¹ and control) by following factorial randomized block design with 3 replications. Cement pots of 1m radius × 1 m height microplot were filled with soil. The soil salinity of different levels were developed by addition of salt water (prepared with dissolving of NaCl, Na₂SO₄ and CaSO₄ in the ratio of 2:2:1). The recommended dose of fertilizer for pearl millet (80:40:30 kg NPK ha⁻¹), finger millet (60:30:20 kg NPK ha⁻¹), foxtail millet (40:20:20 kg NPK ha⁻¹) was applied through inorganic fertilizers (urea, single super phosphate and muriate of potash, respectively). Nitrogen fertilizer was applied in two splits (half at basal and half at 30 days after sowing). Entire dose of phosphorus and potassium were applied as basal at the time of sowing. The data on length of ear head or panicle was measured and their average length was reported as per ear head or panicle basis. Total number of tillers plant⁻¹ was counted from each pot and average was reported as number of tillers plant⁻¹. The air dried ear heads from each pot were threshed, cleaned and weight of the grain was recorded on

the basis of grain yield per pot. The weight of straw from each pot was recorded after complete sun drying until a constant weight.

III. RESULTS AND DISCUSSION

Number of Tillers plant⁻¹

The data demonstrated on yield attributes such as number of tillers plant⁻¹ of different millet crops under varying salinity levels is presented in Table 1. The results indicated that these attributes were significantly influenced by millets, salinity levels and their interaction. Among the millets, finger millet (M₂) recorded the highest number of tillers (5), followed by pearl millet (M₁) of (4), while the lowest (3) was observed in foxtail millet (M₃). Across salinity treatments, the highest number of tillers (5) was recorded in at control. The lowest number of tillers per plant (3) was observed at highest salinity of 5 dS m⁻¹(S₄). The interaction between millets and salinity was found to be significantly highest. The maximum number of tillers per plant (6) was found in the treatment combination M₂S₅, M₂S₁ and M₁S₃. The lowest number of tillers (4) was recorded by the combination M₃S₄.

The superior tillering ability of finger millet may be attributed to its better physiological adaptability and genetic potential for shoot proliferation. The number of tiller per plant decreases with increasing of salinity due to osmotic stress and ion toxicity caused by excess sodium ions in saline condition, which hinders cell division and tiller emergence. These findings align with those of Kumar *et al.* (2018), who reported similar reductions in tillering under salinity. Accumulation of Na⁺ and Cl⁻ ions in shoot tissues causes ion imbalance and toxicity. This affects meristematic activity, especially in basal nodes where tillers initiate. (Parida *et al.* 2005).

Panicle length or ear head length

The data displayed on panicle length of different millet crops as influenced by salinity levels is presented in Table 1. A significant effect of millets, salinity levels and their interaction was observed. In the group of millets, pearl millet (M₁) recorded the highest panicle length of 21 cm, which was at par with foxtail millet (M₃) (18 cm), while finger millet (M₂) registered the lowest panicle length of 9 cm.

With respect to salinity levels, the control (S₅) reported the maximum panicle length (19 cm). The panicle lengths under S₁ (17 cm) and S₂ (17 cm) regarded the same mean values. The shortest panicle length under high salinity S₄ (13 cm).

The interaction between two factors showed significant effect on panicle length. highest. The highest

panicle length (26 cm) was recorded by the treatment combination M₁S₅. The treatment M₁S₂ (23 cm) which was at par with M₁S₃ (21 cm) and M₁S₄ (20 cm). The lowest ear head length (5 cm) was recorded by the combination M₂S₄.

The higher panicle length in pearl millet is a result of its inherent genetic characteristics, efficient resource use, and moderate salinity tolerance, which allow it to maintain

reproductive development even under stress. salinity stress reduces panicle development by impairing reproductive growth. Salinity interferes with flowering and grain formation, leading to reduced ear head elongation. High Na⁺ and Cl⁻ concentrations cause ion toxicity, damaging meristematic tissues responsible for panicle development or earhead development (Parida *et al.* 2005).

Table 1. Effect of salinity levels on yield attributes by different millet crops

Millets	Number of tillers plant ⁻¹						Panicle length (or) ear head length (cm)					
	Salinity levels						Salinity levels					
	S ₁ (2dS m ⁻¹)	S ₂ (3dS m ⁻¹)	S ₃ (4dS m ⁻¹)	S ₄ (5dS m ⁻¹)	S ₅ (control)	Mean	S ₁ (2 dS m ⁻¹)	S ₂ (3dS m ⁻¹)	S ₃ (4dS m ⁻¹)	S ₄ (5dS m ⁻¹)	S ₅ (control)	Mean
M ₁ (Pearl millet)	5	5	4	4	5	4	23	21	20	17	26	21
M ₂ (Finger millet)	6	5	6	5	6	5	10	10	6	5	13	9
M ₃ (Foftail millet)	4	4	4	3	4	3	20	19	18	16	18	18
Mean	5	4	4	3	5		17	17	15	13	19	
	SEm±			CD (P=0.05)			SEm±			CD (P=0.05)		
Millets(M)	0.053			0.156			0.42			1.22		
Salinity levels(S)	0.069			0.201			0.54			1.58		
M×S	0.120			0.348			0.94			2.74		

Grain Yield

The data pertaining to grain yield (g pot⁻¹) was found to be significantly influenced by salinity levels, different millets and also their interaction which was illustrated in the Table 2. Among the three millets, finger millet (M₂) produced the significantly the highest grain yield of 269 g pot⁻¹ trailed by pearl millet (M₁)(211 g pot⁻¹) whereas, the declined grain yield of 195 g pot⁻¹ was recorded by foxtail millet (M₃). The highest grain yield of 251 g pot⁻¹ was recorded at control which was significant over the remaining salinity levels. The lowest grain yield of 200 g pot⁻¹ was observed at the superior salinity level of 5 dS m⁻¹(S₄). The percent reduction in grain yield from the lowest to the highest salinity level was 74 per cent.

The interaction between the varieties and salinity levels indicated that, the maximum grain yield of 292 g pot⁻¹ was noticed in the combination of M₂S₅, which was on par with M₂S₁(285 g pot⁻¹) whereas, the minimum grain yield (176 g pot⁻¹) was obtained in combination of M₃S₄.

Among the millets, finger millet (M₂) records the highest grain yield across salinity levels, indicating its superior adaptability. Its combination of physiological resilience, efficient resource use and stress-tolerant traits makes it the best performer under saline conditions. Finger millet has been genetically reported to have higher yield stability across environments, including under stress conditions like salinity.

The reduction in yield per pot might be due to shrinkage of cell contents, specific ion toxicity, restriction of photosynthesis, ion exclusion and growth limitations originating from nutritional imbalances. Similar findings were reported by Ali *et al.* (2014). Grain yield was greatly influenced by soil salinity. The significant and gradual reduction in grain yield with progressive increase in soil salinity could mainly due to cumulative effect of decrease in plant height, less weight per ear head due its size reduction and less number of filled grains per ear head.

Table 2. Effect of salinity levels on grain and straw yield (g pot⁻¹) by different millet crops

Millets	Grain Yield (g pot ⁻¹)						Straw yield (g pot ⁻¹)					
	Salinity levels						Salinity levels					
	S ₁ (2dS m ⁻¹)	S ₂ (3dS m ⁻¹)	S ₃ (4dSm ⁻¹)	S ₄ (5dS m ⁻¹)	S ₅ (control)	Mean	S ₁ (2dS m ⁻¹)	S ₂ (3dS m ⁻¹)	S ₃ (4dSm ⁻¹)	S ₄ (5dS m ⁻¹)	S ₅ (control)	Mean
M ₁ (Pearl millet)	221	213	204	183	244	211	533	491	446	379	578	485
M ₂ (Finger millet)	285	273	255	242	292	269	612	578	542	416	673	564
M ₃ (Foxtail millet)	205	191	185	175	216	195	391	341	302	286	436	352
Mean	233	226	215	200	251		512	470	430	360	562	
	SEm±			CD (P=0.05)			SEm±			CD (P=0.05)		
Millets (M)	2.09			4.82			3.48			10.14		
Salinity levels (S)	2.70			6.21			4.50			13.10		
M×S	4.69			10.8			7.79			22.69		

Straw Yield

The data on straw yield (g pot⁻¹) of different millet crops under various salinity levels is presented in Table 2. Data pertaining to straw yield (g pot⁻¹) was significantly influenced by salinity levels, different millets and also by their interaction. Within the three millets, finger millet (M₂) produced the highest straw yield (564 g pot⁻¹) succeeded by pearl millet (M₁) (485 g pot⁻¹) whereas the lowest grain yield of 352 g pot⁻¹ was observed by foxtail millet (M₃). The highest straw yield of 562 g pot⁻¹ was noticed at control followed by 512 g pot⁻¹ S₁(2 dS m⁻¹). The lowest straw yield 360 g pot⁻¹ was observed at the superior salinity level of 5 dS m⁻¹(S₄).

The interaction between varieties and salinity levels indicated that the maximum straw yield of (673 g pot⁻¹) was attained in the combination of M₂S₅ followed by M₂S₁(612 g pot⁻¹) whereas, the minimum straw yield (286 g pot⁻¹) was obtained in the combination of M₃S₄.

The superior performance of finger millet may be attributed to its vigorous vegetative growth, greater dry matter accumulation, and relatively higher salt tolerance, allowing it to sustain biomass production even under moderate saline conditions. Salinity was one of the major environmental factors limiting plant growth and yield (Parida and Das, 2005) reported that salinity of soil decreases number of grain per panicle and harvest index. The reduction in yield per pot might be due to excess of soluble salts in the root zone negatively affects plant growth

and yield through osmotic effects, nutritional imbalances, and specific ion toxicities (Tahir et al.2006).

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Translational Modification and pH Optimization of Expression Media for High-Yield Recombinant Human-like Collagen (RHC) Production in *Pichia pastoris* GS115

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Received: 05 Sep 2025; Received in revised form: 03 Oct 2025; Accepted: 07 Oct 2025; Available online: 15 Oct 2025

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Abstract— Collagen plays a vital role in maintaining tissue structure and promoting repair, making it a key component of the extracellular matrix. Due to the limitations associated with extracting collagen from animal sources, interest in recombinant human-like collagen (RHC) has grown substantially. This study presents an approach to enhance RHC production in *Pichia pastoris* GS115 by engineering a novel plasmid (pPIC9K-OSTI/RHC) and refining fermentation parameters. Among the tested conditions, a pH of 6 was found to be optimal, resulting in an RHC high yield of 0.35 g/L. The use of the OSTI α -signal peptide notably improved secretion efficiency, contributing to increased protein output. This work demonstrates a scalable and cost-effective strategy for producing recombinant human-like collagen, laying the groundwork for future applications in tissue engineering, wound healing, and biomedical research.

Keywords— Recombinant Human-Like Collagen (RHC), *Pichia pastoris*, OSTI signal peptide, pH optimization, Protein expression.



I. INTRODUCTION

Collagen is a fundamental fibrous protein that serves as a primary structural element within the extracellular matrix of connective tissues such as skin, bone, tendons, and cartilage. As the most abundant protein in the human body, it plays an essential role in preserving tissue architecture, facilitating wound repair, and influencing cellular behaviors, including adhesion, migration, and proliferation^[1, 2]. Its stability and biological function are largely attributed to its distinctive triple-helical conformation, which is formed by a repeating amino acid motif (Gly-X-Y)_n—where glycine is consistently present, and the X and Y positions are commonly occupied by proline and hydroxyproline^[3, 4]. This structural arrangement enables collagen to withstand mechanical forces and provide tensile strength to connective tissues^[3, 5].

Collagen's exceptional mechanical strength and inherent biocompatibility make it a highly sought-after material for a wide range of biomedical applications, including tissue

engineering, wound repair, and drug delivery systems. As the predominant structural protein within the extracellular matrix, collagen is instrumental in facilitating tissue regeneration and healing, particularly within the scope of regenerative medicine^[6]. Biomaterials derived from collagen have demonstrated considerable potential not only in the restoration of skin, bone, and cartilage but also in accelerating healing processes by promoting cellular adhesion and proliferation^[7].

Conventional methods of extracting collagen from animal-derived tissues present several limitations, including elevated production costs, intricate processing steps, and ethical concerns related to animal welfare and cultural or religious restrictions^[8, 9]. The reliance on animal sources introduces moral challenges, especially in communities where the use of specific animal products is prohibited by religious or cultural beliefs^[10, 11]. Although collagen can also be synthesized through the cultivation of animal cells, this approach is often hindered by low efficiency,

stemming from the high nutritional requirements of the cultures and the relatively modest yields achieved^[8, 12].

Microbial platforms, especially *P. pastoris*, have gained attention as effective systems for producing recombinant collagen, owing to their ability to generate substantial amounts of heterologous proteins. These organisms are non-pathogenic and thrive under relatively simple cultivation conditions, making them attractive for industrial applications^[13-15]. *P. pastoris* uniquely combines features of both prokaryotic and eukaryotic cells, supporting rapid proliferation and high-cell-density fermentation—key advantages for scalable protein manufacturing^[15].

Fine-tuning the conditions for protein expression is essential to achieving high yields in *P. pastoris*. Utilizing specialized media such as Buffered Minimal Methanol Yeast (BMMY) has been shown to significantly boost protein production, as it supplies essential nutrients while supporting effective methanol induction—critical since methanol serves as a key carbon source for this yeast^[16, 17]. Moreover, optimizing fermentation variables like temperature, pH, and nutrient availability can further enhance the synthesis of recombinant proteins in *P. pastoris* systems^[17, 18].

This research aims to enhance the production of recombinant human-like collagen (RHC) in *P. pastoris* by optimizing the BMMY expression medium and employing a newly designed plasmid construct (pPIC9K-OSTI/RHC). The primary goal is to determine the most effective fermentation conditions, particularly the optimal pH, for maximizing protein yield. Through systematic refinement of these parameters, the study seeks to improve both the efficiency and scalability of RHC production, presenting a more sustainable and cost-efficient alternative to conventional collagen extraction methods.

II. MATERIALS AND METHODS

2.1 Reagents, Strains, Plasmids, and Culture Media

Primers, STAR GXL DNA polymerase, and restriction enzymes were obtained from Takara Bio (Dalian, China). Geneticin (G418 sulfate), used as a selective antibiotic, was purchased from Sangon Biotech (Shanghai, China), while other reagents were sourced from Sinopharm Chemical Reagent (Shanghai, China). The *P. pastoris* GS115 strain was maintained in our laboratory. The expression plasmid pPIC9K was custom-synthesized and acquired from Sangon Biotech. The yeast extract peptone dextrose (YPD) medium was composed of 20 g/L glucose, 20 g/L peptone, and 10 g/L yeast extract. Buffered glycerol complex medium (BMGY) included 20 g/L tryptone, 10 g/L yeast extract, 3 g/L K₂HPO₄, 11.8 g/L KH₂PO₄, 13.4 g/L yeast nitrogen base (YNB), and 0.5% (v/v) glycerol. Buffered methanol complex medium (BMMY) shared the same formulation as BMGY, with 0.5% (v/v) methanol added for induction. Minimal dextrose (MD) medium, used for screening histidine auxotrophs, contained 20 g/L glucose, 13.4 g/L YNB, and 20 g/L agar. Solid YPD medium was prepared using 20 g/L glucose, 20 g/L peptone, 10 g/L yeast extract, and 20 g/L agar. All media formulations followed the guidelines provided in the Invitrogen PichiaPink™ Expression System manual.

2.2 Expression of RHC in *P. pastoris*

2.2.1 Construction of Recombinant Plasmid

In this study, three recombinant plasmids were developed: the wild-type pPIC9K, pPIC9K-RHC, and pPIC9K-OSTI/RHC. The pPIC9K plasmid served as the unmodified control, containing no inserted protein-coding sequences. The pPIC9K-RHC variant was engineered to include a gene encoding recombinant human-like collagen (RHC). For the third construct, pPIC9K-OSTI/RHC, both the RHC gene and the OSTI gene (accession number NC_001142.9) from *Saccharomyces cerevisiae* S288C were introduced into the signal peptide's pre-region. The pPIC9K-OSTI/RHC plasmid was synthesized by Sangon Biotech. Comprehensive details regarding the plasmid constructs and their sources are summarized in Table 1. All three plasmids were subsequently expressed in *P. pastoris* GS115 cells.

Table 1: Strain, plasmids, and primers used in this study:

Names	Genotype	References
P. pastoris GS115	His4, host strain	Invitrogen
E. coli Top 10	plasmid-cloning host	Invitrogen
AOX1-F	Forward primer (5'-3'), Seq: GACTGGTTCCAATTGACAAGC	Talen-bio Technology Co., Ltd
AOX1-R	Reverse primer (5'-3'), Seq: GGCAATGGCATTCTGACA	Talen-bio Technology Co., Ltd

pPIC9K	Plasmid	Sangon Biotech Co., Ltd
pPIC9K-OSTI/RHC	pPIC9K plasmid containing the OSTI gene (NC_001142.9) from <i>Saccharomyces cerevisiae</i> S288C pre-region in signal peptide and optimized RHC.	Sangon Biotech Co., Ltd (This Study)
pPIC9K-RHC	pPIC9K plasmid containing RHC	This study

2.2.2 Yeast Transformation and Screening

The expression plasmid was linearized using the *Sal*I restriction enzyme, and the resulting fragments were purified before being introduced into *P. pastoris* GS115 competent cells via electroporation (1500 V, 200 Ω , 50 μ F). Transformed cells were spread onto minimal dextrose (MD) agar plates and incubated at 30 °C for 2–3 days. Colonies were screened using colony PCR with AOX1t-F and AOX1t-R primers to identify successful transformants. To isolate high-copy variants, confirmed pPIC9K-OSTI/RHC-positive colonies were transferred to YPD agar plates containing 3 mg/mL geneticin (G418) and incubated at 30 °C for 48 hours.

2.3 Fermentation Media

2.3.1 Characterization of Fermentation Broth

pH Measurement:

The pH of *P. pastoris* GS115 fermentation was monitored, as it is critical for exogenous protein secretion. Six different pH values (4, 5, 5.5, 6, 6.5, and 7.0) were tested for each culture medium, and the pH was measured every 24 hours until the end of fermentation using a pH meter (Starter 5000 pH ST3100, Changzhou, China)^[19].

Optical Density (OD) Measurement:

During the fermentation process, the growth of *P. pastoris* was monitored by measuring the optical density at 600 nm (OD₆₀₀). For each measurement, 100 μ L of the culture broth was diluted with 900 μ L of sterile distilled water to obtain a 10-fold dilution. The diluted samples were thoroughly mixed, and the absorbance was measured at 600 nm using distilled water as the blank control, using an ultraviolet spectrophotometer (UVmini-1280, Columbia, USA)^[20].

2.3.2 Secretion and Expression of RHC in Shaking Flasks

The recombinant *P. pastoris* GS115 strains carrying the RHC gene were initially cultured in 5 mL YPD medium for 12 hours at 30°C and 220 rpm. A 0.5 mL aliquot of this suspension was then transferred to shaking flasks containing 50 mL BMGY medium, and the culture was grown until the OD₆₀₀ reached approximately 15. The cells were harvested and resuspended in 200 mL of BMMY media to induce RHC expression at 30°C and 220 rpm. Methanol was added to a final concentration of 0.5% (v/v)

every 12 hours to induce protein expression^[21]. The fermentation supernatant was collected for further identification.

2.4 SDS-PAGE Analysis of RHC

To evaluate the expression of RHC, the fermentation supernatant was harvested by centrifugation (7000 $\times$ g, 10 minutes). An 80 μ L aliquot of the supernatant was mixed with 20 μ L of 5 $\times$ SDS loading buffer, then heated at 95°C for 10 minutes. The sample was subjected to SDS-PAGE analysis, using a 5% stacking gel and a 15% resolving gel. The gel was stained with Coomassie Brilliant Blue.

2.5 Protein Purification

Protein extraction was carried out using ammonium sulfate precipitation. Fermented cell suspensions from shaker flasks were subjected to centrifugation at 4000 rpm for 30 minutes at 4 °C using a refrigerated high-speed centrifuge to separate the fermentation supernatant. To eliminate non-target proteins, a 20% ammonium sulfate solution was added, followed by centrifugation to remove the resulting precipitate. Subsequently, a 60% ammonium sulfate concentration was used to precipitate the RHC protein selectively. The collected protein pellet was then dissolved in ultrapure water. Protein concentration in the resulting supernatant was quantified using the bicinchoninic acid (BCA) assay.

III. RESULTS AND DISCUSSION

3.1 Plasmid Construction and Gene Expression

In this study, we constructed three distinct plasmids: pPIC9K, pPIC9K-RHC, and pPIC9K-OSTI/RHC, as shown in Figure 1. To confirm the proper expression of these genes, we performed a PCR experiment. Figure 2(a) displays the band intensity for both the pPIC9K-RHC and pPIC9K-OSTI/RHC genes, which correspond to 10,041 bp and 10,050 bp, respectively. Figure 2(b) shows the plasmid region containing the RHC protein, which spans 1,157 bp. We used AOX1-F and AOX1-R primers to amplify and identify the desired bands during the PCR screening. The band intensity results confirm that the pPIC9K-RHC and pPIC9K-OSTI/RHC plasmids were successfully constructed. Since the pPIC9K plasmid is a wild-type

version without the RHC protein, we omitted the PCR test for this plasmid.

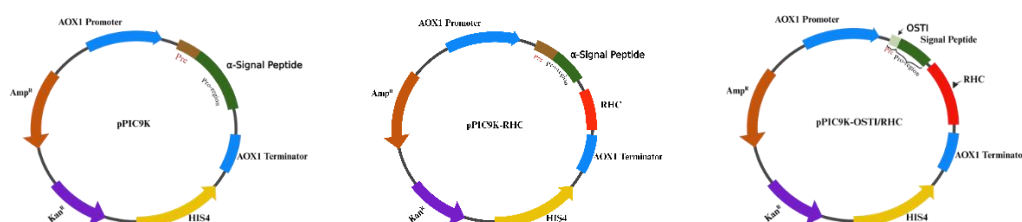


Fig.1: Construction of three different pPIC9K plasmids.

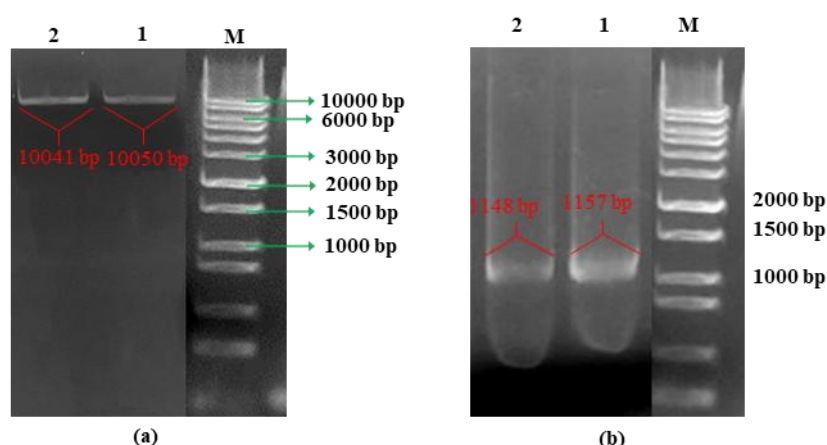


Figure 2: PCR screening: Showing the total base pairs of the whole plasmid. Lane M: 1kb Plus DNA Ladder marker, Lane 1: pPIC9K-OSTI/RHC, Lane 2: pPIC9K-RHC. (a); Showing the base pairs between the AOX1 promoter and AOX1 terminator. Lane M: 1kb Plus DNA Ladder marker, Lane 1: pPIC9K-OSTI/RHC, Lane 2: pPIC9K-RHC. (b)

3.2 Effect of OSTI α -Signal Peptide

Figure 3(a) illustrates the growth of three plasmid variants over time, with the OD₆₀₀ values plotted against time. The growth of pPIC9K-RHC is slightly higher than that of the other variants. Although the first two plasmids show higher growth initially, our desired plasmid, pPIC9K-OSTI/Hss, exhibits superior growth over time. Figure 3(b) confirms that pPIC9K-OSTI/RHC shows the highest band intensity. The protein was identified with a molecular weight of 27 kDa, which conforms with the findings of Ma et al.^[22]

The observed improvement in protein secretion is primarily due to substituting the α -factor pre-region with the OSTI signal peptide derived from *Saccharomyces cerevisiae*

S288C, which enhances the secretion efficiency of recombinant RHC. This signal peptide facilitates co-translational translocation into the endoplasmic reticulum (ER), promoting more efficient protein processing and expression. Such a mechanism is particularly beneficial for the secretion of structurally complex proteins^[23], as it minimizes the likelihood of misfolding and intracellular aggregation, as demonstrated in previous studies^[24]. Incorporation of the OSTI signal sequence into the recombinant plasmid pPIC9K-OSTI/RHC significantly boosts protein levels in the culture supernatant, streamlining downstream purification and improving overall production efficiency^[25].

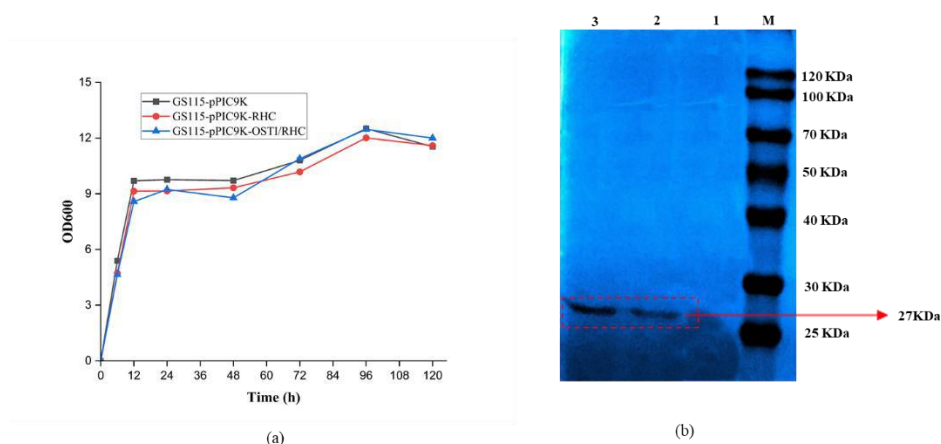


Figure 3: Analysis of the growth of three different *P. pastoris* GS115. (a) SDS-PAGE analysis of three different plasmids. Lane M (Protein Marker Blue Plus II), Lane 1 (pPIC9K), Lane 2 (pPIC9K-RHC), and Lane 3 (pPIC9K-OSTI/RHC). (b)

3.3 Construction of High-Copy Transformants

Figure 5 illustrates the construction of high-copy transformants using the pPIC9K-OSTI/RHC plasmid. To assess the plasmid's growth under different conditions, we treated it with and without an antibiotic and monitored its growth over time. The OD600 value for the pPIC9K-OSTI/RHC with antibiotic reached 13.14 after 96 hours, while the OD600 for the plasmid without antibiotic was

12.01 (Figure 6a). This indicates that the presence of the antibiotic promotes higher growth of the pPIC9K-OSTI/RHC plasmid. To further confirm protein expression in *P. pastoris* G115, we performed an SDS-PAGE analysis for both conditions (Figure 6b). The results from the SDS-PAGE analysis are consistent with our observations, showing a higher band intensity for the pPIC9K-OSTI/RHC with antibiotic treatment compared to the same construct without antibiotic treatment.

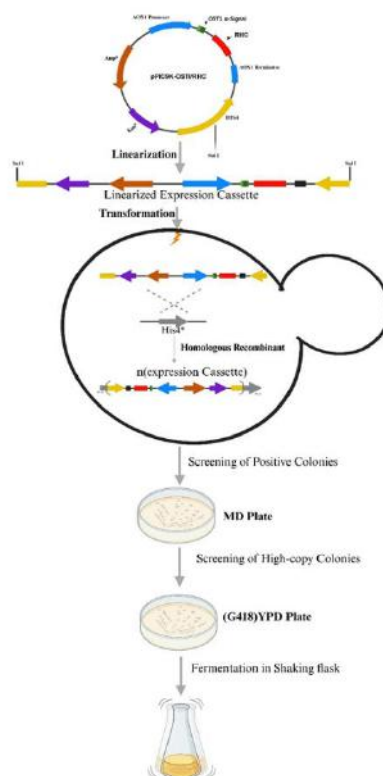


Fig.5: Overview of high-copy plasmid construction process.

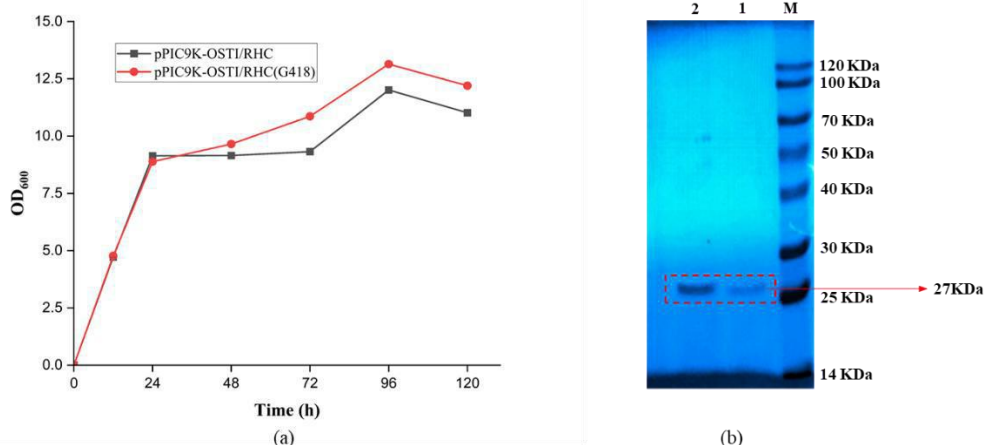


Figure 6: Growth analysis of pPIC9K-OSTI/RHC with and without G418 antibiotic. (a); SDS-PAGE analysis: Lane M (Protein Marker Blue Plus II), Lane 1(pPIC9K-OSTI/RHC without G418), Lane 2 (pPIC9K-OSTI/RHC with G418). (b)

3.4 Expression Media Optimization

After constructing the high-copy transformants with the novel plasmid pPIC9K-OSTI/RHC, we optimized the expression medium (BMMY) by testing six different pH ranges. The OD₆₀₀ values were plotted against time (Figure 7a), and the results indicated that the pPIC9K-OSTI/RHC plasmid exhibited the highest OD₆₀₀ value, 13.5, at pH 6, suggesting optimal growth at this pH. Figure 7b illustrates pH variations over time, showing a decreasing trend, which

indicates an increase in protein production^[26]. To validate these results, we performed SDS-PAGE analysis, which revealed the highest band intensity for the pPIC9K-OSTI/RHC plasmid at pH 6 (Figure 8). Additionally, protein yield measurements after fermentation in a shaker flask confirmed that the highest yield, 0.35 g/L (Figure 9), was obtained at pH 6. These findings strongly support our conclusion that pH 6 is the optimal condition for RHC protein expression using the pPIC9K-OSTI/RHC plasmid.

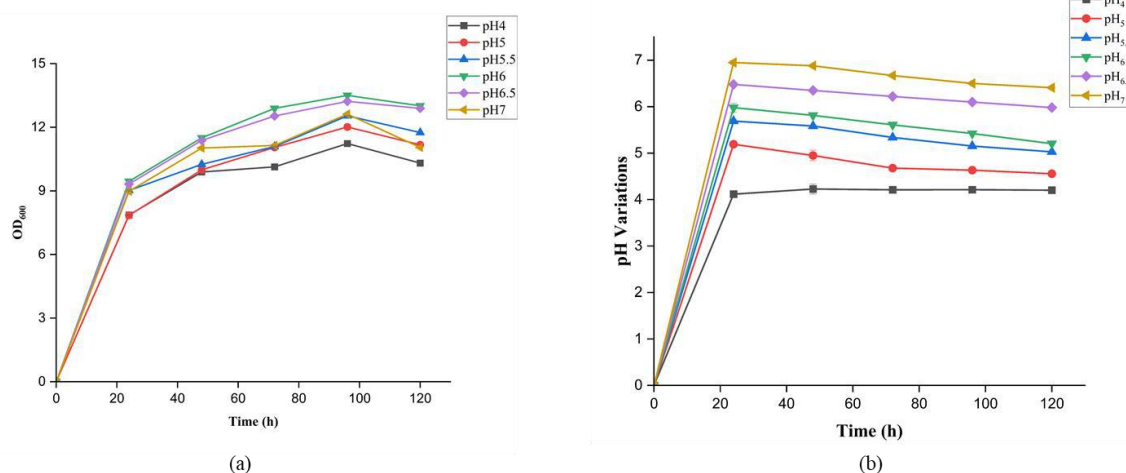


Figure 7: Fermentation media optimization at different pH levels: Analysis of the growth of host strain GS115-pPIC9K-OSTI/RHC at pH 4,5,5.5,6. (a); pH variation observation in fermentation media. (b)

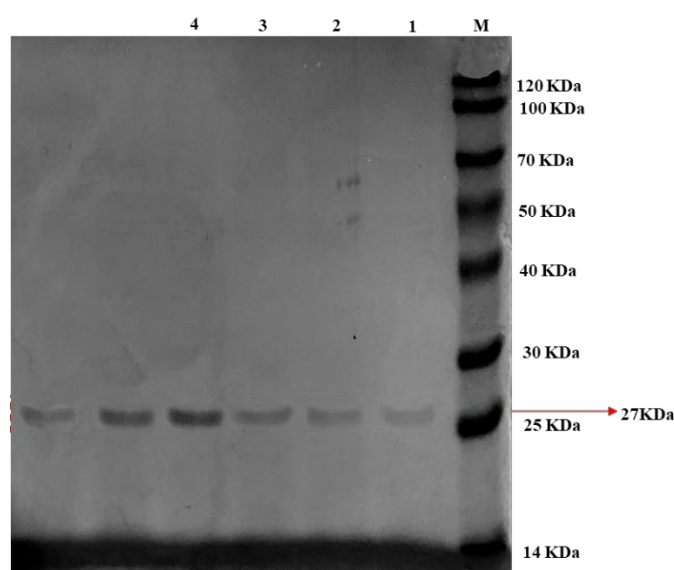


Fig.8: SDS-PAGE analysis of the supernatant. Lane M (Protein Marker Blue Plus II), Lane 1,2,3,4,5 and 6 are respectively pH₄, pH₅, pH_{5.5}, pH₆, pH_{6.5}, pH₇.

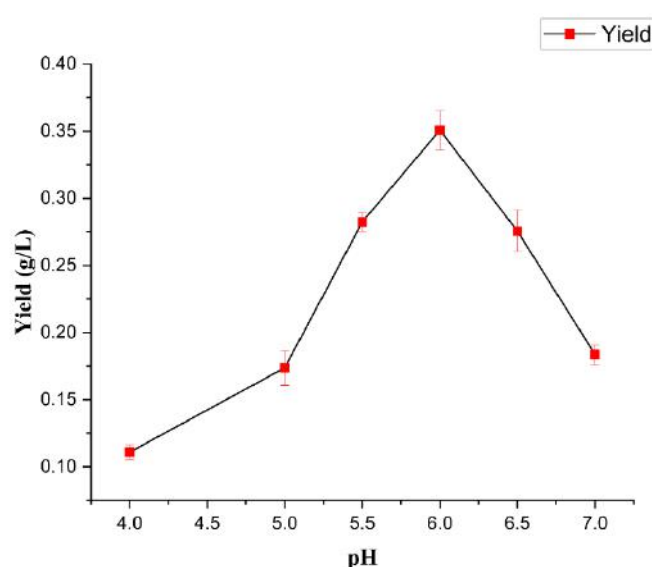


Fig.9: Protein yield for pPIC9K-OSTI/RHC plasmid at various pH.

IV. CONCLUSION

This study successfully developed a high-yield recombinant human-like collagen (RHC) production system using *P. pastoris* GS115 with a novel plasmid construct, pPIC9K-OSTI/RHC. The incorporation of the OSTI signal peptide significantly enhanced the secretory efficiency of RHC, optimizing its expression in yeast cells. We identified pH 6 as the optimal condition for recombinant protein expression, resulting in a remarkable protein yield of 0.35 g/L. The findings confirm that the use of the pPIC9K-OSTI/RHC plasmid, along with the optimized fermentation conditions, provides a cost-effective, scalable, and

sustainable approach to recombinant collagen production. This method holds great potential for applications in tissue engineering, wound healing, and other biomedical fields, offering a viable alternative to traditional methods that rely on animal-based collagen extraction. The results presented here also contribute to the growing body of knowledge on the optimization of protein expression in *P. pastoris*, providing a foundation for further advancements in recombinant protein production.

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Role of Submerged Macrophytes in Restoring Eutrophic Lakes

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Received: 03 Sep 2025; Received in revised form: 04 Oct 2025; Accepted: 08 Oct 2025; Available online: 16 Oct 2025

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Abstract— Eutrophication remains one of the most pressing challenges for freshwater ecosystems, leading to harmful algal blooms, oxygen depletion, and biodiversity loss. Submerged macrophytes, or submerged aquatic plants, play a central role in counteracting eutrophication by functioning as ecosystem engineers. They regulate nutrient dynamics, suppress algal growth, stabilize sediments, and enhance oxygen conditions, thereby facilitating the transition of lakes from turbid, phytoplankton-dominated states to clear-water conditions. This article reviews the ecological roles of submerged macrophytes in restoring eutrophic lakes, highlighting their contributions to nutrient uptake, algae control, oxygen production, habitat provision, sediment stabilization, and allelopathic interactions. A better understanding of these functions underscores the importance of submerged plants as a natural, sustainable, and cost-effective approach to freshwater restoration.



Keywords— Eutrophication, submerged macrophytes, submerged aquatic plants, ecological restoration, freshwater ecosystems, eutrophic lake restoration

I. INTRODUCTION

Freshwater ecosystems are among the most productive yet vulnerable environments on Earth, supporting biodiversity, providing ecosystem services, and sustaining human needs such as drinking water, fisheries, and recreation. However, these systems are increasingly threatened by eutrophication, a process driven by excessive nutrient enrichment, primarily nitrogen (N) and phosphorus (P), originating from agricultural runoff, wastewater discharge, and industrial effluents (Smith et al., 1999; Schindler et al., 2006; Liu et al., 2022). Eutrophication triggers a cascade of ecological problems, including harmful algal blooms, oxygen depletion, fish kills,

biodiversity loss, and the collapse of aquatic food webs (Dodds et al., 2009).

Traditional approaches to lake restoration—such as chemical treatments, sediment dredging, and artificial oxygenation—can provide temporary relief but are often costly, energy-intensive, and ecologically disruptive (Cooke et al., 2005). In contrast, biological approaches that harness natural ecosystem processes are increasingly recognized as sustainable alternatives. Among these, submerged macrophytes, or submerged aquatic plants, function as ecosystem engineers that can profoundly influence nutrient dynamics, water clarity, oxygen balance,

and aquatic community structure (Carpenter & Lodge, 1986; Jeppesen et al., 2005) (Fig. 1).

Submerged macrophytes provide multiple ecological functions that help counteract the drivers and symptoms of eutrophication. They absorb nutrients directly from the water column and sediments, reducing nutrient availability for phytoplankton (Hilt et al., 2006). Their photosynthetic

activity releases oxygen into the water, which supports aerobic microbial processes and aquatic fauna. Structurally, macrophyte beds offer habitat complexity that enhances biodiversity and stabilizes trophic interactions. Moreover, by anchoring sediments and releasing allelopathic compounds, submerged macrophytes suppress phytoplankton dominance and reinforce water clarity (van Donk & van de Bund, 2002; Hilt & Gross, 2008).

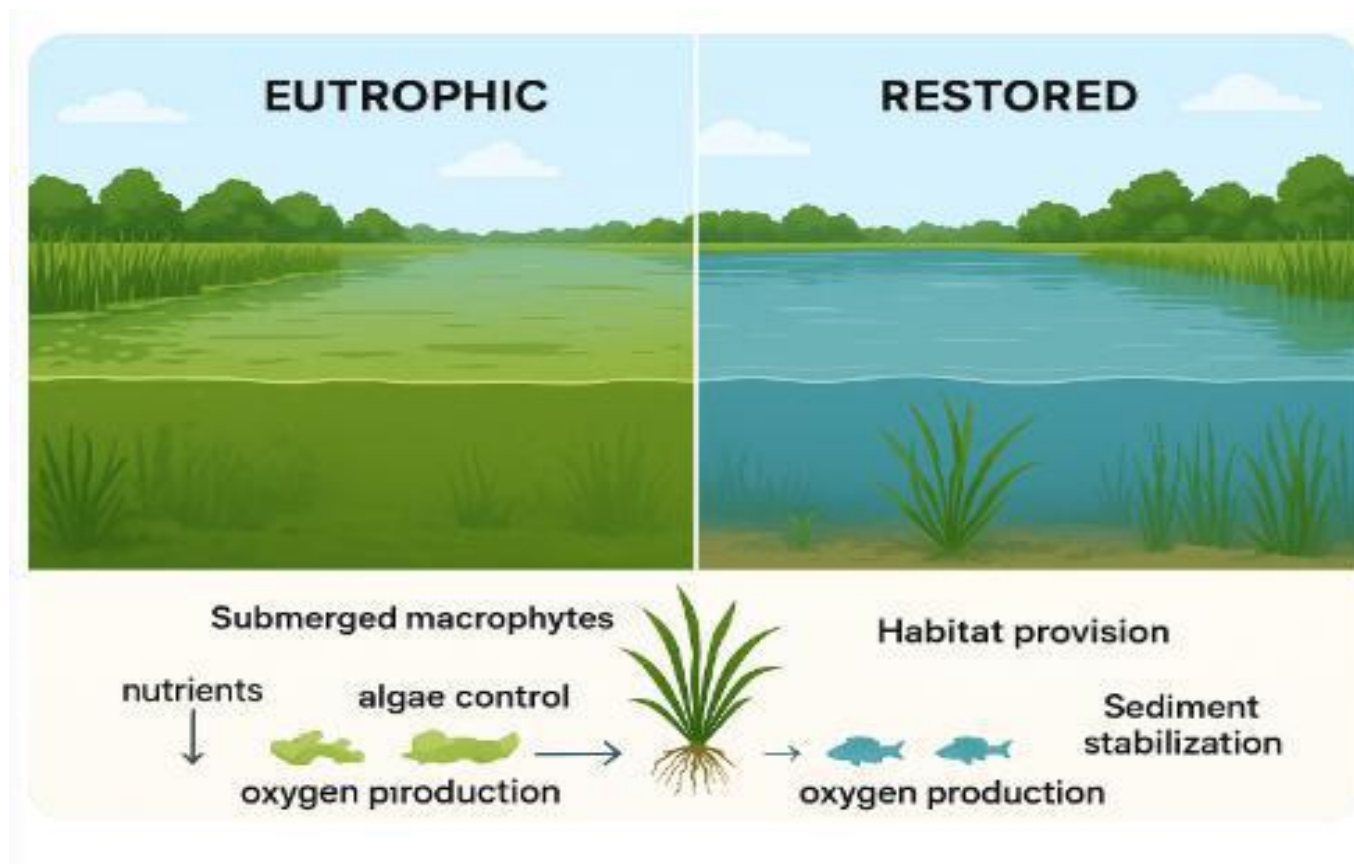


Fig. 1: Submerged macrophytes restore eutrophic lake

This paper focuses on the role of submerged macrophytes in restoring eutrophic lakes, with an emphasis on their ecological functions and feedback mechanisms. By synthesizing evidence from empirical studies, long-term monitoring projects, and theoretical frameworks, we highlight how submerged macrophytes contribute to nutrient cycling, algal control, oxygenation, habitat provision, sediment stabilization, and allelopathy. The goal is to provide a mechanistic understanding of their role in ecological resilience and regime shifts from turbid, algae-dominated states to clear-water, macrophyte-dominated systems.

II. HOW SUBMERGED MACROPHYTES HELP RESTORE EUTROPHIC LAKES

Submerged macrophytes play a pivotal role in reversing and stabilizing eutrophic conditions in freshwater ecosystems. Acting as both nutrient sinks and ecological engineers, these plants contribute to the transition of lakes from a turbid, phytoplankton-dominated state to a clear-water, macrophyte-dominated state (Scheffer et al., 1993; Jeppesen et al., 1998; Yang et al., 2025). Their presence initiates a cascade of positive feedback mechanisms that simultaneously reduce nutrient availability, suppress algal dominance, and enhance habitat quality for diverse aquatic organisms.

At the ecosystem level, submerged macrophytes influence nutrient cycling by directly absorbing nitrogen and phosphorus from both the water column and sediments,

thereby limiting the resources available for phytoplankton blooms (Hilt et al., 2006). This reduction in nutrient concentrations improves water clarity, which further supports macrophyte growth by increasing light penetration. The establishment of macrophyte beds also stabilizes sediments, preventing nutrient resuspension and turbidity (Horppila & Nurminen, 2003). In addition, many submerged macrophytes excrete allelopathic compounds that inhibit the growth of cyanobacteria and other algae, adding a chemical layer of control (Hilt & Gross, 2008).

These plants also contribute to oxygen balance by releasing oxygen through photosynthesis, particularly during daylight hours. This enhances aerobic conditions in the water column and upper sediments, which promotes nitrification and other microbial processes essential for nutrient removal (Ferreira et al., 2018). Beyond water chemistry, submerged macrophytes provide physical habitat complexity, offering refuge for zooplankton that graze on phytoplankton and for fish species that depend on structured vegetation for spawning and feeding (Warfe & Barmuta, 2004). By supporting these trophic interactions, submerged macrophytes indirectly regulate algal populations and reinforce ecosystem stability.

The cumulative effect of these functions creates a reinforcing cycle that strengthens lake resilience. Once

established, submerged macrophyte dominance can buffer lakes against external nutrient inputs, thereby stabilizing the clear-water state. However, the persistence of this state depends on factors such as species composition, nutrient load reductions, and management interventions (Scheffer & van Nes, 2007). This section will explore the specific mechanisms through which submerged macrophytes restore eutrophic lakes, focusing on nutrient uptake, algae control, oxygenation, habitat provision, sediment stabilization, and allelopathy.

2.1 Nutrient Uptake

Submerged macrophytes function as effective nutrient sinks by assimilating both nitrogen (N) and phosphorus (P), the two key drivers of eutrophication, from multiple sources within aquatic ecosystems. These plants are capable of absorbing nutrients directly from the water column through their leaves and shoots as well as from sediments via their root systems, thereby linking benthic and pelagic nutrient cycles (Denny, 1972; Barko & Smart, 1981). This dual nutrient acquisition strategy enables submerged macrophytes to effectively reduce the concentrations of dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP), limiting the resource availability that typically sustains algal blooms (Hilt et al., 2006) (Fig. 2).

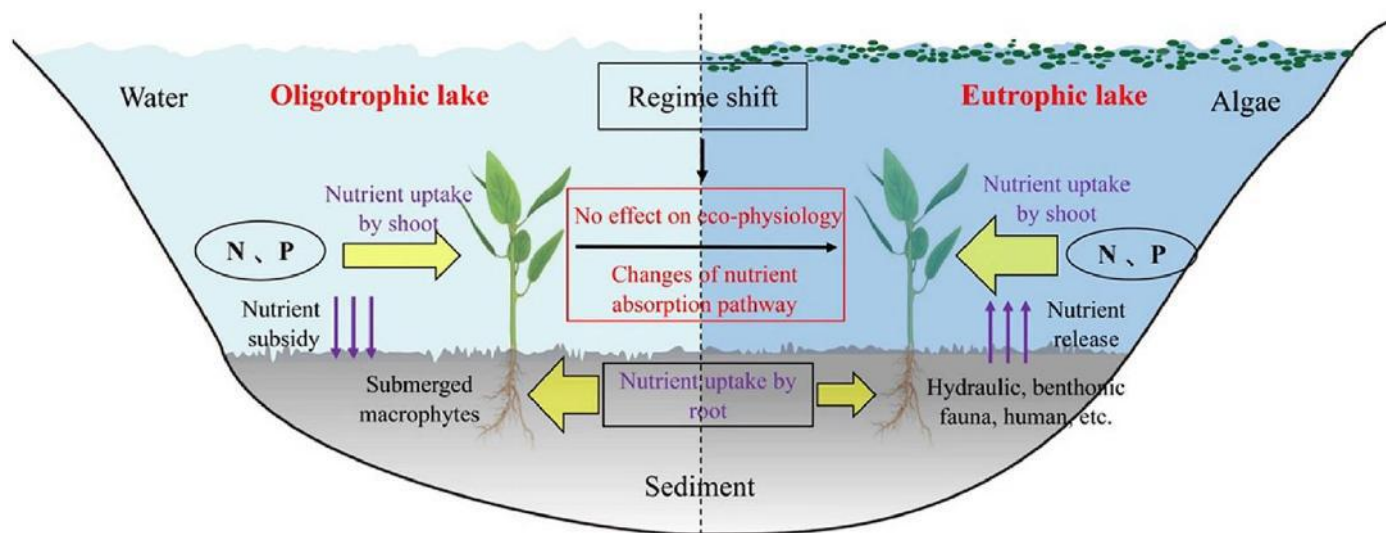


Fig. 2: Nutrient uptake by Submerged macrophytes (Xu et al. 2019)

The ability of submerged macrophytes to draw nutrients from sediments is particularly important in shallow eutrophic lakes, where internal loading often contributes more to eutrophication than external nutrient inputs (Søndergaard et al., 2003; Mi et al., 2008). By intercepting and immobilizing nutrients in their tissues, submerged

macrophytes reduce nutrient recycling between sediments and the water column. Over time, this process contributes to sediment nutrient sequestration, especially when plant biomass is buried or decomposes in situ (Carignan & Kalff, 1980; Yang et al., 2007).

In addition to direct uptake, macrophytes enhance nutrient retention through indirect mechanisms. Their dense canopies slow down water movement, which promotes particle settling and reduces the resuspension of nutrient-rich sediments (van Donk & van de Bund, 2002). Moreover, oxygen released from plant roots into the rhizosphere enhances nitrification and the subsequent denitrification processes carried out by associated microbial communities, thereby facilitating permanent nitrogen removal from aquatic systems (Reddy et al., 1989; Li et al., 2020; He et al., 2010).

Nutrient uptake efficiency varies among species, with fast-growing plants such as *Ceratophyllum demersum* and *Elodea canadensis* showing particularly high assimilation capacities (Hussner et al., 2017). Seasonal dynamics also play a role: nutrient uptake is generally highest during the growing season when photosynthetic rates and biomass accumulation peak (Chambers et al., 2008; Yang, 2011). Restoration efforts can therefore strategically employ nutrient-efficient macrophyte species to accelerate the shift from turbid, algae-dominated states to clear-water, macrophyte-dominated regimes.

Overall, submerged macrophytes reduce nutrient availability through direct assimilation, sediment

stabilization, and microbial facilitation, thereby serving as key biotic agents in reversing eutrophication processes.

2.2 Algae Control

Submerged macrophytes exert strong regulatory effects on algal growth, acting through both direct and indirect mechanisms. By competing with phytoplankton for essential nutrients such as nitrogen (N) and phosphorus (P), macrophytes reduce the resource pool available for algal proliferation, thereby limiting the intensity and duration of algal blooms (Jeppesen et al., 2007; Hilt et al., 2006). This competition is particularly effective in shallow lakes, where macrophytes can intercept nutrient fluxes from both sediments and the overlying water column.

In addition to nutrient competition, submerged plants improve light conditions that indirectly suppress algae. By stabilizing sediments and reducing turbidity, macrophytes increase water clarity and light penetration, which further promotes their own growth and reinforces conditions unfavorable for phytoplankton dominance (Scheffer, 2004). This feedback loop helps maintain a clear-water state, which is typically characterized by reduced phytoplankton biomass and higher ecological stability.

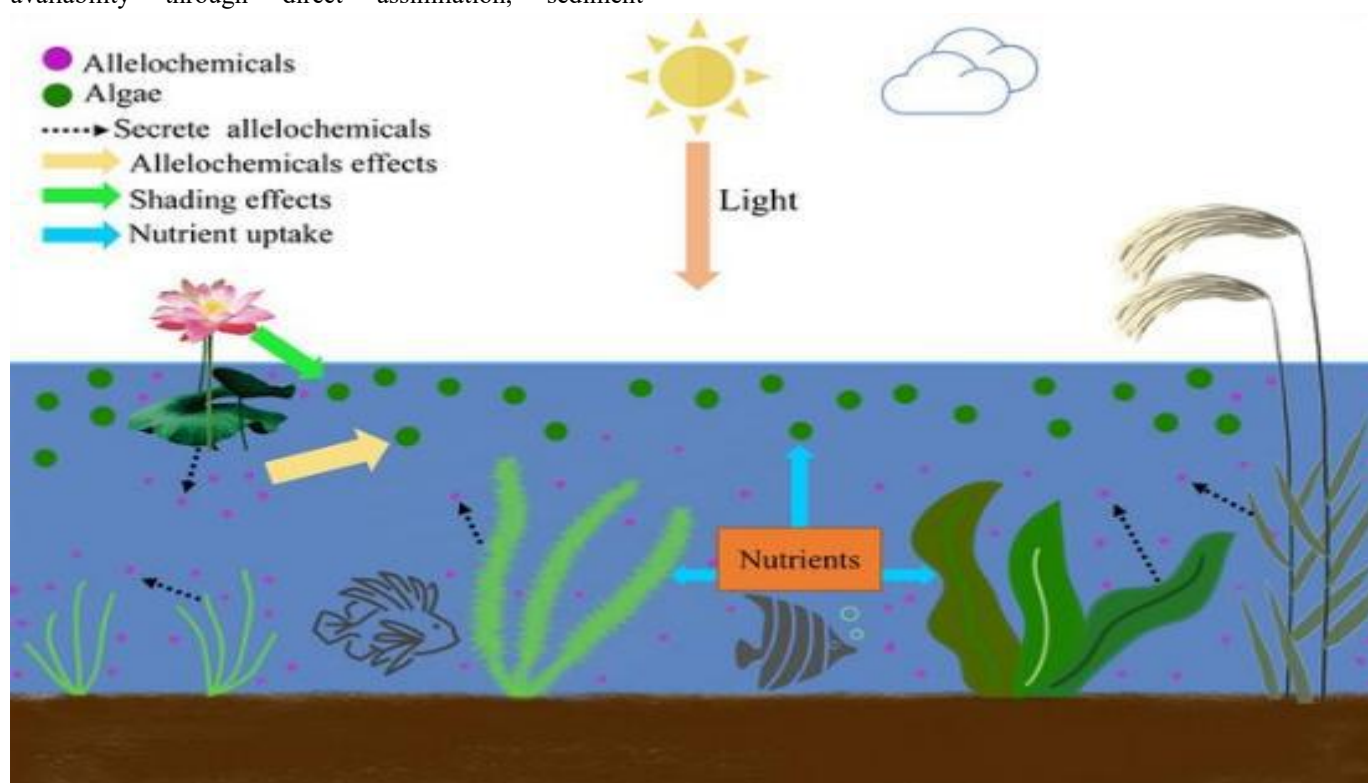


Fig. 3: Algae control by Submerged macrophytes (Wang & Liu, 2023)

Another critical pathway of algal control involves allelopathy, whereby certain macrophyte species release chemical compounds that inhibit the growth and photosynthetic activity of phytoplankton and cyanobacteria (Hilt & Gross, 2008; Gross et al., 2003). These allelochemicals include phenolic compounds and fatty acids that reduce algal cell division or disrupt metabolic processes, directly suppressing harmful algal blooms. For example, *Ceratophyllum demersum* has been shown to excrete allelochemicals capable of reducing cyanobacterial biomass under experimental conditions (Gross et al., 2003).

Submerged macrophytes also contribute to algal suppression through their role in structuring aquatic food webs. Dense plant stands provide refuge for zooplankton, particularly cladocerans such as *Daphnia*, which are efficient grazers of phytoplankton (Timms & Moss, 1984; van Donk & van de Bund, 2002). By offering protection from planktivorous fish, macrophytes indirectly enhance grazing pressure on algae, creating a trophic cascade that further limits algal biomass.

The combined effects of nutrient competition, sediment stabilization, allelopathy, and zooplankton-mediated grazing establish submerged macrophytes as key biological controls of algal populations. Their presence is therefore critical for maintaining ecological balance in eutrophic lakes and for restoring ecosystems that have shifted to turbid, algae-dominated states (Fig. 3).

2.3 Oxygen Production

Submerged macrophytes significantly influence the oxygen dynamics of eutrophic lakes. Through photosynthesis, they release oxygen directly into the surrounding water, counteracting the hypoxic or anoxic conditions that commonly develop in nutrient-enriched systems. This oxygenation effect is most pronounced during daylight hours when photosynthetic activity peaks, providing localized zones of elevated dissolved oxygen that benefit aquatic organisms (Carpenter & Lodge, 1986; Sand-Jensen et al., 1982).

The enhancement of oxygen concentrations by submerged macrophytes contributes to several important ecological processes. First, higher oxygen availability supports aerobic microbial decomposition of organic matter in the water column and sediment, reducing the accumulation of harmful metabolites such as hydrogen sulfide and methane (Barko & James, 1998). Second, oxygen penetration into surface sediments fosters nitrification and coupled denitrification processes, which play a vital role in removing bioavailable nitrogen from aquatic ecosystems (Klapper, 1991). These biogeochemical processes help regulate nutrient cycling and improve overall lake health.

In addition to biochemical benefits, oxygen production enhances the survival and growth of fish and invertebrate communities that are sensitive to oxygen depletion. In eutrophic lakes where oxygen demand is high due to algal decomposition, submerged macrophyte beds can act as refugia, offering zones of improved water quality that sustain higher trophic levels (Jeppesen et al., 2007). Furthermore, oxygen supersaturation in plant stands during daylight may suppress the release of phosphorus from sediments, thereby limiting internal nutrient loading (Körner & Nicklisch, 2002; Yang et al., 2025).

The diurnal fluctuations of oxygen caused by submerged plant activity underscore the need for careful monitoring in restoration projects. While daytime oxygen levels may be elevated, respiration at night can lead to reductions in dissolved oxygen, particularly in dense plant stands. Nevertheless, when managed appropriately, the net contribution of submerged macrophytes to oxygen enrichment is strongly positive, reinforcing their role as ecosystem engineers in restoring eutrophic lakes.

2.4 Habitat Provision

One of the most critical ecological roles of submerged macrophytes is the creation of structural habitat complexity that supports diverse aquatic communities. The three-dimensional architecture of macrophyte beds provides shelter, feeding grounds, and breeding areas for a wide array of organisms, ranging from microorganisms to higher trophic levels such as fish (Diehl & Kornijów, 1998; Thomaz & Cunha, 2010). This structural heterogeneity increases habitat availability and fosters higher biodiversity compared to unvegetated lakebeds.

For invertebrates, macrophytes serve both as physical refugia and as surfaces for periphyton colonization, which constitutes an important food resource (Cheruvilil et al., 2000). Aquatic insects, mollusks, and crustaceans utilize submerged vegetation for protection from predation and as oviposition sites. Zooplankton communities also benefit, as dense macrophyte stands reduce fish predation pressure, thereby supporting larger-bodied grazers such as *Daphnia* that help regulate phytoplankton biomass (Timms & Moss, 1984).

Fish communities are particularly influenced by macrophyte presence. Submerged vegetation provides spawning substrates for many species, nursery habitats for juvenile fish, and ambush sites for piscivores (Stahra et al., 2012). For example, studies have shown that lakes with extensive macrophyte coverage often sustain higher fish biomass and species diversity compared to lakes where vegetation is absent or sparse (Engel, 1990) (Fig. 4).

Beyond individual species benefits, the habitat provisioning role of submerged macrophytes enhances the stability and

resilience of lake ecosystems. By supporting multiple trophic levels, they contribute to more complex and interconnected food webs that can buffer against environmental fluctuations and anthropogenic disturbances (Philippov et al., 2022). This is particularly relevant in eutrophic systems, where biodiversity tends to decline due to algal dominance and oxygen depletion. Restoring macrophyte beds thus reintroduces essential habitat complexity that promotes ecological balance.

The extent and quality of habitat provision in aquatic ecosystems depend strongly on plant species composition, density, and spatial distribution. Species with finely divided leaves, such as *Myriophyllum spicatum*, generally provide more surface area and refugia for invertebrates and small fish compared to broad-leaved species like *Vallisneria* (Kilgore et al., 1990; Gettys et al., 2023). Restoration strategies should therefore consider both functional diversity and structural complexity when selecting macrophytes for reintroduction.



Fig. 4: Native *Potamogeton* plants as fish habitat provision in Lake Ototoa, New Zealand

2.5 Sediment Stabilization

Submerged macrophytes play a crucial role in stabilizing sediments, which is fundamental for improving water clarity and reducing internal nutrient loading in eutrophic lakes. Their root and rhizome systems anchor sediments to the lakebed, thereby reducing resuspension caused by wind-induced turbulence, waves, or bioturbation from benthic organisms such as carp (Zhang et al., 2002; van Donk & van de Bund, 2002). This stabilization decreases turbidity and helps prevent the release of nutrients—particularly phosphorus—back into the water column.

The importance of sediment stabilization extends beyond physical processes to include significant chemical interactions. By reducing sediment disturbance, macrophytes limit the diffusion of soluble reactive phosphorus and ammonium from anoxic sediments (Horppila & Nurminen, 2003; Yang, 2011). Additionally,

oxygen released from macrophyte roots can oxidize the sediment-water interface, promoting the formation of iron and manganese oxides that bind phosphorus and decrease its mobility (Barko & James, 1998; Sand-Jensen et al., 1982). These processes collectively reduce the risk of internal loading, which often sustains eutrophication even when external nutrient inputs have been reduced.

Sediment stabilization also creates positive feedback for macrophyte growth. Reduced turbidity increases light penetration, enhancing photosynthesis and allowing macrophyte populations to expand further into deeper zones (Scheffer, 2004). This expansion, in turn, leads to greater stabilization effects, reinforcing a clear-water state.

Field studies have demonstrated that lakes with healthy macrophyte stands experience markedly lower sediment resuspension rates compared to lakes dominated by phytoplankton or devoid of vegetation (Hilt et al., 2006).

For example, restoration projects in shallow European lakes have shown that reintroduction of macrophytes such as *Vallisneria* and *Potamogeton* significantly improved water clarity by reducing resuspended particulate matter and internal phosphorus cycling (Amador et al., 2024).

However, the efficiency of sediment stabilization varies depending on plant morphology, density, and distribution. Species with extensive root and rhizome systems, such as *Vallisneria* and *Potamogeton*, tend to be more effective in stabilizing sediments compared to rootless species like *Ceratophyllum demersum*. Therefore, species selection should carefully consider sediment stabilization as a functional trait when planning lake restoration projects.

2.6 Allelopathic Effects

Beyond nutrient uptake, oxygenation, and sediment stabilization, submerged macrophytes can influence eutrophic lake dynamics through allelopathic interactions. Allelopathy refers to the release of biochemically active compounds by plants that inhibit the growth or reproduction of other organisms, such as phytoplankton and cyanobacteria (Gross, 2003). This mechanism adds another layer of control against harmful algal blooms, making submerged macrophytes effective biotic regulators in lake ecosystems.

Research has shown that various macrophyte species, including *Ceratophyllum demersum*, *Myriophyllum spicatum*, and *Elodea canadensis*, release secondary metabolites such as phenolic compounds, fatty acids, and terpenoids into the water (Hilt & Gross, 2008; Gross et al., 2003; Yang et al., 2025). These substances can disrupt algal cellular processes, including photosynthesis, enzyme activity, and cell division, leading to reduced algal biomass and altered community structure. For example, *Myriophyllum spicatum* has been demonstrated to inhibit cyanobacteria through the release of hydrolysable tannins and polyphenolic compounds (Gross et al., 1996).

Allelopathic effects are particularly valuable in eutrophic systems where nutrient concentrations remain high, as they can directly suppress algal growth even when nutrient competition alone is insufficient. In controlled mesocosm experiments, macrophyte exudates have been observed to significantly reduce the dominance of cyanobacteria, thereby improving water clarity and enabling macrophyte reestablishment (Gross et al., 2003). This creates a positive feedback loop that favors the persistence of a clear-water state.

The strength of allelopathic effects, however, can vary depending on plant species, biomass density, environmental conditions, and the sensitivity of algal taxa present. Light,

temperature, and microbial degradation of allelochemicals can also influence their efficacy (Hilt & Gross, 2008). Consequently, while allelopathy represents a promising mechanism for phytoplankton control, its practical application in restoration projects requires further investigation and adaptive management.

In summary, allelopathy adds a unique biological mechanism to the suite of ecological functions provided by submerged macrophytes. By directly suppressing algal growth, allelopathic plants strengthen the stability of restored systems and may reduce reliance on chemical or mechanical algal control methods.

III. CONCLUSION

Submerged macrophytes play indispensable roles in restoring and maintaining the ecological balance of eutrophic lakes. Through nutrient uptake, algae control, oxygen production, habitat provision, sediment stabilization, and allelopathic interactions, these plants function as ecosystem engineers that reinforce the transition from turbid, phytoplankton-dominated states to clear-water conditions. Their capacity to regulate nutrient cycling, enhance biodiversity, and promote ecosystem resilience underscores their significance as a natural solution for eutrophication management.

Although challenges remain—such as species-specific responses to environmental change, vulnerability to external nutrient loading, and ecological thresholds that limit macrophyte recovery—their ecological functions provide a strong foundation for sustainable restoration strategies. Understanding these mechanisms not only advances theoretical ecology but also informs applied management practices, bridging the gap between fundamental science and practical restoration.

Future research should continue to refine knowledge of species-specific functional traits, feedback dynamics, and climate resilience, while integrating submerged macrophytes into broader ecosystem models. In doing so, the role of these plants can be fully realized as a cornerstone in safeguarding freshwater ecosystems against the persistent threat of eutrophication.

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Biological control of Plant-parasitic nematodes in Bananas using *Trichoderma atroviride* and cocoa-based organic amendments

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Received: 01 Sep 2025; Received in revised form: 02 Oct 2025; Accepted: 07 Oct 2025; Available online: 16 Oct 2025

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Abstract— This study evaluates the combined use of the endophytic fungus *Trichoderma atroviride* (strains Endo 1 and Endo 2) and cocoa (*Theobroma cacao*) organic amendments for managing plant-parasitic nematodes in three banana cultivars: Boniface, Grand Naine, and Williams. Field experiments were conducted on a commercial banana farm in Costa Rica using a randomized block design. Treatments involved applying fungal spores and cocoa amendments bi-monthly over a six-week interval. Results revealed no statistically significant differences in total nematode populations among treatments; however, the combined application of *T. atroviride* and organic amendments reduced nematode populations more effectively than control plots. Improvements were also observed in root health and plant growth parameters over time. This suggests a potential, though not conclusive, synergistic effect of these biocontrol agents. Further long-term studies are recommended to better understand their independent and combined effects on nematode management in bananas.



Keywords— endophytic fungi, organic amendments, plant-parasitic nematodes, root health, banana cultivation

I. INTRODUCTION

Bananas (*Musa* spp.) play a vital role in global food security and serve as a staple food for over 400 million people. Bananas rank among the most significant global food crops, both in production and trade, with annual consumption exceeding 116.2 million tons. Latin America and the Caribbean account for approximately 80% of worldwide exports and nearly 30% of global production, which significantly influences their social development (UN, 2019; FAO, 2016; Dita *et al.*, 2011).

Banana-producing regions face significant threats from climate change as well as outbreaks of pests and diseases (van Asten *et al.*, 2011). Among the most critical diseases affecting bananas are the black leaf streak disease caused by the ascomycete fungus *Mycosphaerella fijiensis* (Morelet), the Panama disease as a result of *Fusarium oxysporum* Scheele f. sp. *Cubense* infestation, the banana bunchy top virus (BBTV) transmitted by the aphid *Pentalonia nigronervosa* (Coquerel), the Moko disease caused by the bacterium *Ralstonia solanacearum* (Smith), and several others such as reduced yield, toppling, reduced number, size and

yellowing of the leaves, etc., caused by plant-parasitic nematodes (PPN). The most common PPN are *H. multicinctus* (Cobb), *M. incognita* (Kofoed and White), *M. javanica* (Treub), *Pratylenchus goodey* (Sher and Allen), and *R. similis* (Cobb and Thorne) (Jones, 2007; Soto, 2008; Ploetz et al., 2003). Plant-parasitic nematodes inflict substantial economic losses on commercial banana plantations (Pocasangre et al., 2015; Soto, 2008; Ploetz et al., 2003). On average, worldwide losses due to PPN in bananas are estimated to be around 19.7%, with potential losses rising to 80% based on environmental conditions and agricultural practices used in the plantations (Araya, 2003; Niere et al., 1999). In Costa Rica, the primary drivers of a yield reduction of 30% to 50% in banana crops are *R. similis* and *H. multicinctus* (Castro et al., 2005).

Currently, conventional control methods for PPN involve two to three applications of nematicides annually. Traditional control methods include effective cultural practices such as soil management, optimal drainage, the use of cover crops, and the application of organic matter (Pocasangre, 2013). Research on the use of biological control agents such as bacteria, endophytic fungi, and mycorrhiza is currently underway. These biological agents form microbial communities in the rhizosphere with the capacity to improve root health and overall plant development through suppression of banana PPN (Sikora et al., 2008; Sikora and Pocasangre, 2006; Zum Felde et al., 2005). Endophytic fungi are organisms with the ability to colonize internal plant tissue in a commensalism, mutualistic, or pathogenic relationship (Riveros, 2010; Sikora and Schuster, 1998). Several genera of fungi with antagonistic properties toward plant pathogens have been identified. Among those are *Trichoderma* and *Fusarium*, which are abundant in plant tissues and present high competition against PPN in bananas and plantains (Chaves, 2007; Zum Felde et al., 2006; Pocasangre et al., 2000).

To date, there is no evidence that specific nematode control can be achieved solely through the use of organic amendments (Timper, 2014). To anticipate and prevent yield losses in bananas due to PPN, more studies concerning the effects of biocontrol agents and cultural practices should be conducted. Therefore, this study aims to evaluate the combined effects of *Trichoderma atroviride* strains and cocoa-based organic amendments on the biocontrol of plant-parasitic nematodes in three Cavendish banana cultivars.

II. MATERIALS AND METHODS

Field site

To investigate these objectives, field trials were conducted from February to November 2015 (10 months) at the

EARTH University commercial banana farm (Las Mercedes of Guácimo, Limón province), in the humid tropical region of Costa Rica (GPS coordinates 10° 12' 45" N 83° 35' 39" W), at 39 m above sea level. Meteorological stations at the farm recorded an average annual temperature of 25 °C, a relative humidity of 90%, and an average precipitation of 4315 mm (EARTH University, 2014).

Experimental design

The experimental area of approximately one hectare was divided into three equal blocks arranged in a continuous layout. Each block was further subdivided into three sub-blocks, each measuring 13.2 m by 45 m, separated by tertiary drains, resulting in a total of 9 sub-blocks. These sub-blocks were then divided into three small plots of the same size, leading to a total of 27 small plots. Each small plot was divided into three sections, which created nine sections per sub-block, corresponding to nine treatments, each treatment repeated three times (Table 1). Each sub-block contained six rows with 20 plants each, totaling approximately 120 plants. The plants were spaced 2.4 m apart between rows and 2.2 m apart within rows (Ochoa and Spiegeler, 2014).

Treatments

The experiment consisted of evaluating the combined application of *T. atroviride* strains Endo 1 (E1) and Endo 2 (E2) with organic amendments (OA). Fungi and OA were applied in front of the daughter plants from three cultivars of Cavendish bananas: Boniface (B), Grand Naine (GN), and Williams (W). Control plots were established for each cultivar without fungi or organic amendments. Treatments are shown in Table 1.

Table 1. The effects of the combined application of *T. atroviride* strains Endo 1 and Endo 2 with organic amendments on three cultivars of Cavendish bananas (Boniface, Grand Naine and Williams).

Treatment	Description
BE1OA	Boniface + Endo 1 + Organic Amendments
BE2OA	Boniface + Endo 2 + Organic Amendments
BC	Boniface Control
GNE1OA	Gran Nain + Endo 1 + Organic Amendments
GNE2OA	Gran Nain + Endo 2 + Organic Amendments
GNC	Gran Nain Control
WE1OA	Williams + Endo 1 + Organic Amendments
WE2OA	Williams + Endo 2 + Organic Amendments
WC	Williams Control

Fungal inoculum

The *T. atroviride* strains E1 and E2 were prepared from inocula preserved in the Cryo Bank of the Natural Sciences laboratory at EARTH University. Propagules from each fungal strain were cultured on 9-cm-diameter Petri dishes containing potato dextrose agar (PDA). Two weeks after incubation at 24 °C, mycelia were scraped from PDA dishes, washed with sterile distilled water, filtered with a piece of cloth to obtain a microspore solution, and transferred into 5 L jugs. Subsequently, the concentration of microspores in the final solution was determined using a hemocytometer under a light microscope.

Organic amendment

Cocoa fruit husks, as a remnant of the harvest (under organic plantations), were chopped into smaller pieces before being composted. The pH and temperature of the compost were monitored at two-week intervals until it was ready for use. The mixture was stirred every third night and watered as needed. The organic material was allowed to sit for three months and cured for an additional two weeks before being utilized.

Field application of *T. atroviride* strains and organic amendment

Five hundred milliliters of 4.0×10^4 cfu/mL *T. atroviride* E1 and E2 microspores and 1.5 kg of organic amendments were applied twice on 17/03/2015 and 30/04/2015 at the stem base of each selected plant.

Plant growth measurements

Plant measurements were carried out three times at a three-month interval (on 15/02/2015, 26/05/2015, and 25/08/2015). The first measurement was carried out one month before *T. atroviride* and organic amendment applications. Five of the 12 previously identified plants were randomly selected from each plot to measure plant height in meters and pseudostem circumference in centimeters. Height was taken from the plant base to the start point of the leaf flag, and pseudostem circumference was measured one meter from the plant base (Rosales *et al.*, 2008).

Root sampling and root health analyses

Twelve plants were selected from each plot, marked with identification tags, and grouped into four sets of three plants for root sampling. A small hole measuring 13 cm long, 13 cm wide, and 30 cm deep was excavated 10 cm from the succession sucker using a shovel, and roots were collected in plastic bags. Root samples were taken within 59 days interval between the first (29/04/2015) and second sampling (27/06/2015) and 54 days between the second and third samplings (10/09/2015). Roots were washed with tap water to remove soil and debris, weighted, and cut to separate functional roots from non-functional roots, and the root

health index (RHI), root diameter, and necrosis index (NI) were assessed (Rosales *et al.*, 2008). The RHI is a parameter that measures the non-functional root percentage of a sample. A scale of 1 to 9 was used to evaluate RHI, where 1 represents a lower percentage of non-functional roots and 9 represents the highest percentage of non-functional roots, as shown in Table 2. For measuring root diameter and NI, five roots from each sample were randomly selected. The root diameter was measured using a graduated Vernier caliper, and the average diameter for the five roots was calculated. To assess root NI, the roots were cut into 5-cm pieces and dissected longitudinally with a scalpel. The root NI was estimated by measuring the percentage of damage in five root segments. Roots with no necrotic tissue received a score of 0%, roots with a quarter of the tissue affected by necrosis received a score of 5%, those with half of the tissue necrotic received a score of 10%, and a 20% score was attributed to fully necrotic root segments.

Table 2. Scale parameters used to measure root health index (Rosales *et al.*, 2008).

Root health index (RHI)	Non-functional root percentage ranges (%)
1	0 - 10
2	11 - 20
3	21 - 30
4	31 - 40
5	41 - 50
6	51 - 60
7	61 - 70
8	71 - 80
9	> 80

Nematode extraction, identification and quantification

Ten grams of roots were weighed and blended at low speed for 10 seconds and then at high speed for five seconds with a Vitamix E310 Explorian (120V) blender. The blend was sieved through 60 µm, 140 µm, and 500 µm mesh sieves, and the resulting suspension was collected into a beaker to obtain a 100 mL root suspension in water. Two milliliters of aliquots were taken from the 100 mL and transferred into a counting plate. The number of *H. multicinctus*, *M. incognita*, and *R. similis* were counted under a light microscope (Rosales *et al.*, 2008).

Statistical analysis

Results were analyzed using InfoStat statistical software (Di Rienzo *et al.*, 2020) through analysis of variance (ANOVA) for each evaluated variable. An LSD Fisher comparison test

at a 5% significance level was performed on variables that showed significant differences between treatments.

III. RESULTS

Effects on plant growth

Plant height and plant pseudostem girth increased over time, though treatments and controls did not differ significantly ($p > 0.05$) (Figure 1). Banana Cultivars showed a similar growth tendency over time. Plant height and pseudostem girth of Bonifacio, Grand Naine, and Williams were statistically similar over time ($p > 0.05$) (Figure 2). Associations of E1+OA and E2+OA with the cultivars showed no significant differences for both plant height and pseudostem girth variables ($p > 0.05$). Treatment BE1OA had greater growth in height, while WE1OA had lesser growth throughout time. On the other hand, treatments BE1OA and GNE1OA showed higher total averages of plant pseudostem girth superior to 47 cm (Table 3). Treatments with greater average height showed high pseudostem girth, which showed a positive and direct relationship between both growth parameters.

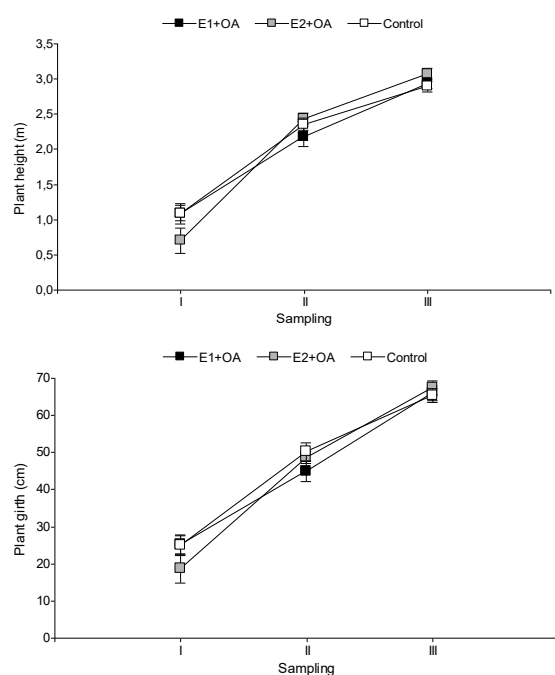


Fig.1. Effect of combined endophytic fungi and organic amendments (E1+OA and E2+OA) on plant height and pseudostem girth during 113 days.

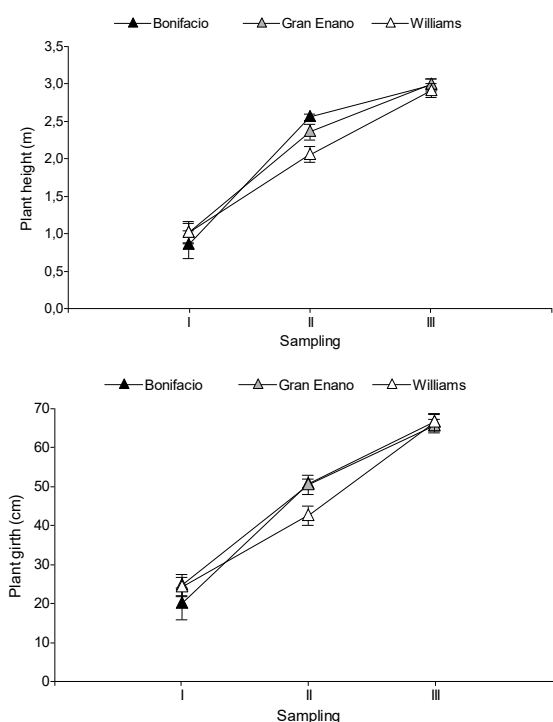


Fig.2. Banana height and pseudostem girth behavior over time.

Table 3. Effect of treatments on plant height and plant pseudostem girth.

Treatment	Height (m)	Pseudostem girth (cm)
BE1OA	2.27 a	48.38 a
BE2OA	1.93 a	41.98 a
BC	2.13 a	46.60 a
GNE1OA	2.07 a	47.22 a
GNE2OA	2.18 a	46.80 a
GNC	2.12 a	46.71 a
WE1OA	1.86 a	40.70 a
WE2OA	2.03 a	46.16 a
WC	2.09 a	46.78 a

† Averages with similar lowercase letters are not significantly different ($p > 0.05$).

Effects on root health: root health index and necrotic index

The associations (E1+OA and E2+OA) showed no significant differences ($p > 0.05$) regarding RHI and NI when compared to control. Both associations reduced RHI and NI, being more efficient for the E2+OA (Figure 3).

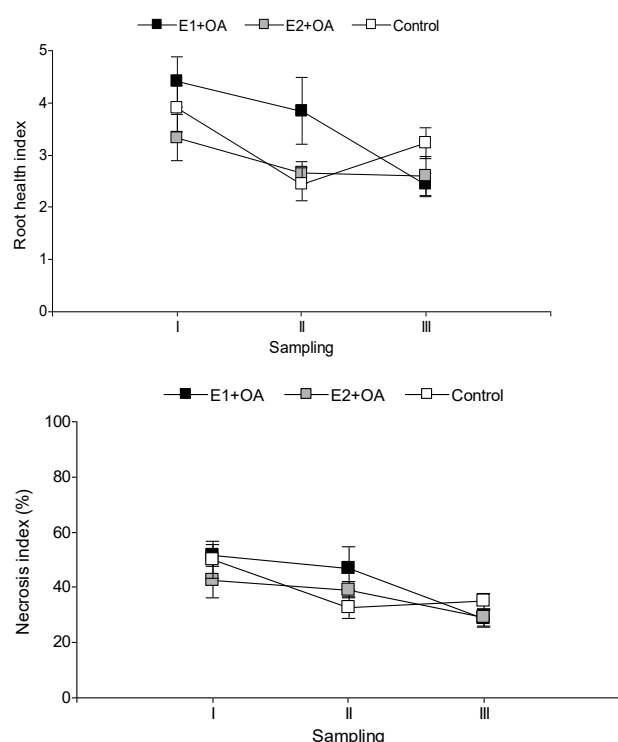


Fig.3. Effect of combined endophytic fungi and organic amendments (E1+OA and E2+OA) on banana root health and necrosis indices during three root sampling periods ($p > 0.05$).

The RHI and NI did not differ significantly among cultivars. The Bonifacio cultivar had a higher percentage of RHI than Williams and Grand Nain (Figure 4). The RHI showed a declining tendency over time. Treatments such as BE2OA, BC, and WE2OA indicated a lower total average of 2.7, 2.7, and 2.8 for RHI, respectively. Treatments GNE2OA and WE1OA showed a significant improvement in root health, lowering its RHI from 4.44 in the first sampling to 2.22 in the third sampling, achieving a 50.0% reduction (Table 4).

Table 4. Effect of treatments on the root health index.

Treatment	Days after application		
	0	59	113
BE1OA	4.78 a	3.56 a	2.78 ab
BE2OA	3.00 a	2.44 a	2.67 ab
BC	2.78 a	2.78 a	2.67 ab
GNE1OA	3.89 a	4.67 a	2.33 a
GNE2OA	4.44 a	2.56 a	2.22 a
GNC	4.56 a	2.22 a	3.00 ab
WE1OA	4.44 a	2.56 a	2.22 a
WE2OA	2.56 a	3.00 a	2.89 ab
WC	4.33 a	2.33 a	4.00 b

† Averages with similar lowercase letters are not significantly different ($p > 0.05$).

Overall, there was a reduction in NI as a result of each treatment throughout time. In the treatments BC, BE2OA, and WE2OA, the average root area with necrotic damage decreased by 33.46%, 33.98%, and 36.94%, respectively. Treatment WE1OA showed the greatest reduction in necrotic damage, with its NI decreasing from 59.89% during the first sampling period to 25.67% in the third sampling, resulting in a 57.14% reduction. For the Bonifacio cultivar, the E2+OA treatment yielded the best result.

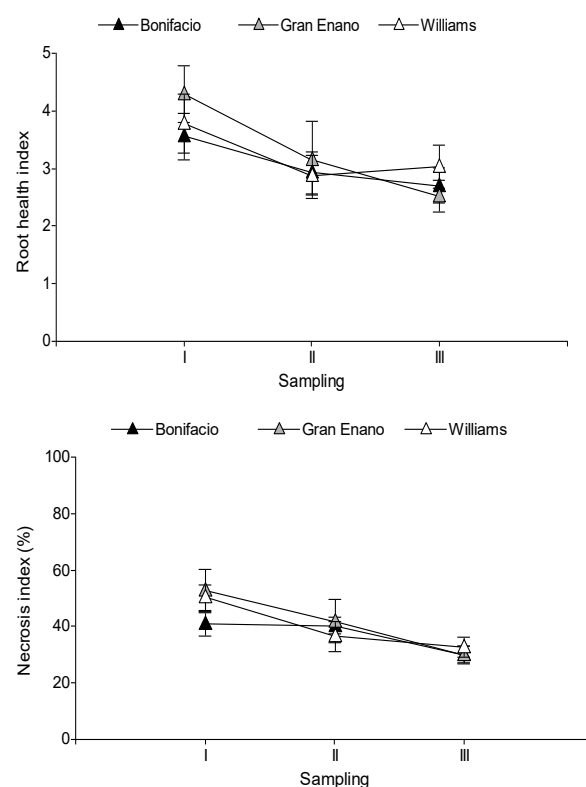


Fig.4. Root health and necrosis indices of banana cultivars over time.

Effects on root health: functional root weight and dead root weight

There was an increase in FRW over time for all treatments. Treatments and controls showed no significant difference throughout time. However, the plots treated with E2+OA and control had the highest functional roots compared to those treated with E1+OA (Figure 5). The FRW of banana cultivars increased over sampling time. Although there were no significant differences among cultivars, Bonifacio and Williams had the highest FRW, followed by the Grand Naine (Figure 6). While there were no significant differences in FRW among the banana cultivars and their interactions with *T. atroviride* during the first and third sampling periods, a significant difference was noted during

the second sampling period. Treatments BE2OA and WC had the highest FRW over time (Table 7). Regarding dead root weight, treatments were significantly different during the first and third samplings and remained similar in the second sampling. Treatment GNE2OA had the highest and most significant reduction of the total percentage of dead roots of 74% (Table 8). Though FRW increased, there were no significant differences over time.

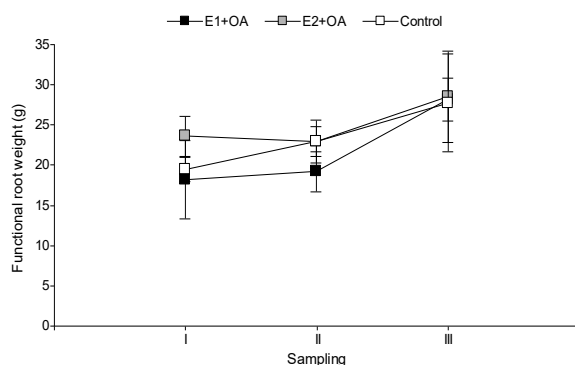


Fig.5. Effect of combined endophytic fungi and organic amendments (E1+OA and E2+OA) on functional root weight over three sampling periods.

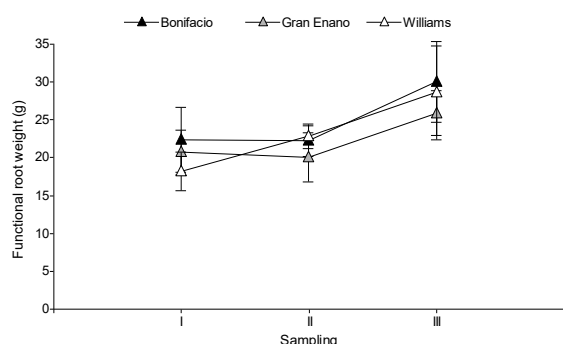


Fig.6. Functional root weight behavior for Bonifacio, Gran Naine, and Williams over time.

Table 7. Functional root weight of each treatment throughout time.

Treatment	Sample (g)		
	I	II	III
BE1OA	24.56 a	19.36 ab	27.09 a
BE2OA	22.08 a	28.90 b	37.79 a
BC	20.50 a	18.41 ab	25.14 a
GNE1OA	19.28 a	15.49 a	31.35 a
GNE2OA	25.55 b	18.79 ab	26.17 a
GNC	17.32 a	25.76 ab	20.06 a
WE1OA	10.73 a	22.75 ab	26.05 a

Treatment	Sample (g)		
	I	II	III
WE2OA	23.13 a	21.13 ab	21.63 a
WC	20.71 a	24.61 ab	38.07 a

† Averages with similar lowercase letters are not significantly different ($p > 0.05$).

Table 8. Effect of treatments on dead root weight throughout time.

Treatment	Days after application		
	0	42	84
BE1OA	17.58 abc	17.32 a	10.68 ab
BE2OA	12.22 ab	17.23 a	9.86 ab
BC	11.70 ab	14.38 a	10.71 ab
GNE1OA	8.31 a	13.63 a	8.04 ab
GNE2OA	23.40 c	14.93 a	6.00 a
GNC	14.46 abc	8.41 a	10.84 ab
WE1OA	8.50 a	16.91 a	7.15 a
WE2OA	9.18 a	11.70 a	11.29 ab
WC	19.69 bc	8.70 a	14.31 b

† Averages with similar lowercase letters are not significantly different ($p > 0.05$).

Effects on plant-parasitic nematodes

The root population densities of *R. similis*, *M. incognita*, and *H. multicinctus* varied depending on the treatments applied. Although there were no significant overall differences in *R. similis* population densities, the combined application of E1+OA and E2+OA significantly reduced the *R. similis* populations at 84 and 42 days after application compared to the control (Figure 7). In the case of *M. incognita*, there were differences among treatments during the three sampling periods. The E1+OA and E2+OA treatments resulted in slight reductions in the population, with decreases of 33% and 36%, respectively, compared to the control. The population densities of *M. incognita* remained constant in the control treatment throughout all three sampling periods (Figure 7). For *H. multicinctus*, no significant differences between treatments were observed. However, the application of E1+OA consistently suppressed the population. Both E2+OA and control treatments showed a reduction in *H. multicinctus* densities from the first to the second sampling period; however, the population density increased again from the second to the third sampling (Figure 7).

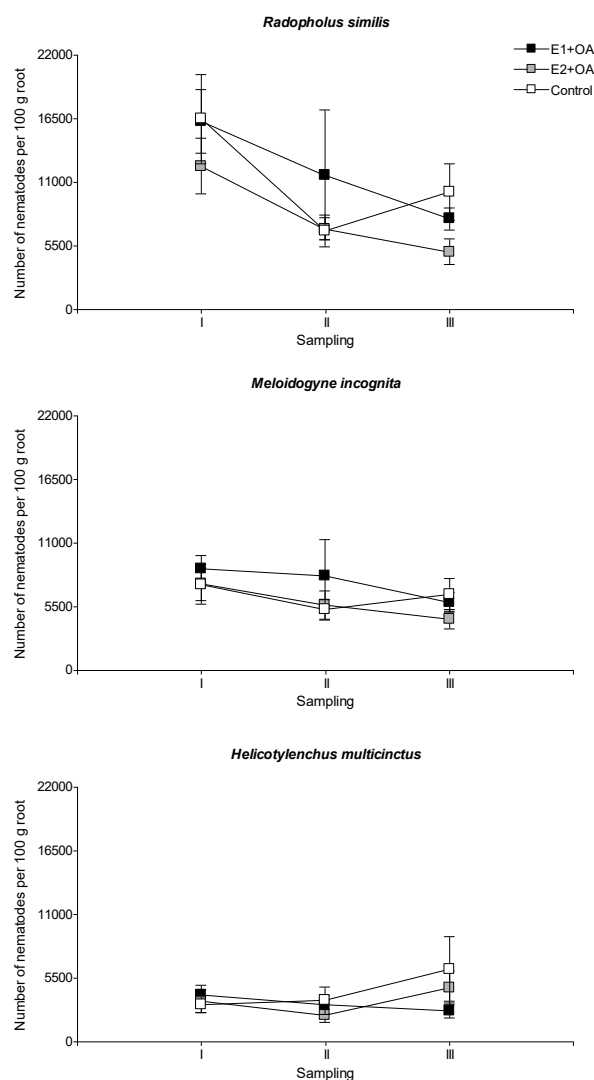


Fig.7. Effect of combined endophytic fungi and organic amendments (E1+OA and E2+OA) on plant-parasitic nematode populations during three sampling periods ($p > 0.05$)

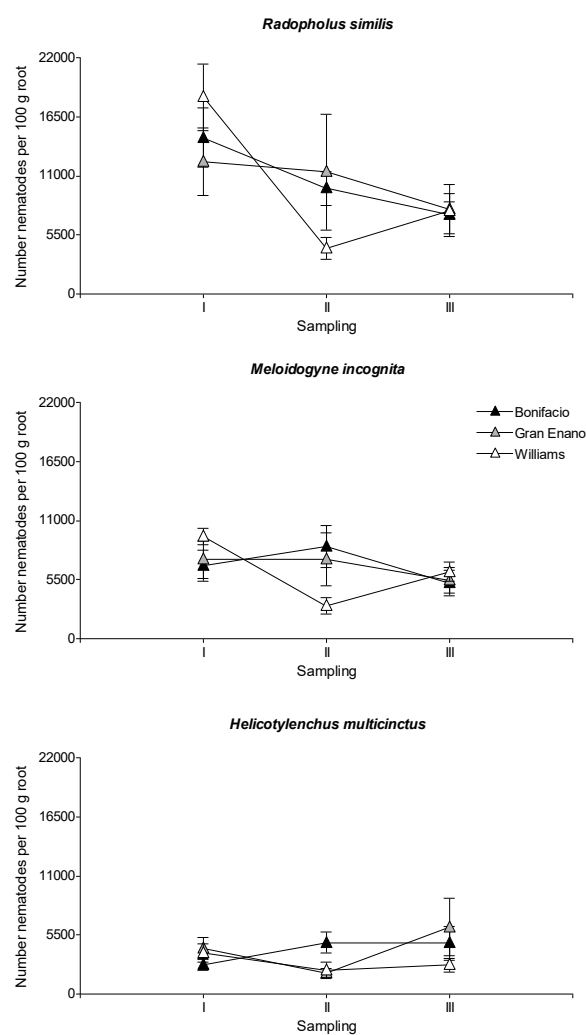


Fig.8. Plant-parasitic nematode populations behaviour on B, GN, and W cultivars throughout time (averages with similar lowercase letter are not significantly different; $p > 0.05$).

The GN cultivar exhibited the lowest incidence of *R. similis*, followed by the B and W cultivars (see Figure 8). However, these differences were not significantly different from the control group. While there were no significant variations in the *M. incognita* population among the banana cultivars, both the B and GN cultivars demonstrated a greater reduction in this phytonematode between the second and third sampling periods. The behaviors of *M. incognita* and *R. similis* were similar in the W cultivar. For the *H. multicinctus* nematode, its population increased over time in the B cultivar. In contrast, the GN cultivar showed a decrease in population between the first and second samplings, followed by an increase between the second and third samplings. The *H. multicinctus* population in the Williams cultivar remained stable throughout the study.

Regarding the effects of fungal strains and cocoa amendment application on the total PPN densities, there were no significant differences between treatments. However, the association between *T. atroviride* and cocoa organic amendment reduced the total population PPN. Treatment E1+OA had more impact in reducing PPN (41%), followed by treatment E2+OA and control with 34% and 23% reductions, respectively. There were no significant differences in total PPN between B, GN, and W cultivars. The total number of PPN was slightly reduced for both B and GN over time, while for the W cultivars, it decreased between the first and second samplings followed by an increase between the second and third samplings (Figure 9).

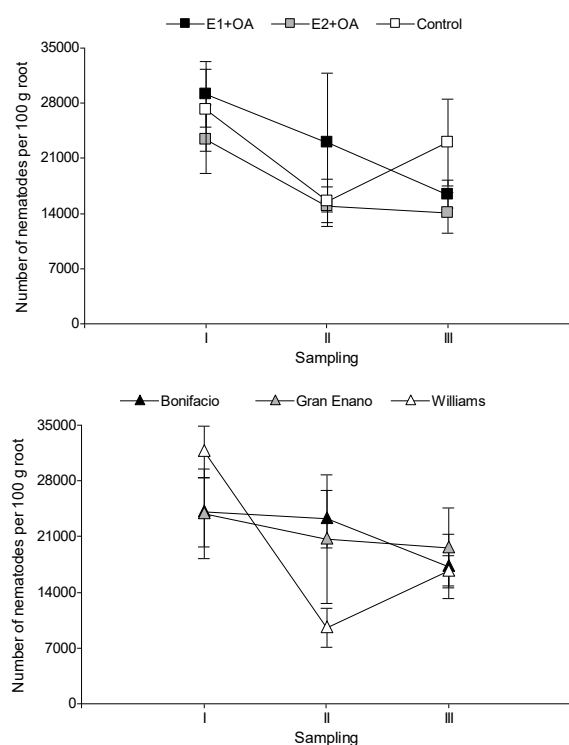


Fig.9. Effect of combined endophytic fungi and organic amendments (E1+OA and E2+OA) and banana cultivars on total plant-parasitic nematode populations during three sampling periods ($p > 0.05$)

Table 4. Population density of PPN per 100g of B, GN, and W cultivars under different treatments.

Treatment	PPN/100 g root			Total
	<i>R. similis</i>	<i>M. incognita</i>	<i>H. multicinctus</i>	
BE1OA	12611 a	8926 b	3889 c	25426 d
BE2OA	7370 a	5630 b	3981 c	16981 d
BC	11759 a	5981 b	4389 c	22130 d
GNE1OA	12000 a	7074 b	3130 c	22204 d
GNE2OA	8315 a	5500 b	3871 c	17686 d
GNC	11241 a	7630 b	5426 c	24296 d
WE1OA	11185 a	6815 b	2907 c	20907 d
WE2OA	8704 a	6407 b	2596 c	17707 d
WC	10426 a	5611 b	3241 c	19278 d

† Averages with similar lowercase letter are not significantly different ($p > 0.05$).

No significant differences in plant-parasitic nematode (PPN) populations over time ($p > 0.05$) were observed among the treatments. However, interesting interactions were noted between cultivars and control treatments. The treatments BE2OA, GNE2OA, and WE2OA exhibited lower populations of *R. similis*, averaging fewer than 9,000

nematodes per 100 grams of roots. For *M. incognita* the treatments GNE2OA, WC, and BE2OA recorded the lowest population densities, with an average below 6,000 nematodes per 100 grams of roots. In contrast, *H. multicinctus* had populations of fewer than 5,000 nematodes per 100 grams of roots across all treatments. Additionally, the treatments WE1 and WE2 demonstrated even lower PPN populations, averaging below 3,000 nematodes per 100 grams of roots over time (see Table 4).

IV. DISCUSSION

Growth Promotion

Height and pseudostem circumference increased during the experiment, which coincides with the normal plant growth dynamics promoted by division and cell expansion with time (Taiz and Zeiger, 2006). The growth rate of each banana cultivar is a function of its genetics and the influence of abiotic conditions such as nutrition, climate, and crop cultural management.

Treatments showed no statistical differences with respect to plant height and pseudostem circumference. These results are not in line with those who found that banana plants treated with *T. atroviride* presented greater plant height than the absolute control (Cassambai et al., 2012; Chaves et al., 2009; Menjivar et al., 2006). In another study conducted by Pocasangre et al. (2006), a six-centimeters plant height increase was recorded in a banana plant nursery protected with *T. atroviride* and *Fusarium oxysporum*. Pocasangre et al. (2004) found that endophytic fungi application in banana plants promotes growth and leads to an increase in length and root weight, in addition to an increase in pseudostem diameter. Radical biomass production and phytonematode biocontrol provided by endophytic fungi increase the radical efficiency of nutrient exploration (Sikora and Pocasangre, 2006). This factor is largely due to the antagonistic characteristics of *T. harzianum* 1295-27, which promote phosphate solubilization and other micronutrient availability necessary for plant growth (Altomare et al., 1999).

There is evidence that *T. atroviride* produces indole-3-acetic acid (IAA), which is used to promote plant growth (Gupta et al., 2014). Association of organic amendments with endophytic fungi increases secretion and production of organic acids such as gluconic, citric, and fumaric acids and phenols, which solubilize and increase the availability of nutrients such as phosphorus, zinc, iron, and manganese, which are necessary for plant development (Yedidia et al., 2011; Altomare et al., 1999). Apart from nutrition improvement, organic amendments also provide a favorable environment for endophytic fungi colonization on plant tissue, thus ensuring maximum growth promotion (Stirling,

2014). The difference in the results of this experiment might be related to incompatibilities between fungi and organic amendments. Therefore, it is necessary to study the compatibility of *T. atroviride* and cacao fruit husk organic amendments so as to obtain expected results (Guetsky *et al.*, 2001).

In this study, there was a noticeable reduction in PPN and root damage in the third sample compared to the earlier samplings, indicating that fungi and amendments need time to establish properly in the rhizosphere, and their effect is not immediate but more sustainable over time. Therefore, it is advisable to monitor similar experiments for an extended period to comprehensively determine the biocontrol effects of the fungi and organic amendments on phytonematodes over an extended period.

Root Health

Root health depends on many factors and is highly related to phytonematode population density. In this research, it was quite notorious that the necrosis index, as well as root health, improved substantially in the third sample in comparison with the first sample that reported the highest root damage percentages. Root health improvement and a decrease in root necrosis were observed over time. Pocasangre *et al.* (2004) stated that radical health improvement could be associated with biocontrol and root biomass production enhanced by endophytic fungi and organic amendments, which increase radical nutrient exploration efficiency. Root health improvement implies a greater ability of the plant to tolerate pathogens.

Although no statistically significant differences were obtained between treatments in terms of functional and dead root parameters, functional root weight increases and a decrease in dead root weight were registered over the three samples. This indicates that endophytic fungi and organic amendments have the possibility of improving radical health, regardless of the banana cultivar. Similar results were found by Ochoa and Spiegelner (2014), who obtained functional root weight increases in Boniface and Grand Nain cultivars with Endo 1 application. Similarly, Menjivar *et al.* (2006) found that banana plants inoculated with endophytic fungi on Bananita and Carmen farms in Costa Rica had a superior functional root weight than the absolute control, although there were no significant differences within treatments. In the same investigation, *Trichoderma* sp. and *Fusarium* sp. fungi had statistically superior functional root weight than the absolute control at the FORMOSA farm in Costa Rica. The functional root weight parameter is closely related to plant growth and shows water and nutrient absorption capacity as well as plant anchorage. Rosales (2008) established that there exists a direct relationship between functional root weights and banana

productivity. The weight values of functional roots of BE2OA (37.8 g per plant) and GNE1OA (31.4 g per plant) are in the root weight ranges reported by Rosales (2008), who studied the relationship between functional roots and productivity in six banana-growing cantons in Costa Rica. In three cantons of higher banana productivity, functional root weight obtained in Siquirres was between 36 g and 135 g per plant; in Matina, it was between 35 g to 143 g per plant; and in Talamanca, it was between 31 g to 114 g per plant.

For the dead roots variable, treatments showed values that differed from the absolute control for the first and third samples. The third sample stands out a lot, having treatments with the lowest dead root weight values: GNE2OA with 6.00 g, WE1OA with 7.15 g, and GNE1OA with 8.04 g. Similar results were found by Menjivar *et al.* (2006), who obtained 7.1 g of dead roots in the Valery banana cultivar applied with *T. atroviride* on the FORMOSA farm in Costa Rica. This reduction in the number of dead roots coincides with the increase in functional root weight obtained in this research, which shows a positive effect of treatments on radical health. Dead root weight is a radical health indicator that reflects the damage caused by nematodes, rot caused by excess water in the soil, and physical damage to the roots. Per 10 g of dead roots, banana productivity is reduced by 88 boxes/ha on average (Rosales, 2008).

The association of *T. atroviride* with organic amendments did not affect root diameter and total root weight. These results differ from those obtained by Cassambai *et al.* (2012), Chaves *et al.* (2009), and Chaves (2007), who found a significant increase in the total root weight of bananas in greenhouses with *Trichoderma* sp. application. Several studies have shown that endophytic fungi promote growth in banana plants through increased root system development (Chaves *et al.*, 2009; Chaves, 2007; Pocasangre *et al.*, 2004; Meneses, 2003; Pocasangre, 2003, 2002). Endophytic fungi inoculations increased the total weight of Grand Nain roots by 39 % (Meneses, 2003). The differences in the obtained results of this study may be caused by failure or miniature fungi colonization of root tissue, modified ecology with the use of cocoa-based organic amendments, and nitrogen fertilizer and herbicide application in the experimental field (Guetsky *et al.*, 2001). According to Luc *et al.* (2005), antagonistic agents' potential can be increased by the use of organic amendments and green manure in various ways. Amendments play a central role in soil fertility improvement in physical, chemical, and biological aspects. This provides an ideal environment in the rhizosphere that stimulates the colonization of plant tissue by endophytic fungi (Stirling, 2014). Good colonization of *Trichoderma*

sp. allows this fungus to cause substantial changes in plants' metabolism, radical health promotion, and nutrient availability, hence increasing crop growth. Khan *et al.* (2012) also reported an increase in root biomass compared to the absolute control treatment. In the same experiment, Khan *et al.* (2012) postulate that organic amendments improve organic matter composition in the soil, which promotes radical health improvement.

Effects on plant-parasitic nematodes

Although there were no statistically significant differences between the treatments, notable variations in the *R. similis* population were observed among the samples. Specifically, the *R. similis* population in the third sample was lower than in the first, indicating a 49% reduction between these two samples. This suggests that endophytic fungi and the applied organic amendments require time to colonize and exert their biocontrol effects. According to Pocasangre (2000), endophytic fungi exhibit antagonistic effects on *R. similis* in the roots of several banana genetic groups, including Grand Nain, Williams, Gros Michel, FHIA 01, and FHIA 23, leading to a population reduction of 79% to 90%. In a further study, Pocasangre (2002) reported a decrease in the *R. similis* population of between 21% and 83% with the application of *Trichoderma* sp. and *Fusarium* spp. on in vitro plants of the Grand Nain cultivar. Additionally, a field study conducted by Pocasangre *et al.* (2006) demonstrated that inoculation with *T. atroviride* on banana plants reduced the *R. similis* population. This reduction is associated with a significant decrease in the number of females in the root systems of the affected plants (Sikora, 1992). Studies on endophytic bacteria and mycorrhiza have shown that these bacteria enhance plant-mycorrhiza interactions, which in turn stimulate vegetative growth and activate plant defense mechanisms against attacks by phytonematodes (Niere *et al.*, 1999; Alamri *et al.*, 2022). The low effectiveness of endophytic fungi observed in this study may be attributed to rhizosphere soil contamination caused by management practices, such as the application of nematicides and fertilizers. These practices are known to affect biodiversity and disrupt interactions within the soil (Altieri, 1992; Tiwari, 2024), which can significantly reduce the antagonistic potential of the microflora in the rhizosphere (Carroll, 1990).

While there were no statistically significant differences between treatments concerning the reduction of *M. incognita* populations, the combination of endophytic fungi with organic amendments reduced the population of this phytonematode by 33% to 36%. Similarly, there was a trend towards reduced *H. multicinctus* populations with the E1+OA application. Boniface and Grand Naine cultivars also exhibited trends of phytonematode population reduction, despite no statistically significant differences

between them. These findings are consistent with those reported by Ochoa and Spiegeler (2014), who studied phytonematode biocontrol using *T. atroviride* in Boniface, Grand Nain, and Williams cultivars. Other research has shown that applying endophytic fungi can achieve phytonematode population reductions ranging from 15% to 90% in bananas (Cassambai *et al.*, 2012; Chaves *et al.*, 2009; Chaves, 2007; Pocasangre *et al.*, 2004). To optimize the control of plant-parasitic nematodes, it is essential to identify synergistic effects between biocontrol agents and different banana cultivars (Hallmann and Sikora, 2011; Sikora *et al.*, 2010). In this study, no statistically significant differences were observed in the overall total population of plant-parasitic nematodes (PPN) when using the endophytic fungus *T. atroviride* and organic amendments. However, it has been documented that applying organic amendments increases the presence of nematode antagonists in the soil (Stirling, 2011; Luc *et al.*, 2005). Decomposing organic matter produces secondary metabolites such as phenols, nitric acid, and ammonia gas, which can interfere with nematode activity (Hallmann and Sikora, 2011). Khan *et al.* (2012) discovered that combining the *T. harzianum* fungus with organic amendments from neem (*Azadirachta indica*) leaves reduces the population of *M. incognita* in eggplants. Similar findings were reported by Stirling *et al.* (2005), who combined *T. harzianum* with sawdust amendments, successfully decreasing the *M. javanica* population in tomatoes over a two-year experiment. Additionally, incorporating sugar cane residue amendments into the field 23 weeks before planting resulted in an 85% reduction in the *Pratylenchus zeae* nematode population in sugar cane roots 24 weeks after planting (Stirling *et al.*, 2005). These studies indicate that high carbon-to-nitrogen (C/N) ratio amendments act slowly in suppressing nematode populations. Consequently, the organic amendments used in this study did not achieve the expected nematode control, likely due to insufficient time for decomposition and the influence of other agricultural practices, such as chemical fertilizers and insecticides.

V. CONCLUSIONS

This study indicates that combining *Trichoderma atroviride* strains with cocoa-based organic amendments has potential for managing plant-parasitic nematodes in banana cultivation. While no statistically significant reductions were observed, trends toward improved root health and reduced nematode populations were notable, particularly over time. These findings suggest a delayed but possibly sustained biocontrol effect. Further research is needed to isolate the independent and combined impacts of these treatments and to assess their compatibility under varying agricultural conditions.

ACKNOWLEDGMENT

We gratefully acknowledge EARTH University and Staff for their unwavering support and provision of resources throughout the course of this research. Also, we would like to express our sincere gratitude to Dr. Ivânia Sofia Grasiña Esteves [Dept. of Life Sciences (NEMATO-lab) Faculty of Sciences and Technology University of Coimbra, Portugal] for her invaluable assistance in the editing and proofreading of this manuscript.

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Trend Analysis and Seasonal Variability of Market Arrivals and Prices of Mustard in Haryana

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Received: 08 Sep 2025; Received in revised form: 06 Oct 2025; Accepted: 11 Oct 2025; Available online: 18 Oct 2025

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Abstract— This study analyses long-term trends and seasonal variations in market arrivals and prices of mustard in Haryana, focusing on four major markets - Rewari, Bhiwani, Hisar, and Narnaul, over the period 2001–2020. Secondary tri-master data on arrivals and prices were examined using compound growth rates, trend equations, and seasonal indices to capture temporal and seasonal dynamics. Results indicate that arrivals remained largely stable until 2017, followed by a notable increase after implementation of e-NAM, with Rewari consistently recording the highest volumes. The compound growth rate of arrivals was positive in all markets except Hisar, with Narnaul registering the highest growth (12.9%). Prices showed a steady upward trend across all markets, with a state average growth rate of 7.4 per cent per annum and a high coefficient of determination ($R^2 = 0.96$). Seasonal analysis revealed a marked concentration of arrivals during March - June, peaking at 168.4 per cent above the annual average in 2019-20, while the lean seasons of July - October and November - February recorded substantial deficits. Prices exhibited an inverse seasonal relationship, with the lowest index in March - June 2012-13 (-1718.3%) and the highest in July - October 2012-13 (995.7%). These findings underscore the need for improved storage facilities, staggered marketing, and effective policy interventions; such as rural warehouses, pledge finance schemes, and local processing units – to stabilise prices, reduce post-harvest distress sales, and enhance farmer income.



Keywords— Mustard, market arrivals, price trends, seasonal variation, compound growth rate, e-NAM

I. INTRODUCTION

Agricultural economics and statistical analysis frequently come together to investigate the fascinating relationship between seasonal variability and market dynamics. The mustard (*Brassica juncea*) market in the Indian state of Haryana's four districts - Rewari, Bhiwani, Hisar, and Narnaul, is a prime example of this fascinating topic. A thorough analysis of the seasonal patterns and variations in market arrivals and prices in these districts might yield important insights into the larger agricultural ecosystem. Mustard occupies a central place in Haryana's agricultural environment.

Haryana's mustard market is defined by its own seasonal dynamics, with year-round significant swings in market arrivals and pricing. A number of variables, such as climatic trends, planting and harvesting dates, consumer

demand, and governmental regulations, affect these seasonal variations. To make informed decisions about production, marketing, and price stability measures, farmers, policymakers, and other stakeholders in the agricultural sector must have a thorough understanding of the patterns and seasonal fluctuations in mustard market arrivals and pricing.

The purpose of this research study is to give a thorough analysis of the seasonal variability and trends in mustard market arrivals and pricing in Haryana. This study will provide light on historical trends and variations in mustard market arrivals and pricing by utilizing analytical techniques and secondary data sources. This will provide insights into the underlying variables influencing these dynamics. In doing so, it will provide important information that will direct government policies, market

interventions, and agricultural practices in order to maintain the stability and sustainability of mustard commerce and cultivation in the area.

II. REVIEW OF LITERATURE

A review of the literature provides valuable insights into existing research, methodologies, and findings related to the trend analysis and seasonal variability of market arrivals and prices of mustard in Haryana. This review highlights the key themes and contributions of prior studies in this field.

Seasonal Variability in Agricultural Markets:

Research on seasonal variations in agricultural markets is a foundational theme. Studies have consistently emphasized the importance of understanding the seasonality in crop production and its impact on market arrivals. These findings serve as the basis for exploring mustard in Haryana, a region marked by distinct agricultural seasons (Ahn et al., 2018).

Mustard Market Dynamics: Research specifically focused on the mustard market in Haryana indicates that it is not immune to the effects of seasonal fluctuations. Studies have revealed that mustard arrivals and prices are influenced by a variety of factors, including climate, planting and harvesting schedules, and government policies. These dynamics have led to concerns about the economic stability of mustard cultivation (Gupta et al., 2019).

Price Trends and Inflation Analysis: Several studies have undertaken price trend analysis to understand the broader inflationary trends associated with mustard. This research has highlighted the role of mustard in the economic landscape, with its price trends reflecting evolving consumer demand and supply dynamics (Jain & Verma, 2020).

Regional Variability and Comparative Analysis:

Some studies have taken a regional approach, comparing different districts or states within India to examine how market arrivals and prices of mustard vary. This approach is particularly relevant to the present research, which focuses on the specific districts of Rewari, Bhiwani, Hisar, and Narnaul (Kumar et al., 2019).

Government Policies and Interventions:

Government policies play a pivotal role in shaping the mustard market. Previous research has shed light on the impact of government interventions, such as price support schemes and procurement policies, on mustard prices and arrivals. Understanding these interventions is crucial for assessing their effectiveness (Rawat & Yadav, 2018).

Data Sources and Statistical Analysis: Studies have stressed the importance of robust data sources and advanced statistical methodologies for conducting trend analysis and seasonal variability studies. Accurate and comprehensive data are the foundation of sound statistical analysis (Sharma et al., 2019).

Implications for Stakeholders: Research in this area has consistently underlined the implications of seasonal variations and price trends for various stakeholders, including farmers, traders, policymakers, and consumers. The findings are instrumental in making informed decisions and crafting effective policies (Choudhury et al., 2019).

Data and Methodology:

The study is based on secondary data of price & arrival of mustard crop of four markets Rewari, Bhiwani, Hisar and Narnaul for the period 2000-2020 taken tri-masterly. First tri-master is from March-June, Second from July-October and third November-February. To measure the variability in the collated data, the compound growth rates (CGR) of prices and arrival of mustard in selected grain markets were estimated using the following model:

$$Y = ab^t$$

where, Y = prices and arrival of mustard in selected grain markets

a = constant, b = (1+r), r=compound growth rate, t= time

Line graphs were also used to show the trends in prices and arrival of mustard in selected grain markets.

Time-trend analysis

Tri-master data of prices and arrival of mustard in selected grain markets have been used by considering time (Trimaster) as an independent variable and has been regressed against price & arrival to get the trend equation the form

$$Y = a + bt$$

where, Y = price/arrival, a = Intercept, b = Slope and t = time

Measurement of seasonal variation

The seasonal variation can be assumed to be the difference between the actual value and the trend (three-season moving average) value.

Monthly data on general prices and market arrivals were utilized to analyze the seasonal patterns of price and arrival in the selected markets. To estimate seasonal indices, the ratio-to-moving average method was applied. In the markets of Rewari, Bhiwani, Hisar and Narnaul,

districts, Mustard price & arrivals occurred throughout the year, making them suitable for seasonal analysis. A 3-month moving average was employed to calculate the seasonal indices.

The ratio-to-moving average method involves the following steps:

Step 1: A centered 3-month moving average was calculated from the original time series data. This moving average captures both trend and cyclical variations.

Step 2: The original values were divided by their corresponding centered moving averages. This process isolates the seasonal and irregular components as shown in the formula:

$$\frac{Y}{MA} = \frac{T \times S \times C \times 1}{T \times C} = S \times 1$$

Step 3: To eliminate the irregular component, the resulting values were averaged across the same months over multiple years. These averages were then multiplied by 100 to obtain the seasonal indices.

III. RESULTS AND DISCUSSIONS

This section presents the empirical findings of the study on trends and seasonal variations in the market arrivals and prices of mustard in Haryana over the period 2001-2020. The analysis integrates both temporal and seasonal dimensions, supported by compound growth rates, seasonal indices, and graphical trends. Results are

discussed in the context of market dynamics, highlighting patterns across the four selected markets - Rewari, Bhiwani, Hisar, and Narnaul - and at the state level. The discussion further interprets these findings in light of economic implications, with a focus on supply-demand interactions, market seasonality, and potential policy interventions to stabilise prices and optimise farmer returns.

Trend in Market Arrivals of Mustard

The analysis of mustard arrivals in the selected markets of Haryana from 2001 to 2020 (Fig. 1) revealed two distinct phases. Between 2001 and 2017, arrivals exhibited a relatively constant trend with only minor fluctuations, indicating a stable supply chain during this period. From 2017 onwards, however, there was a marked increase in arrivals, possibly due to improved production, enhanced procurement facilities post-e-NAM implementation in 2016 by Haryana, thus giving favourable price expectations. These findings are in coherence with other studies (Bhatia et al., 2022).

Table 1: Compound annual growth rate of prices and arrival of mustard in selected grain markets

Sr. No.	Particular	CGR Arrivals
1	Bhiwani	4.4
2	Rewari	1.8
3	Hisar	-0.6
4	Narnaul	12.9
5	Haryana	2.7

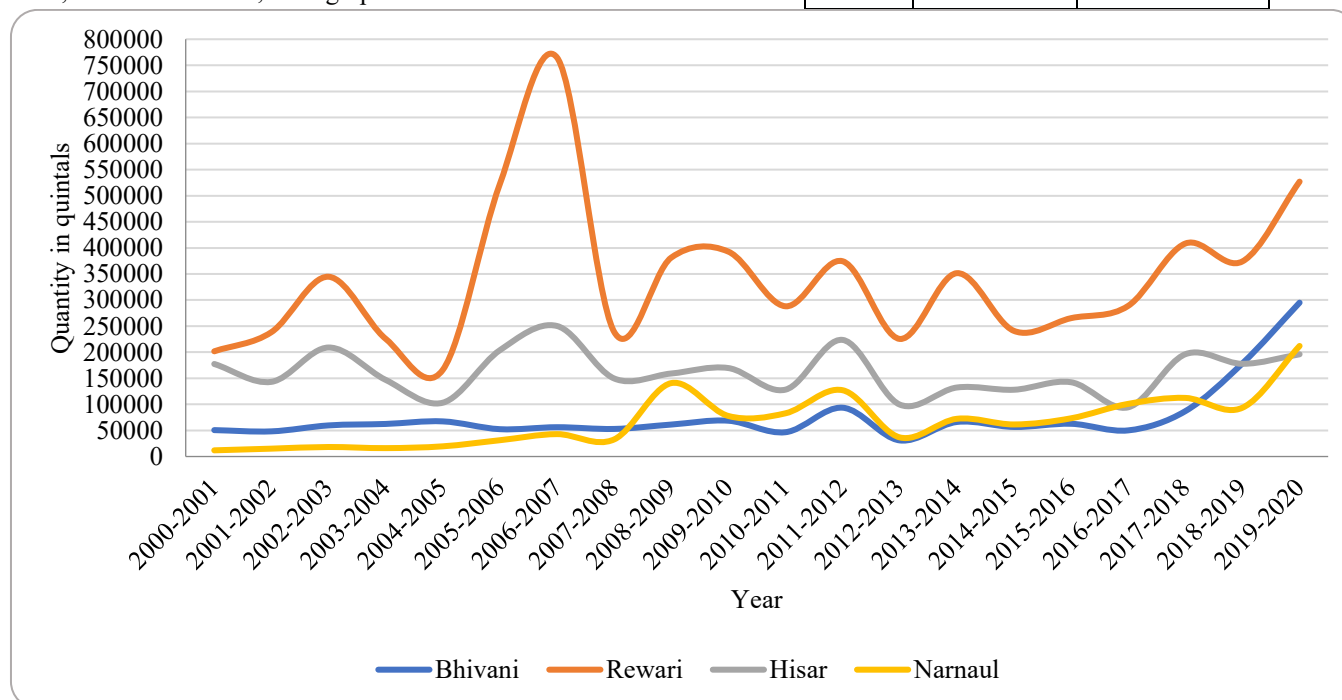


Fig 1. Trends in arrival of mustard in selected grain markets from 2001 to 2020

Among the four markets - Rewari, Bhiwani, Hisar, and Narnaul, Rewari consistently recorded the highest arrivals, underscoring its role as one of the major markets for mustard in the state. The compound growth rate (Table 1) showed positive arrival growth in Bhiwani (4.4%), Rewari (1.8%), Narnaul (12.9%), and Haryana overall (2.7%). Hisar alone recorded a slight negative growth (-0.6%). The particularly high growth in Narnaul highlights its growing role in mustard marketing, which may be linked to improved production of the crop, market infrastructure, etc.

Trend in Market Prices of Mustard

Mustard prices across all selected markets showed a steady and consistent increase during 2001-2020 (Fig. 2). The high coefficient of determination ($R^2 = 0.96$) indicates that time accounted for most of the variation in prices, reflecting strong temporal predictability. Compounded annual growth rate values for prices ranged from 6.5 per cent in Narnaul to 8.0 per cent in Rewari, with a state average of 7.4 per cent per annum (Table 2). The market price trend was found to be similar to market arrival i.e., a sharp increase in prices as well post-e-NAM

adoption by Haryana in 2016. The similar trend was observed by Bhatia et al. (2022).

Table 2: Compound annual growth rate of prices of mustard in selected grain markets

Sr. No.	Particular	CGR Prices
1	Bhiwani	7.9
2	Rewari	8.0
3	Hisar	7.3
4	Narnaul	6.5
5	Haryana	7.4

The simultaneous growth in both arrivals and prices suggests that demand for mustard has expanded sufficiently to absorb increased supply without exerting long-term downward pressure on prices. For producers, this trend translates into better price realisation over time, while for consumers it reflects a gradual rise in edible oil costs.

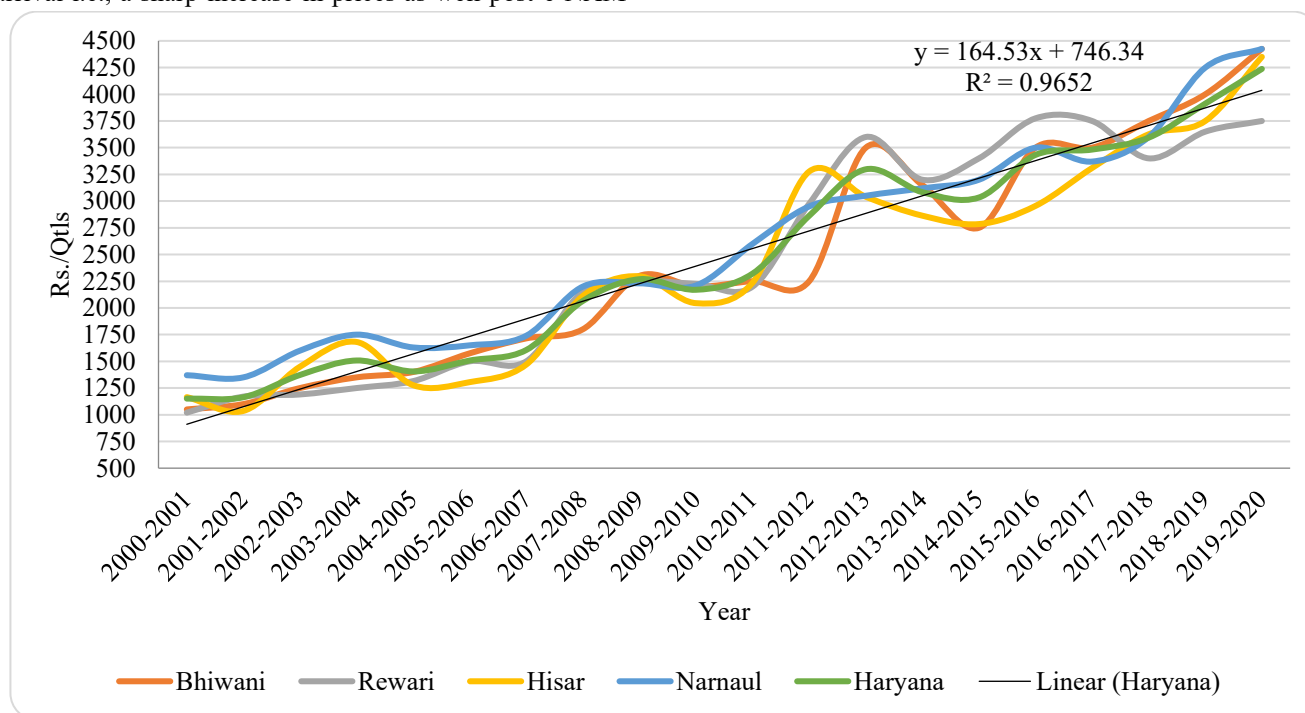


Fig 2. Trends in prices of mustard in selected grain markets from 2001 to 2020

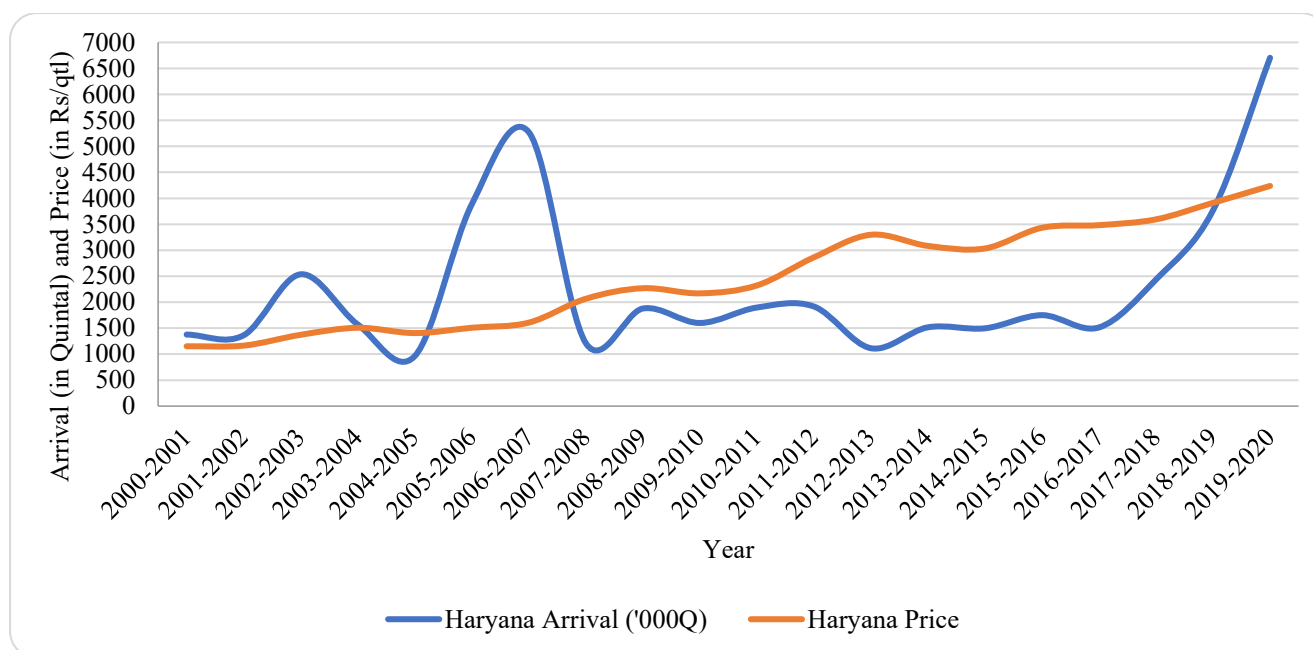


Fig 3. Trends in Prices Arrival of Mustard in Haryana from 2000 to 2020

Seasonal variation in arrivals of mustard

Seasonal indices (Table 3; Fig. 4) revealed a highly pronounced concentration of mustard arrivals during the March – June period, coinciding with the harvest and immediate post-harvest months. During these months, arrivals frequently exceeded the annual average, with indices above 100 in the majority of years. Across the two decades, March – June arrivals were consistently above the annual average, with the highest seasonal index recorded in 2019-20 (168.4%), followed closely by 2006-07 (145.6%) and 2005-06 (114.7%). These figures reflect substantial surpluses entering the market immediately after harvest. In contrast, the July – October and November –

February periods exhibited negative seasonal variations almost every year. The sharpest declines occurred in November – February 2018-19 (-92.3%), November – February 2005-06 (-80.3%), and July – October 2019-20 (-72.1%), indicating minimal market arrivals in these lean months. Several years also displayed wide swings within the same season. As observable, March – June arrivals rose from 48.1 per cent in 2004-05 to 114.7 per cent in 2005-06, before peaking at 145.6 per cent in 2006-07, showing considerable year-to-year volatility. The observations of this study are found consistent with other studies as well (Bhatia et al., 2022; Sarkar et al., 2021).

Table 3: Seasonal variation in arrivals of mustard during the year 2000-2020

Year	Season	Variation	Year	Season	Variation
2000-01	Mar-June	73.6	2010-11	Mar-June	73.6
	July-Oct	-24.7		July-Oct	-32.0
	Nov-Feb	-33.7		Nov-Feb	-64.5
2001-02	Mar-June	60.5	2011-12	Mar-June	115.8
	July-Oct	-26.1		July-Oct	-51.8
	Nov-Feb	-49.9		Nov-Feb	-29.2
2002-03	Mar-June	89.3	2012-13	Mar-June	50.0
	July-Oct	-40.0		July-Oct	-21.9
	Nov-Feb	-33.0		Nov-Feb	-46.6
2003-04	Mar-June	58.2	2013-14	Mar-June	85.3
	July-Oct	-25.2		July-Oct	-36.5

	Nov-Feb	-27.2		Nov-Feb	-40.4
2004-05	Mar-June	48.1	2014-15	Mar-June	68.8
	July-Oct	-20.9		July-Oct	-30.8
	Nov-Feb	-62.5		Nov-Feb	-39.3
2005-06	Mar-June	114.7	2015-16	Mar-June	70.8
	July-Oct	-51.3		July-Oct	-30.4
	Nov-Feb	-80.3		Nov-Feb	-40.5
2006-07	Mar-June	145.6	2016-17	Mar-June	72.6
	July-Oct	-62.2		July-Oct	-31.3
	Nov-Feb	-37.6		Nov-Feb	-63.3
2007-08	Mar-June	62.3	2017-18	Mar-June	113.7
	July-Oct	-27.8		July-Oct	-50.9
	Nov-Feb	-58.3		Nov-Feb	-59.7
2008-09	Mar-June	105.1	2018-19	Mar-June	107.2
	July-Oct	-47.0		July-Oct	-46.0
	Nov-Feb	-51.4		Nov-Feb	-92.3
2009-10	Mar-June	92.1	2019-20	Mar-June	168.4
	July-Oct	-39.6		July-Oct	-72.1
	Nov-Feb	-41.8			

This seasonality pattern suggests that market supply is heavily skewed toward the harvest period, leading to seasonal gluts and lower prices. Such

fluctuations highlight the absence of effective staggered marketing and storage strategies, compelling farmers to sell bulk quantities immediately after harvest.

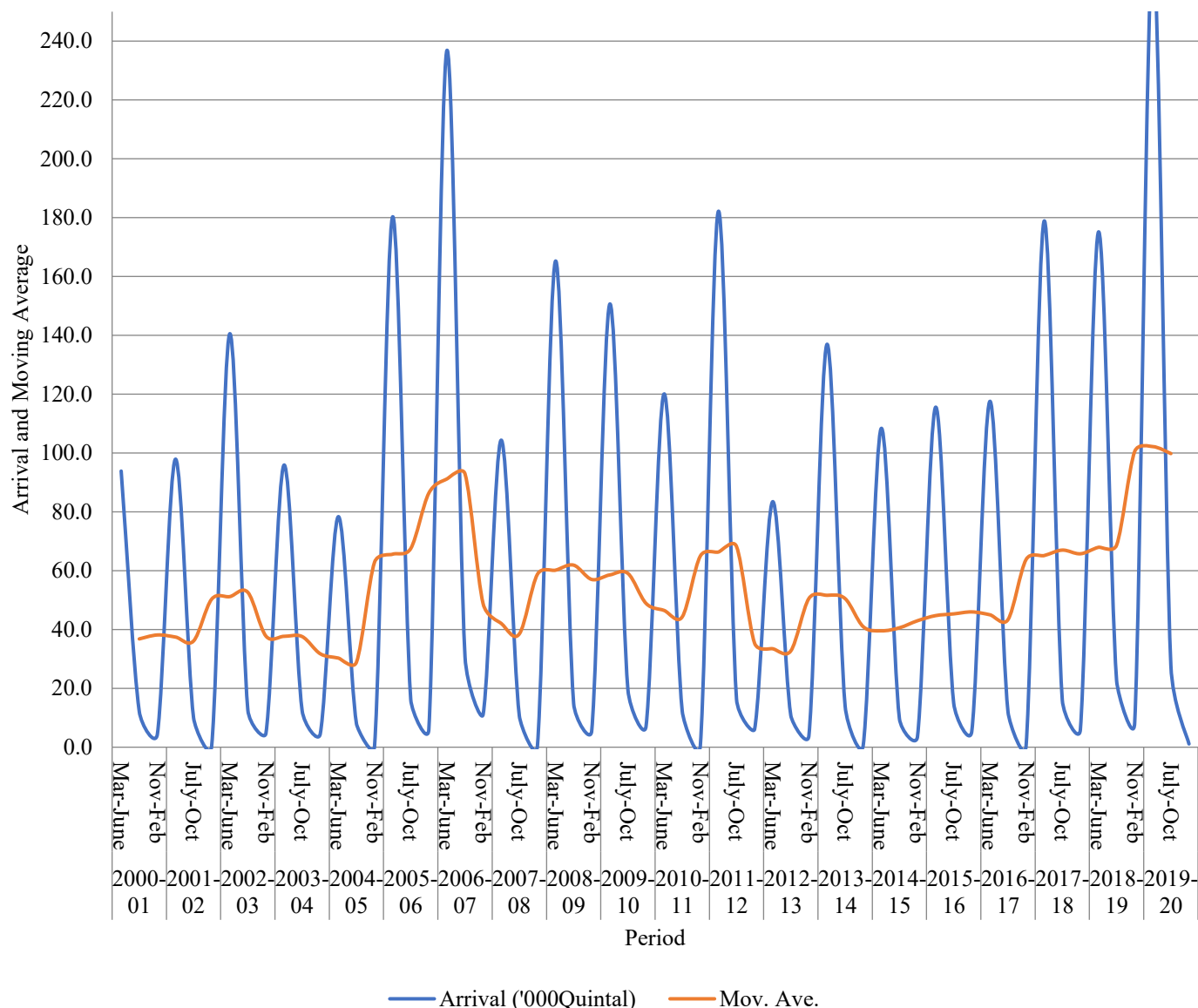


Fig 4. Seasonal variation in price of mustard during the year 2000 to 2020 Seasonal Variation in Market Prices

Seasonal price indices (Table 4; Fig. 5) displayed a near-perfect inverse relationship with arrivals. Prices were generally lower in the high-arrival months (March–June) and higher in the lean seasons (July – October and November – February). The lowest seasonal price index occurred in March – June 2012-13, at -1718.3 per cent, followed by March – June 2009-10 (-530.2%) and 2010-11 (-571.0%). These substantial negative deviations highlight the downward pressure of abundant supply during harvest.

In contrast, the highest positive seasonal price variation was recorded in July – October 2012-13, when prices were 995.7 per cent above the annual average, followed by July – October 2009-10 (431.7%) and July – October 2008-09 (331.5%). November – February also showed strong positive deviations in certain years, with the

highest being 827.0 per cent in 2011-12, 395.8 per cent in 2001-02, and 323.5 per cent in 2005-06. The findings of this study are corroborated by studies conducted by Bhatia et al. (2022), Sarkar et al. (2021) and Singhal (1985).

Over the entire period, March – June price index remained below the annual average in all years, while July – October and November – February price index was above 100 in most years, reflecting scarcity-driven price premiums. The magnitude of seasonal price swings, especially the extreme highs in certain lean months, underscores the vulnerability of mustard prices to supply-side seasonality. This inverse relationship underscores the influence of supply on price formation and were recorded in similar studies by Sarkar et al. (2021) and Kumawat and Kumar (2006). This pattern highlights the economic

opportunity for farmers to improve income by storing produce post-harvest and selling during high-price months.

Table 4: Seasonal variation in price of mustard during the year 2000 to 2020

Year	Season	Seasonal Variation	Year	Season	Seasonal Variation
2000-01	Mar-June		2010-11	Mar-June	-571.0
	July-Oct	100.0		July-Oct	202.6
	Nov-Feb	320.8		Nov-Feb	104.7
2001-02	Mar-June	-345.8	2011-12	Mar-June	-147.8
	July-Oct	-25.0		July-Oct	55.0
	Nov-Feb	395.8		Nov-Feb	827.0
2002-03	Mar-June	-348.0	2012-13	Mar-June	-1718.3
	July-Oct	12.6		July-Oct	995.7
	Nov-Feb	187.8		Nov-Feb	190.8
2003-04	Mar-June	-127.4	2013-14	Mar-June	-283.4
	July-Oct	-73.5		July-Oct	-42.5
	Nov-Feb	-9.3		Nov-Feb	189.4
2004-05	Mar-June	-77.9	2014-15	Mar-June	-234.7
	July-Oct	253.2		July-Oct	64.8
	Nov-Feb	57.3		Nov-Feb	136.7
2005-06	Mar-June	-326.0	2015-16	Mar-June	-141.8
	July-Oct	151.8		July-Oct	-44.7
	Nov-Feb	323.5		Nov-Feb	115.5
2006-07	Mar-June	-369.7	2016-17	Mar-June	-98.6
	July-Oct	32.0		July-Oct	35.0
	Nov-Feb	111.9		Nov-Feb	148.7
2007-08	Mar-June	-104.5	2017-18	Mar-June	-151.6
	July-Oct	-46.7		July-Oct	-24.5
	Nov-Feb	44.1		Nov-Feb	238.2
2008-09	Mar-June	-148.5	2018-19	Mar-June	-232.7
	July-Oct	331.5		July-Oct	-15.3
	Nov-Feb	139.2		Nov-Feb	170.0
2009-10	Mar-June	-530.2	2019-20	Mar-June	-158.3
	July-Oct	431.7		July-Oct	-20.0
	Nov-Feb	144.3		Nov-Feb	

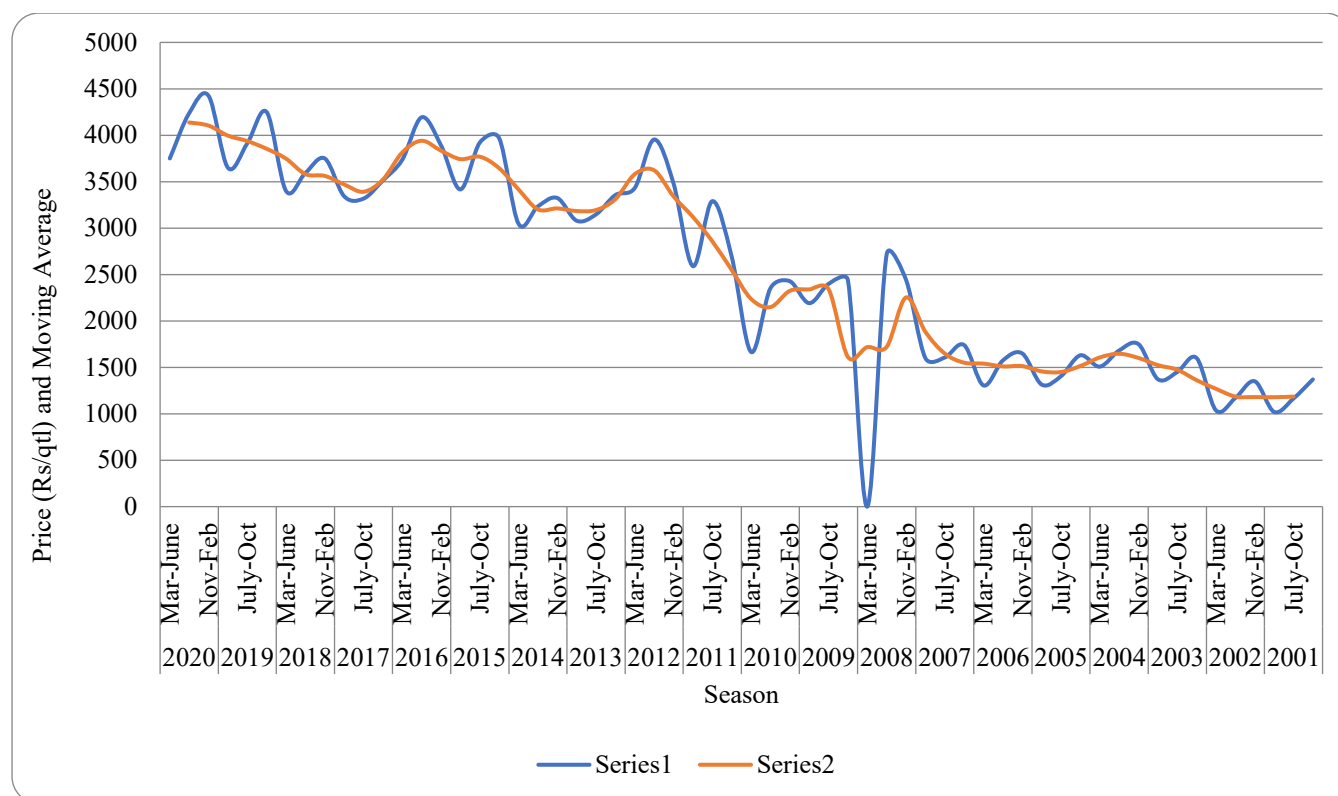


Fig 5. Seasonal variation in price of mustard during the year 2000 to 2020

IV. CONCLUSION

The study revealed that mustard arrivals in Haryana remained largely stable from 2001 to 2017, followed by a notable increase in the subsequent years, with Rewari emerging as the leading market in terms of arrivals. Prices exhibited a consistent upward trend across all selected markets, supported by high growth rates and strong predictability, indicating sustained demand for mustard and its products. Seasonal analysis confirmed a pronounced concentration of arrivals during March - June, leading to price suppression in the harvest period, while prices peaked during the low-arrival months of November - February.

These findings highlight the critical role of storage and marketing infrastructure in addressing seasonal gluts and improving farmer price realisation. Policy measures such as the establishment of rural warehouses, implementation of pledge finance schemes, dissemination of market intelligence, and promotion of mustard oil processing facilities can help stabilise markets and enhance value addition. Strengthening these areas will not only improve the economic resilience of mustard growers in Haryana but also contribute to the overall efficiency and sustainability of the state's oilseed economy.

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Market Dynamics of Guava in Haryana: Analyzing Price Spread and Marketing Efficiency

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Received: 06 Sep 2025; Received in revised form: 04 Oct 2025; Accepted: 09 Oct 2025; Available online: 18 Oct 2025

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Abstract— This research paper analyzes the price spread and marketing efficiency of guava in Haryana through different distribution channels, shedding light on the complexities and nuances of guava supply chains. Four distinct marketing channels were identified, varying in the involvement of intermediaries between producers and consumers. Channel I, involving only producers and consumers, exhibited the highest marketing efficiency due to the absence of intermediaries. Conversely, Channel IV, the most intricate channel with multiple intermediaries, displayed the lowest marketing efficiency. Furthermore, the study delineates significant constraints faced by guava farmers in Haryana, with nematode infestation, inadequate sapling quality, and fruit fly damage emerging as the top challenges. These insights into market dynamics and challenges faced by farmers provide valuable knowledge to enhance guava cultivation and marketing strategies in the region. The study also identifies a need for targeted interventions to address specific constraints and improve the overall guava supply chain in Haryana, ultimately benefiting both producers and consumers.



Keywords— Guava marketing, price spread, marketing efficiency, supply chain, Haryana

I. INTRODUCTION

Guava farming is an essential component of agricultural practices, contributing significantly to the horticultural economy. However, guava farmers encounter various challenges related to price spread and marketing, ultimately affecting their overall productivity and livelihoods. The term "price spread" refers to the difference or spread between the price at which farmers sell their produce and the price at which consumers purchase the same product in the market. Understanding and addressing price spread issues are crucial for achieving fair returns for farmers and ensuring affordable prices for consumers. The arrivals and prices for both are negatively correlated and accordingly it was observed in Pune, Ahmednagar and Nashik APMC markets for guava crop (Kumbharet *et al.*, 2014).

In India, the area under guava cultivation during 2023-24 was 361.44 thousand hectares with a production of 5368.05 thousand metric tonnes. During the same

period, Haryana had an area of 16.75 thousand hectares and production of 183.64 thousand metric tonnes (GoI, 2024).

In the context of guava farming, marketing encompasses the processes involved in getting guava produce from the farmer to the end consumer. Effective marketing involves stages such as transportation, storage, processing, packaging, and distribution, all aimed at meeting consumer demand efficiently and at reasonable prices. However, guava farmers often face complexities and limitations in the marketing process, hindering their ability to reach wider markets and maximize profits. Furthermore, guava farmers grapple with an array of constraints that impact their ability to cultivate, market, and sell their produce effectively. These constraints may include biological challenges like pest infestations and crop diseases, technical barriers such as lack of knowledge about modern farming techniques, limited access to irrigation facilities, inadequate infrastructure, market fluctuations, and insufficient market information.

Addressing these constraints is crucial to enhance the sustainability and profitability of guava farming, ensuring a consistent supply of quality produce to consumers while empowering farmers economically. Finding solutions to these challenges can significantly improve the livelihoods of guava farmers, foster agricultural growth, and contribute to the overall development of the horticulture sector.

II. METHODOLOGY

The present study was conducted in Hisar, Nuh and Yamunanagar districts of Haryana state, which was selected purposively on basis of highest area and production under guava cultivation. Each district represents a distinct agro-climatic zone and socio-economic context, providing a broader understanding of guava cultivation practices across different regions. From each district 20 farmers were selected. A total of 60 farmers were interviewed for collection of data. Primary data pertaining to the year 2022-23 were collected from selected respondents by conducting personal interviews with help of specifically designed schedule. The different market functionaries such as contractors, commission agents, retailers and consumers were randomly selected from the market in the study area. The data collected from the different market functionaries were analyzed to estimate the marketing costs and margins through important marketing channels. The market channels of guava were examined by selecting a random sample of such intermediaries as pre harvest contractors, wholesalers, commission agents and the retailers. Information regarding the marketing pattern/ channels of guava was collected from the producers and marketing agencies involved in the marketing of guava through different channel. Information was also obtained from the market intermediaries involved in the purchase of guava with in the village and in the market. The relevant data were collected with the help of a pre tested, well designed schedule. Information regarding marketing aspects of guava was collected from the producers and the retailers in order to find out the producer's share in the price paid by the consumers. The main channels in operation in the marketing of guava were studied to work out the price spread. To estimate the marketing costs and margins through important marketing channels was used for computing the marketing margins. From the gross margins, the costs incurred by the concerned agencies were deducted to arrive at the net margins. The marketing efficiency of different marketing channels were worked out by using the following method.

(a) Shepherd's Method (ME), (Acharya and Agarwal, 2011)

$$ME = \frac{RP}{MC}$$

Where, RP = Retailer's sale price or consumer's purchase price

MC = Total marketing costs

(b) Acharya's Method (MME), (Acharya and Agarwal, 2011)

$$MME = \frac{FP}{MC + MM}$$

Where, FP = Net price received by farmer

MC = Total marketing costs

MM = Total net margins of intermediaries

(c) Conventional method, (Acharya and Agarwal, 2011)

$$Efficiency = \frac{O}{I} \times 100$$

Where, O= output is the value added

I= input is the real cost of marketing

E= marketing efficiency

III. RESULT AND DISCUSSION

The findings of the study are discussed below:

Price spread of guava in Haryana

Price spread of guava in Haryana is presented in Table 3. The table outlines different distribution channels for guava supply chains, delineating the flow of guava fruit from producers to consumers through various intermediaries. Channel I having two participants; Producer and Consumer, is the simplest distribution channel, where the producer directly sells the product to the end consumer without involving any intermediaries. Channel II having participants are Producer, Retailer and Consumer. In this channel, the producer sells products to retailers, who then sell them to the end consumers. The retailer acts as an intermediary between the producer and the consumer. Channel III having participants are Producer, Commission Agent cum Wholesaler, Retailer and Consumer. This channel involves an additional intermediary, the commission agent cum wholesaler. The producer sells products to the commission agent cum wholesaler, who then sells them to retailers, and the retailers, in turn, sell to the consumers. Channel IV has participants: Producer, Pre-harvest Contractor, Commission Agent cum Wholesaler, Retailer and Consumer. Channel IV is the most complex distribution channel mentioned. It involves multiple intermediaries before reaching the end consumer. The producer initially

engages a pre-harvest contractor, who oversees the pre-harvest activities. The produce is then sold to a commission agent cum wholesaler, who further sells it to retailers. Finally, retailers sell the product to the end consumers. Similar findings were also reported by (Bairwaet *et al.*, 2012).

Table 3: Price spread in guava via different marketing channels

Channel – I	Producer– Consumer
Channel – II	Producer – Retailer – Consumer
Channel – III	Producer – Commission agent cum wholesaler – Retailer – Consumer
Channel – IV	Producer – Pre-harvest contractor- Commission agent cum wholesaler – Retailer – Consumer

Table 4 showed the marketing efficiency of guava in Haryana under different marketing channels. Marketing efficiency according to Acharya's method (Modified Measure of Marketing Efficiency) under different marketing channels i.e. channel I, channel II, channel III and channel IV were 13.07, 1.48, 0.65 and 0.30

respectively. From this efficiency index it could be observed that channel I was the most efficiency among all marketing channels. This is because of the fact that in channel I intermediaries are not involved and hence this channel was most efficient than all other channels. Moreover, marketing efficiency increased with the decreased in market intermediaries between producer and consumer.

The marketing efficiency according to Conventional method under different marketing channels i.e. channel I, channel II, Channel III and Channel IV were 1.00, 2.37, 3.50 and 5.50 respectively. From this efficiency index it could be observed that channel IV was the most efficiency among all marketing channels. The marketing efficiency according to Sephard's method under different marketing channels i.e. Channel I, Channel II, Channel III and Channel IV were 14.07, 5.89, 5.77 and 7.13 respectively. From this efficient index it could be observed that channel I was the marketing efficiency among all marketing channels. Similar findings were also reported by Chaluvadi *et al.*, (2019), Sainet *et al.*, (2013), Singh *et al.*, (2020)

Table 4: Overall average marketing efficiency of guava under different marketing channels

S. N o.	Particulars	Channel I	Channel II	Channel III	Channel IV
		Producer – Consumer	Producer –Retailer – Consumer	Producer – wholesaler – Retailer – Consumer	Producer – Pre-harvest contractor- wholesaler – Retailer – Consumer
1	Consumer purchase price(RP)	3861.87	4397.74	4879.32	5571.81
2	Total marketing cost (MC)	274.40	747.03	845.63	782.00
3	Total net margin of intermediaries (MM)	0.00	1024.26	2113.95	3519.61
4	Net price received by farmers	3587.47	2626.45	1919.74	1270.20
5	Value added	274.40	1771.29	2959.58	4301.61
Marketing efficiency					
A	Conventional method	1.00	2.37	3.50	5.50
B	Shepherd's method	14.07	5.89	5.77	7.13
C	Achary's method	13.07	1.48	0.65	0.30

The results of table 5 provides an insightful overview of various constraints faced by farmers in guava cultivation, outlining the farmers' responses as a percentage and the corresponding ranking of each constraint based on their perceived severity. The most pressing challenge, as identified by a significant majority

of farmers (87%), is the nematode problem in guava plants, securing the top rank (I). Nematodes, which are microscopic worms, can cause substantial damage to guava plants, affecting their growth, yield, and overall health. Following closely is the constraint of a lack of quality saplings, acknowledged by 81 per cent of farmers,

earning it the rank of II. This constraint emphasizes the crucial role of high-quality saplings in successful guava cultivation, highlighting concerns regarding the availability or quality of saplings for planting. Damage caused by fruit flies during the rainy season emerged as the third most prominent constraint, with 77 per cent of farmers recognizing its impact, granting it the rank of III. Fruit-fly infestation can lead to a reduction in guava fruit quality and quantity, posing a significant challenge during specific weather conditions. The fourth-ranked constraint, identified by 73 per cent of farmers, is the lack of technical knowledge about pruning and crop regulation. Farmers highlighted the importance of adequate knowledge and skills in proper pruning and crop regulation practices for optimizing guava production. Lack of canal water for irrigation facilities, ranking V, was acknowledged by 62 per cent of farmers. Insufficient irrigation, especially from reliable water sources like canals, can adversely affect guava plants' growth and productivity. Lastly, the constraint of lacking market information ranked VI, with 23% of farmers recognizing its significance. Having adequate knowledge about markets and consumer demand is crucial for successful market participation and selling produce at a fair price. Similar findings were also reported by Singh *et al.*, (2022), Singh *et al.*, (2013)

Table 5: Constraints faced by Guava farmers in Haryana
n=60

Constraints	Farmers response (%)	Rank
Nematode problem in guava plants	52(87)	I
Lack of quality sapling	49 (81)	II
Damage due to fruit-fly in rainy season	46(77)	III
Lack of technical knowledge about pruning and crop regulation	44 (73)	IV
Lack of canal water for irrigation facilities	37(62)	V
Lack of market information	14(23)	VI

IV. CONCLUSION

This study was conducted for marketing of guava in Haryana. In total, 60 farmers were sampled from Mewat, Yamunanagar and Hisar district of Haryana.

According to Acharya's method channel I was the most efficiency among all marketing channels. This is because of the fact that in channel I intermediaries are not involved and hence this channel was most efficient than all other channels. Moreover, marketing efficiency increased with the decreased in market intermediaries between producer and consumer. Major problems faced by the guava growers were damage due to Nematode problem in guava plants (87%) followed by lack of quality sapling (81%). In constraints underscores the multifaceted challenges that guava farmers face, ranging from biological factors like nematode infestations and fruit-fly damage to issues concerning resource availability (such as quality saplings and irrigation water) and knowledge gaps. Addressing these constraints effectively is vital to enhancing guava cultivation and ensuring better yields and economic returns for the farmers.

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Trends and Profitability Analysis of Garlic Cultivation in Haryana

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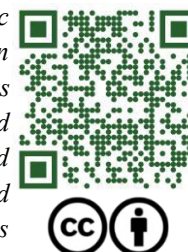
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Received: 12 Sep 2025; Received in revised form: 09 Oct 2025; Accepted: 12 Oct 2025; Available online: 19 Oct 2025

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Abstract— Garlic is an important spice crop in India, valued for its culinary, medicinal and economic contributions. This study examines the growth trends, instability and profitability of garlic cultivation in Haryana over a 22-year period (1999–00 to 2020–21) using time-series data, compound annual growth rates (CAGR) and Cuddy-Della Valle Index (CDVI), the analysis revealed significant increases in area and production, while productivity growth remained moderate. Instability in garlic production decreased indicating greater production stability. Primary survey data from 30 farmers across key districts showed that garlic cultivation is economically viable, with an average gross return of ₹290,000/ha and net returns of ₹120,000/ha. Despite challenges such as market price fluctuations and pest management, government initiatives and improved agronomic practices have positively influenced garlic production and farmer incomes in Haryana.



Keywords— Garlic cultivation, Haryana, profitability, spice crops, farmer income

I. INTRODUCTION

Garlic (*Allium sativum* L.) is a globally significant spice crop, cultivated extensively for its distinctive flavor, high nutritional value, and diverse medicinal applications. As a member of the Alliaceae family, garlic has been utilized not only as a culinary ingredient but also as a therapeutic agent for centuries. Numerous studies have highlighted its bioactive compounds—particularly allicin—which are associated with antimicrobial, antioxidant, and cardiovascular health benefits (Lanzotti, 2006; Rahman, 2007). Due to these properties, garlic enjoys high consumer demand in both domestic and international markets, making it an economically important crop for farmers and agribusinesses alike. India is among the leading garlic-producing countries globally, contributing significantly to

world supply. The crop occupies a prominent position in India's horticultural sector, providing livelihood support to a large number of small and marginal farmers while also contributing to export earnings. According to the National Horticulture Board (2022), garlic production in India has shown consistent growth in recent years, reflecting both rising market demand and expanded cultivation. Within India, the state of Haryana has experienced notable growth in garlic production, with several districts emerging as major contributors to the crop's regional output. This expansion is largely supported by government-led initiatives, most prominently the launch of the National Horticulture Mission (NHM) in 2005–06, which aimed to accelerate horticultural development by improving infrastructure, providing input subsidies, and promoting

scientific cultivation practices (MoA&FW, 2020). Despite these positive developments, garlic cultivation in Haryana continues to face challenges related to production variability, market instability, and input cost fluctuations. The lack of consistent yield levels and unpredictable price movements expose farmers to economic uncertainty, which may affect their long-term interest in garlic farming. Furthermore, while government schemes have contributed to the expansion of horticulture in general, there is limited empirical evidence assessing their specific impact on garlic cultivation at the state or district level. Understanding the patterns of growth, the degree of instability, and the economic viability of garlic farming is therefore essential for designing more targeted interventions to improve farmer outcomes. In response to these gaps, this study undertakes a comprehensive evaluation of garlic cultivation in Haryana. It aims to analyze the growth trends in area, production and productivity over a defined period, with a focus on major garlic-producing districts. Additionally, it assesses the extent of instability in garlic cultivation across different years and regions, using established statistical methods. A key objective of the research is to conduct an in-depth economic analysis, including cost of cultivation, gross and net returns and benefit-cost ratios to evaluate the profitability of garlic farming under current market and policy conditions.

II. MATERIALS AND METHODS

Secondary data

The study was conducted in Haryana state. Time-series data for 22 years (1999-00 to 2020-21) on area, production and productivity of garlic were collected from different published and unpublished sources like, Department of Horticulture Govt. of Haryana, Directorate of Agriculture Govt. of Haryana, State Statistical Abstract of Haryana etc.

Selection of districts

Those districts were selected that was together contributed more than 60 percent share in total production of the concerned crop and provided in the table 1

Table 1: Selection of districts for garlic

Districts	Production (tonne)	% Share of production
Karnal	16078	38.91
Yamuna Nagar	7933	19.20
Jind	2447	5.92

Other districts	14862	35.97
Total	41321	100.00

Primary data

The primary data were collected through a personal interview method with the help of pre-tested schedule from the selected registered farmers under Bhavantar Bharpayee Yojna (BBY). For garlic crop, two districts Karnal and Yamuna Nagar were selected purposively on account of highest production under crop cultivation. Then, one highest production block was selected from each of the selected districts. From Karnal district, Indri block and from Yamuna Nagar district, Radaur block were selected. From each selected block, further one village was selected having maximum number of farmers registered under BBY for garlic crop. In this way from Indri block, Muradgarh village and from Radaur block, Rajheri village were selected. From each of the selected villages, 15 farmers were randomly selected and interviewed. Thus, a total of 30 farmers were selected for this study.

Analytical tools:

The Compound Annual Growth Rate (CAGR)

The CAGR was obtained using the formula

$$Y_t = Y_0 (1+r)^t \text{ ----- (1)}$$

Y_t = refers to area/production/yield of "t" time

Y_0 = refers to base year area/production/yield

The logarithmic transformation of equation (1) is given as,

$$\ln Y_t = \ln Y_0 + t \ln (1+r) \text{ ----- (2)}$$

where, Y_t is the value of the variable at t^{th} period for which growth of area/ production/ yield is calculated, r is the compound annual growth rate.

Now, let $(\ln Y_0) = \beta_1$ and $\ln (1+r) = \beta_2$, the above equation becomes $\ln Y_t = \beta_1 + \beta_2 t$

β_1 and β_2 are estimated through the Ordinary Least Square (OLS) method and the compound annual growth rate (r) is obtained by

$$r = (\text{antilog } \beta_2 - 1) \times 100 \text{ ----- (3)}$$

Instability Index (Cuddy-Della Valle Index)

The Coefficient of Variation (CV) is measure the variation over-estimates the level of instability in the time series data that are characterized by long term trends. The use of Cuddy-Della Valle Index (CDVI) corrects the coefficient of variation for the trend component in the time series data (Della Valle, 1979). So, the CDVI is used in the

present study to measure the instability in the area and production.

$$CDVI = CV \times \sqrt{(1 - \bar{R}^2)} \quad (4)$$

where, CDVI is the instability index in percent

$$CV = (\sigma / \bar{X}) \times 100$$

$\bar{R}^2$ is the coefficient of determination from a time trend regression adjusted for the number of degrees of freedom.

The ranges of instability are as follows:

Low instability = between 0 to 15

Median instability = greater than 15 and lower than 30

High instability = greater than 30 (Sihmar, 2014)

Trend analysis

The trend in area, production and productivity of selected crop were computed for the series data of last 30 years, i.e. 1991-92 to 2020-21. To trace the path of process different trend functions were used. Among the competitive trend functions, the best function was selected based on their goodness of fit adjusted for the number of degrees of freedom (measured in terms of $\bar{R}^2$ value and significance of the coefficients).

1. Linear function

This function assumes a linear relationship between X and Y. The goal of linear trend analysis is to determine how well the data fits this linear model and to make predictions based on this relationship.

The mathematical equation for a linear trend line (also known as regression line) is given by

$$Y_t = a + bX_t + \varepsilon_t$$

Where,

‘ Y_t ’ is the dependent variable (area or production or productivity),

‘ X_t ’ is the independent variable (time in years),

‘a’ is the intercept,

‘b’ is the regression coefficient,

‘ ε_t ’ is the error term.

The values of ‘a’ and ‘b’ were estimated by applying the ordinary least squares (OLS) approach

2. Quadratic function

This function shows curved relationship between Y and X, rather than the straight-line relationship assumed in linear trend analysis. Quadratic data function is useful where there is peak or trough in the data of past periods i.e., when there is a non-linear trend in the data that can be approximated by a quadratic function.

Quadratic fit (or quadratic regression) can be expressed by the following mathematical equation

$$Y_t = a + bX_t + cX_t^2 + \varepsilon_t$$

Where,

‘ Y_t ’ is the dependent variable (area or production or productivity),

‘ X_t ’ is the independent variable (time in years),

‘a’ is the intercept,

‘b’ and ‘c’ are regression coefficients of X,

‘ ε_t ’ is the error term

The values of ‘a’, ‘b’ and ‘c’ were estimated by applying the ordinary least squares (OLS) approach.

3. Logarithmic function

This equation reflects a logarithmic relationship between X and Y. It is commonly used when the rate of change in Y varies logarithmically with X i.e., Y changes by a certain proportion each time X increases by a constant ratio.

The equation for a logarithmic trend line (or logarithmic regression) is

$$Y_t = a + b \ln(X_t) + \varepsilon_t$$

Where,

‘ Y_t ’ is the dependent variable (area or production or productivity),

‘ X_t ’ is the independent variable (time in years),

‘a’ is the intercept,

‘b’ is the regression coefficient,

‘ ε_t ’ is the error term

The values of ‘a’ and ‘b’ were estimated by the method of ordinary least squares (OLS)

4. Exponential function

This function signifies an exponential relationship between X and Y. It is used when the growth or decay of Y is proportional to its current value, leading to rapid changes over time or across values of X.

The equation for an exponential trend line (or exponential regression) is typically represented as

$$Y_t = a \cdot e^{bX_t} + \varepsilon_t$$

Where,

‘ Y_t ’ is the dependent variable (area or production or productivity),

‘ X_t ’ is the independent variable (time in years),

‘a’ is the coefficient (also known as the initial value or constant multiplier),

'b' is the exponent (also known as the growth rate or decay rate),

'e' is the base of the natural logarithm, approximately equal to 2.71828.

The values of 'a' and 'b' were estimated by applying the ordinary least squares (OLS) approach.

Selection of the suitable function was done by using values of adjusted R-square.

Adjusted R² – Criteria

It explains the variation of dependent variable due to variation in independent variable in the model. Assumption is that every independent variable in the model explains the variation in the dependent variable. But in reality, some dependent variables are not explained by some independent variables.

The coefficient of determination (R²) gives some information about the goodness of fit of a model. In regression, the R², or the coefficient of determination is a statistical measure of how well the regression line approximates the real data points. The value of R² lies between 0 and 1. The R² value of 1.0 indicates that the regression line perfectly fits the data i.e., model explains all variation of the dependent variable. It provides a measure of how well outcomes are likely to be predicted by the model. Mathematically R-square is given as sum of residuals (SSres) divided by total sum of squares (SStot) and then subtract it from 1. Here, total variation is measured by SStot. SSres measures unexplained variation and SSreg measures explained variation.

$$R^2 = 1 - \frac{\text{Error sum of square}}{\text{Total sum of square}} = 1 -$$

$$\frac{\sum_{t=1}^n (Y_t - \hat{Y}_t)^2}{\sum_{t=1}^n (Y_t - \bar{Y})^2}$$

$$R^2 = 1 - \frac{SS_{res}}{SS_{tot}}$$

$$R^2 = \frac{SS_{reg}}{SS_{tot}}$$

Where,

Y_t is the actual value,

$\hat{Y}_t$ is the predicted value,

$\bar{Y}$ is the mean value.

Adjusted R² ($\bar{R}^2$) measures the proportion of variance than can only be explained by independent variables that significantly contribute to the explanation of the dependent variable. In regression analysis, it penalizes use of independent variables that not help in predicting the dependent variable.

Mathematically, adjusted R-squared can be computed in terms of sum of squares. The degree of

freedom is the only difference between R-square and Adjusted R-square.

$$\bar{R}^2 = 1 - \frac{SS_{res}/df_e}{SS_{tot}/df_t}$$

In the above equation, df_t is degrees of freedom (n-1) of estimate of population variance of the dependent variable and df_e is degrees of freedom (n-p-1) of estimate of population error variance. Adjusted R² is calculated by using R² value as follows

$$\bar{R}^2 = 1 - \frac{(1-R^2)(n-1)}{n-p-1}$$

Where,

R² is the sample R-square,

p is number of independent variables,

n is total sample size.

Cost Concept

To calculate cost for major vegetables CACP (Commission for Agricultural Costs and Prices) cost concept were used (Kumar et al. 2019).

Cost A1 = Value of hired human labour + Value of hired and owned bullock labour + Value of hired and owned machine labour + Value of seed (both farm or purchased seed) + Value of manures and fertilizers (owned and purchased) + Depreciation + Irrigation charges + Land revenue + Interest on working capital + Miscellaneous expenses

Cost A2 = Cost A1 + rent paid for leased in land

Cost B1 = Cost A1 + interest on working capital (excluding land)

Cost B2 = Cost B1 + rental value of owned land + rent for leased in land

Cost C1 = Cost B1 + imputed value of family labour

Cost C2 = Cost B2 + imputed value of family labour

Cost C3 = Cost C2 + 10 per cent of Cost C2 as management cost

Result and Discussion

Growth in area, production and productivity of garlic in Haryana

The table 2 detailing the growth rates of area, production, and productivity of garlic in Haryana from 1999 to 2021 provides valuable insights into the agricultural trends across different districts, including Karnal, Yamuna Nagar, Jind, Other districts, and the overall state of Haryana. The Compound Annual Growth Rate (CAGR) values represent the average annual growth rate over the period, while the significance levels indicate whether these growth rates are statistically significant at the 1 per cent

level (p -value < 0.01) and 5 per cent level (p -value < 0.05). The results demonstrate varied growth trends across districts and overall for Haryana. In terms of area, Karnal showed a growth rate of 2.73 per cent, Yamuna Nagar had a higher growth rate of 6.20 per cent and Jind exhibited the highest growth rate at 9.62 per cent. Other districts demonstrated a moderate growth rate of 3.70 per cent, while Haryana as a whole experienced an overall growth rate of 4.07 per cent. This indicates that Jind showed the most significant expansion in area, with Yamuna Nagar and other districts also contributing notably to the growth in garlic cultivation. Regarding production, the highest growth was observed in Jind, with a CAGR of 12.64 per cent, followed by Yamuna Nagar at 9.49 per cent. Karnal showed a more

moderate growth rate of 4.61 per cent, while Other districts and Haryana saw respective growth rates of 5.76 per cent and 6.13 per cent respectively. Haryana as a whole experienced a relatively strong increase in production, indicating steady growth across the state. For productivity, Karnal had a CAGR of 1.83 per cent, Yamuna Nagar showed a more substantial increase at 3.10 per cent, and Jind had a growth rate of 2.75 per cent. Other districts showed a productivity growth of 1.99 per cent, while Haryana overall had a growth rate of 1.98 per cent. Yamuna Nagar led in productivity growth, reflecting efficient cultivation practices, followed by the other districts contributing positively to overall state productivity.

Table 2: Growth rates of area, production and productivity of garlic in Haryana: 1999-2021

Aspect	Period	Karnal		Yamuna Nagar		Jind		Other districts		Haryana	
		Mean	CAGR	Mean	CAGR	Mean	CAGR	Mean	CAGR	Mean	CAGR
Area (ha)	Overall Period	883.05	2.73**	618.36	6.20**	134.36	9.62**	1525.95	3.70*	3175.36	4.07**
Production (tonne)	Overall Period	8676.64	4.61**	5955.41	9.49**	1284.27	12.64**	13358.55	5.76**	28956.68	6.13**
Productivity (t/ha)	Overall Period	9.91	1.83**	9.5	3.10**	9.59	2.75	8.71	1.99**	9.13	1.98**

Note 1: CAGR = Compound Annual Growth Rate (per cent).

Note 2: **, * are significant at 1 and 5 per cent respectively.

Note 3: Data of garlic are available from the year 1999-00. So only overall period has taken from 1999 to 2021

Kumar et al (2019) concluded in his studies that growth in the area, production and yield of garlic in Haryana vis-à-vis India was estimated using the compound growth function. The necessary secondary data were collected for a period of 27 years from 1990-91 to 2016-17. During 1990-91 to 2016-17, in Haryana growth rates showed a significant positive growth in area, production and yield under garlic. Likewise, India as a whole country also showed a significant positive growth in area, production and yield under garlic. Lal and Kait (2021). Also indicate the similar pattern in growth in area, production and productivity. The results indicate that there is a significant positive growth rate of 2.20%, 4.50% and 2.20% in the area, production and productivity of ginger in the district of Panchkula.

Trends in area, production and productivity of garlic in Haryana

From figure 1 the trends in area, production and productivity of garlic were presented. The trend analysis of garlic cultivation in Haryana from 1999–2000 to 2020–21 revealed significant growth in production and productivity, while the area under cultivation showed moderate and slightly fluctuating behaviour. The polynomial equation for area has an R^2 value of 0.76, indicating that 76 per cent of the variation in the area trend is explained by time, suggesting moderate growth in the area under garlic cultivation.

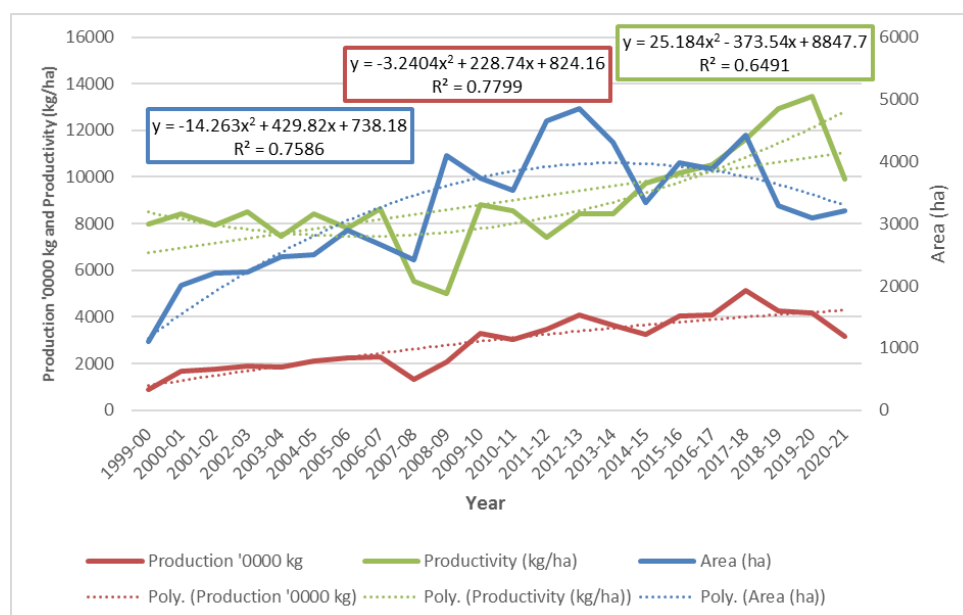


Fig.1: Trends in area, production and productivity of garlic in Haryana

The polynomial equation for production has an R^2 value of 0.78, showing that 78 per cent of the variation in the production trend is explained by time, reflecting a significant increase in garlic production during the study period. For productivity, the polynomial equation has an R^2 value of 0.65, indicating that 65 per cent of the variation in productivity is explained by time, showing a relatively strong upward trend in productivity. The R^2 values for area, production, and productivity suggest that the variations in these trends are statistically significant ($P < 0.01$), with productivity showing a particularly strong correlation with time, followed by production and area.

Instability in area, production and productivity of garlic in Haryana

The data for the instability analysis of area, production, and productivity across districts of Haryana based on the Coefficient of Variation (CV) and Cuddy-Della Valle Index (CDVI) given in Table 3. In terms of area instability, during the period, very high instability was observed in Yamuna Nagar and Jind (CV: 51.06%, CDVI: 42.51%) and (CV: 57.51%, CDVI: 38.90%), indicating volatile land allocation in the early years.

Table 3: Instability in area, production and productivity of garlic in Haryana: 1991-2021 (per cent)

Aspect	Period	Karnal		Yamuna Nagar		Jind		Other districts		Haryana	
		CV	CDVI	CV	CDVI	CV	CDVI	CV	CDVI	CV	CDVI
Area	Overall Period	23.83	18.38	51.06	42.51	57.51	38.90	37.98	33.18	32.06	24.09
Production	Overall Period	39.69	27.53	64.35	44.35	71.49	43.44	40.49	27.95	39.43	19.28
Productivity	Overall Period	32.80	31.36	30.36	21.14	49.91	50.03	19.89	15.84	24.88	21.48

Note 1: Period-I: (1991-92 to 2004-05); Period-II: (2005-06 to 2020-21); and Overall period: (1991-92 to 2020-21).

Note 2: CV = Coefficient of Variation; CDVI = Cuddy-Della Valle Index.

Note 2: Data of garlic are available from the year 1999-00 so only overall period is taken from 1999 to 2021

Whereas Karnal (CV: 23.83%, CDVI: 18.38%), other districts (CV: 37.98%, CDVI: 33.18%), and Haryana (CV: 32.06%, CDVI: 24.09%), recorded medium area instability

In terms of production, Karnal (CV: 39.69%, CDVI: 27.53%) and Haryana (CV: 39.43%, CDVI:

19.28%). Other districts (CV: 40.49%, CDVI: 27.95%) recorded medium instability. However, instability sharply declined in the period. Yamuna Nagar (CV: 64.35%, CDVI: 44.35%) and Jind (CV: 71.49%, CDVI: 43.44%), high production instability in the overall period.

The instability analysis of garlic productivity across the major districts of Haryana showed medium instability in Karnal (CV: 32.80%, CDVI: 31.36%), Yamuna Nagar (CV: 30.36%, CDVI: 21.14%), Jind (CV: 49.91%, CDVI: 50.03%), Haryana (CV: 24.88%, CDVI: 21.48%), and other districts (CV: 19.89%, CDVI: 15.84%).

Cost in garlic cultivation

The cost concepts involved in garlic cultivation in the study area are presented in Table 4. Cost A1 in the cultivation of garlic was ₹434,971.12 per hectare. Cost A1 includes all the paid-out costs from the farmer's side, covering expenses such as seeds, fertilizers, pesticides,

labor, and irrigation. Cost A2 was ₹523,582.37 because the farmers had taken land on rent for garlic cultivation in the study region. Cost B1 was calculated as ₹434,971.12 per hectare, corresponding to Cost A1, while Cost B2, which includes both the operational cost and the rent for the land, was observed to be ₹523,582.37. Cost C1, on an overall average, was ₹434,971.12 per hectare, which is equivalent to Cost A1 and Cost B1. Further, Cost C2, was calculated to be ₹523,582.37 per hectare, matching Cost A2 and Cost B2. For Cost C3, which accounts for management-related overheads, it equals Cost C2 plus 10 per cent of Cost C2, resulting in a total of ₹564,918.18 per hectare for the overall farm size.

Table 4: Cost of cultivation in garlic (₹/ha)

S.no.	Particular	Cost
1	Cost A1	434971.12
2	Cost A2	523582.37
3	Cost B1	434971.12
4	Cost B2	523582.37
5	Cost C1	434971.12
6	Cost C2	523582.37
7	Cost C3	564918.18

Cost and returns of garlic cultivation

The distribution of costs and returns per hectare for garlic farming is given in Table 5. The gross returns per hectare from garlic cultivation were estimated at ₹693,550, with the return over variable costs amounting to

₹280,191.88. The net return per hectare from garlic was ₹128,631.82. The average production was 130 quintals per hectare, with a total cost of cultivation of ₹564,918.18 per hectare. Of this, variable costs amounted to ₹413,358.12 (73.17%) of the total cost. Seed costs were ₹135,850 (24.05%), which is a

Table 5: Cost and returns of garlic production in Karnal and Yamuna Nagar districts of Haryana

Sr. No.	Inputs	No./Quantity per ha	Value (₹/ha)	Per cent of total cost
1	Preparatory tillage	8	15684.00	2.78
2	Pre-sowing irrigation		1370.85	0.24
3	Seed (Kg)/Nursery raising	905	135850.00	24.05
4	Seed treatment		0.00	0.00
5	Sowing/Transplanting		30875.00	5.47
6	Ridging and layout operation		17043.00	3.02
7	FYM (q)	330	19760.00	3.50
8	Fertilizer nutrients		0.00	0.00
	a) Urea(Kg/ha)	310	1857.00	0.33
	b) DAP/SSP/NPK(Kg/ha)	311	8398.00	1.49
	c) Muriate of potash(Kg/ha)	123.5	3829.00	0.68

	d)ZnSO ₄ (Kg/ha)	9	1111.50	0.20
	e)Sulphur (Kg/ha)	24.7	1235.00	0.22
	f)Other nutrient(Kg/ha)	95	7657.00	1.36
	Total Fertilizer Invest		24087.50	4.26
9	Fertilizer application cost		2000.70	0.35
10	Irrigation	8	11077.95	1.96
11	Hoeing/Weeding		0.00	0.00
	(a)Chemical	1	3952.00	0.70
	(b)Manual	2	24700.00	4.37
12	Earthing up		0.00	0.00
13	Plant protection	7	25815.00	4.57
14	Harvesting/Picking charges		72865.00	12.90
15	Miscellaneous		1235.00	0.22
	Total (1 to 15)		386316.00	68.38
16	Interest on working capital @7%		27042.12	4.79
17	Variable cost		413358.12	73.17
18	Packaging charges		2470.00	0.44
19	Transportation		17290.00	3.06
20	Management charges @10%		41335.81	7.32
21	Risk factor @ premium of MBBY		1853.00	0.33
22	Rental value of land		88611.25	15.69
23	Total cost		564918.18	100.00
24	Production(q/ha)	130	0.00	
25	Price Received (₹/q)		5335.00	
26	Gross Return(₹/ha)		693550.00	
27	Return over variable cost		280191.88	
28	Net return(₹/ha)		128631.82	
29	Cost of Production (₹/q)		4345.52	
30	B :C Ratio		1.23	

significant portion of the total costs due to the use of high-quality seed. Farm yard manure costs were ₹19,760 (3.50%), while fertilizer costs stood at ₹24,087.50 (4.26%). Plant protection chemicals accounted for ₹25,815 (4.57%), and interest on working capital was ₹27,042.12 (4.79%). Packaging charges were ₹2,470 (0.44%), and transportation costs were ₹17,290 (3.06%). Management charges, calculated at 10% of the variable cost, were ₹41,335.81 (7.32%), and the rental value of land was ₹88,611.25 (15.69%). The risk factor, calculated as a premium for the MBBY (Mukhya Mantri Bagwani Bima Yojna), was ₹1,853 (0.33%) of the total cost, providing insurance coverage for

the farmers under the scheme. The cost of production per quintal was ₹4,345.52. The Benefit-Cost Ratio (B:C ratio) was 1.23, indicating that garlic cultivation in the study area was profitable, with every rupee spent yielding a return of ₹1.23.

III. CONCLUSION

The study on garlic cultivation in Haryana over a period revealed significant changes in area, production, and productivity. Overall, Haryana witnessed a CAGR of 4.07 per cent in area, 6.13 per cent in production, and 1.98 per

cent in productivity, with the most substantial growth observed in Jind district. In terms of area and production instability, during the period, very high instability was observed in Yamuna Nagar and Jind districts. At the farm level, the cost of cultivation per hectare was ₹5.64 lakh (Cost C3), yielding an average production of 130 quintals and gross returns of ₹6.93 lakh, resulting in net returns of ₹1.28 lakh per hectare. These findings underscore the importance of policy interventions like NHM in enhancing both economic returns and production stability in garlic farming while also emphasizing the need for continued investment in infrastructure and farmer support systems.

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Comparison Analysis and Application of GlobeLand30 and CNLUCC Datasets in Land Use Dynamic Analysis in Dongguan City

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Received: 08 Sep 2025; Received in revised form: 05 Oct 2025; Accepted: 13 Oct 2025; Available online: 21 Oct 2025

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Abstract— Accurate analysis of land use dynamics is fundamental for sustainable development and refined territorial spatial management in high-density urban areas. Although the GlobeLand30 and CNLUCC datasets have been widely used in land use studies, they fundamentally differ in classification systems, design objectives, and accuracy, and there remains a lack of systematic performance comparison and applicability assessment for highly dynamic urban areas. This study takes Dongguan City (2000–2020) as a case study, employing dynamic degree models, transition matrices, and spatial analysis methods to systematically compare the performance differences between the two datasets across three dimensions: classification system compatibility, spatiotemporal consistency, and application scenario suitability. The findings reveal that CNLUCC, with its management-oriented three-tier classification system, excels in identifying industrial land (accuracy up to 92%) and is suitable for localized fine-scale management scenarios such as territorial spatial planning. In contrast, GlobeLand30, with its globally consistent observation standards, achieves higher overall accuracy (85.72%) and proves particularly valuable for global change studies and cross-border comparisons. Based on the more accurate GlobeLand30 data analysis, Dongguan's land use exhibits a typical urbanization pattern characterized by "spatial polarization—boundary expansion—functional restructuring," with concurrent expansion of construction land and restoration of ecological land. Therefore, CNLUCC is recommended for territorial management, while GlobeLand30 is preferred for cross-border studies, and a hybrid validation strategy can be adopted for critical land category analyses. The comparative framework developed in this study provides a scientific basis for selecting land use data and guiding sustainable planning in similar high-density urban areas worldwide.



Keywords— CNLUCC (China Land Use/Land Cover Change); Globeland30; Land Use/Land Cover Change (LUCC) ; Dynamic Degree Analysis; Transfer Matrix

I. INTRODUCTION

Land Use/Land Cover Change (LUCC) research is a crucial component of global environmental change studies. Its data, serving as a spatial representation of the interaction between human activities and the natural environment, provides fundamental information for understanding human-land relationships. High-quality land use data can accurately reflect the spatiotemporal characteristics of surface cover changes, revealing the extent and methods of human interventions in natural systems—such as urbanization, agricultural development, and ecological restoration—thereby offering decision-making support for regional sustainable development. Particularly in rapidly urbanizing regions like the Pearl River Delta (PRD), where human-land conflicts are prominent, accurately identifying land use dynamics holds significant practical importance for optimizing territorial spatial patterns, ensuring ecological security, and improving resource utilization efficiency [1].

As a forefront of China's reform and opening-up and a vital engine for economic development, the PRD region has undergone rapid urbanization. Dongguan, as a core city in the PRD, has experienced particularly dramatic land use transformations over the past two decades. Between 2000 and 2020, Dongguan's urbanization rate surged from 40% to 92%, while the proportion of construction land increased from 15% to 45%. Meanwhile, the area of cropland decreased by 60%, intensifying the conflict between cropland loss and construction land expansion. Such drastic changes not only exert pressure on the regional ecological environment but also pose severe challenges to the sustainable utilization of land resources.

Remote sensing technology, with its advantages of macroscopic, rapid, and periodic observation, has become the primary means of obtaining large-scale, long-term land use information. Currently, with the conceptualization and practical application of remote sensing big data, land use data widely used in academic research includes global standardized datasets (such as GlobeLand30) and national or regional datasets (such as China's CNLUCC). Among them, the GlobeLand30 dataset is a global 30-meter resolution land cover dataset, representing a significant achievement of China's National High-Tech Research and Development Program (863 Program) on global land cover remote sensing mapping and key technology research. It

adopts a single-layer observational framework with 10 first-level categories, directly mapping based on physical land cover characteristics to ensure global-scale consistency. On the other hand, CNLUCC (China Multi-Period Land Use Remote Sensing Monitoring Dataset) primarily uses Landsat remote sensing imagery from the United States as its main data source. It employs a three-tier hierarchical management framework (6 first-level categories → 15 second-level categories → 30 third-level categories), with progressive subdivision based on land use control logic, making it more aligned with China's land management practices.

In recent years, these two datasets have been widely used in academic research, but each exhibits distinct strengths and limitations. In the academic community, GlobeLand30 has garnered broader attention and application due to its global coverage and consistent data standards, particularly in fields such as global change research and cross-border comparative analysis. Multiple studies indicate that GlobeLand30's overall accuracy typically exceeds 80% and continues to improve with version updates (the V2020 version achieved an overall accuracy of 85.72% with a Kappa coefficient of 0.82). On the other hand, the CNLUCC dataset is more widely utilized in domestic academic circles, demonstrating clear advantages in areas closely tied to national policies, such as territorial spatial planning, ecological conservation and restoration, and resource management. However, its accuracy shows some variability across different regions and application scenarios (with an overall accuracy of approximately 76.41%).

However, these two datasets exhibit significant differences in classification systems, spatial resolution, update frequency, and accuracy, directly impacting the reliability of land-use change analysis and the effectiveness of subsequent policies. Particularly in high-density urban areas like Dongguan, where land-use structures are complex and changes are rapid, different datasets may yield divergent conclusions about the same phenomenon. For instance, in Dongguan's case, CNLUCC achieved a 92% accuracy in identifying industrial land, whereas GlobeLand30 missed detecting 61.4% of wetlands. Such discrepancies create confusion for researchers when selecting data and compromise the comparability and

reliability of research findings.

Therefore, this study focuses on two datasets, CNLUCC and GlobeLand30, and quantitatively evaluates their performance in analyzing land use changes in Dongguan City from three dimensions: classification system, spatiotemporal consistency, and application scenario matching. By combining dynamic degree models and transition matrices, the study aims to clarify their respective advantages and limitations, providing a basis for data selection and result interpretation in related research.

II. STUDY AREA AND DATA

2.1 Overview of the Study Area

Dongguan City is located on the eastern bank of the Pearl River Estuary in the central-southern part of Guangdong Province (longitude $113^{\circ}31' \sim 114^{\circ}15'E$, latitude $22^{\circ}39' \sim 23^{\circ}09'N$) (Figure 1). As a key node city in the Guangdong-Hong Kong-Macao Greater Bay Area, it covers an area of 2,460.08 square kilometers. As a typical representative of high-density cities in the Pearl River Delta, its urbanization process and land use patterns exhibit rapid, intensive, and highly dynamic characteristics. By 2020, Dongguan's urbanization rate had reached 92%, with a permanent population of approximately 10.437 million. In 2024, its GDP amounted to 1,290.018 billion yuan, showcasing a developed economy dominated by manufacturing, a young population structure, and a low level of aging [2].

In terms of land use, Dongguan has undergone a dramatic transformation process. Between 2000 and 2020, the proportion of construction land surged from 15% to 45%, while farmland area decreased by 60%. The conflict between farmland loss and construction land expansion is prominent, reflecting the strong occupation of land resources by rapid urbanization. Currently, Dongguan has entered a new development phase centered on "stock renewal and connotation enhancement," completely shifting away from the traditional model of relying on new land supply and blind expansion. Instead, it focuses on tapping spatial potential through urban village redevelopment, industrial park upgrades, and the redevelopment of inefficient land use. The 2025 land supply plan clearly reflects this policy direction, with industrial and mining

storage land becoming the absolute dominant supply category (266.13 hectares, accounting for 55.93%), while residential land supply accounts for only 9.3% of the total annual supply (approximately 441,000 square meters). This highlights Dongguan's strong intent to safeguard manufacturing space and promote the integration of industry and urban development.

In terms of policy, according to the "Dongguan Territorial Spatial Master Plan (2021-2035)," the city's urban development boundary area is strictly controlled within 1,433.37 square kilometers, with increasingly refined land use management. By 2035, the plan aims to maintain a cultivated land area of no less than 101,100 mu and a permanent basic farmland protection area of no less than 100,000 mu, strengthening protection through the "Field Chief System" and the construction of cultivated land aggregation zones. The ecological protection redline area will be no less than 361.08 square kilometers (including a marine ecological protection redline of 26.74 square kilometers), focusing on protecting the ecological barrier in the southeastern mountainous areas and the wetland system of the Dongjiang River basin, while systematically advancing ecological restoration projects [3].

Topographically, Dongguan City generally presents a pattern of higher elevation in the southeast and lower in the northwest. The southeastern part is dominated by low mountains and hills (accounting for 44.5% of the city's land area), with the main peak of Yinpingzui Mountain reaching an altitude of 898.2 meters. The northwestern part consists of the alluvial plain of the Dongjiang River (43.3% of the land area), characterized by flat terrain and a crisscrossing network of waterways. The southwestern part belongs to the river alluvial plain, significantly influenced by tidal movements. This interlaced geographical pattern of "hills-plains-water bodies" has profoundly shaped the spatial differentiation of land use: ecological conservation is prioritized in the southeastern mountainous areas, while urban and agricultural development is concentrated in the northwestern plains. Dongguan is currently employing multiple approaches, including territorial spatial planning, urban renewal, and comprehensive land remediation, to reconcile land-use conflicts and optimize spatial structure, making it a typical case study for achieving high-quality development alongside high-level resource protection.

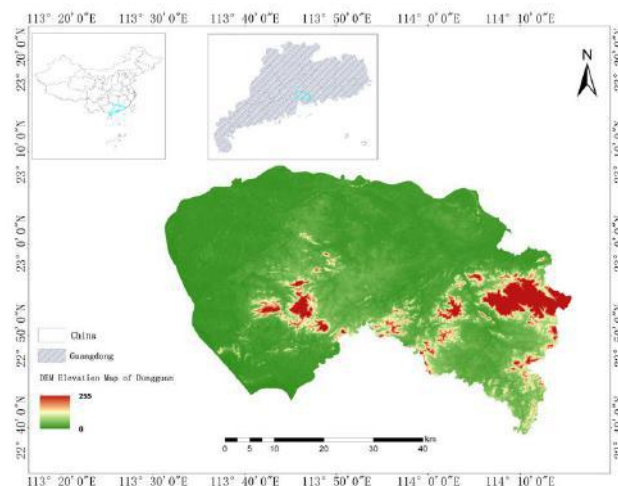


Fig.1 Location and Elevation Map of Dongguan

2.2 Data Sources and Preprocessing

2.2.1 Data Sources

The data for this study primarily comes from the global land cover dataset (GlobeLand30), the China Multi-period

Land Use Remote Sensing Monitoring Dataset (CNLUCC dataset) [3], and the Geospatial Data Cloud (Table 1). Three periods of remote sensing images for Dongguan City in 2000, 2010, and 2020 were selected, with both datasets having a spatial resolution of 30 m.

Table 1 Data Sources of This Study

Data Category	Data Sources	Purpose
Land use data	Chinese Academy of Sciences Resources and Environment Science Data Center (CNLUCC) https://www.resdc.cn/DOI/DOI.aspx?DOIID=54	Generate land uses dynamic degrees and transfer matrix data
	GlobeLand30 Global Land Cover Data http://www.globallandcover.com/	
DEM	Geospatial Data Cloud https://www.gscloud.cn/search	Generate elevation map of Dongguan City
Administrative Boundary (Vector Data)	National Platform for Common GeoSpatial Information Services https://www.tianditu.gov.cn/	Generate the study area boundary map

2.2.2 Data Preprocessing

This study utilized ArcGIS 10.4 software to preprocess and analyze GlobeLand30, CNLUCC, and Landsat remote sensing images, primarily for extracting spatiotemporal information on land use changes in Dongguan City. The specific workflow is as follows:

(1) Due to the diversity of data sources, their coordinate systems and pixel sizes are inconsistent.

Therefore, the Extract by Mask tool in the Spatial Analyst Tools was used to mask the data according to the study area. Additionally, the Define Projection and Project tools in the Data Management Toolbox were employed to unify all data to the geographic coordinate system WGS_1984 and the projected coordinate system WGS_1984_World_Mercator, while also standardizing pixel sizes to ensure spatial consistency.

(2) The original images underwent preprocessing, including background value removal and image mosaicking. The vector boundary of Dongguan City was then used for mask extraction to obtain land use data within the study area.

(3) ArcGIS was utilized for geometric calculations to generate a land use transfer matrix. The "Table to Excel" tool was employed to export the data, and the area of each land type during different periods, as well as the land use dynamic degree, were calculated.

III. RESEARCH METHODOLOGY

This study focuses on general data sources such as GlobeLand30 and CNLUCC. Through land use dynamic degree analysis (DDA), transfer matrix calculation, and classification accuracy assessment, it explores the appropriateness and precision of land use in the study area. By comparison, the study aims to scientifically evaluate the rationality, feasibility, and differences of the research data (Figure 2).

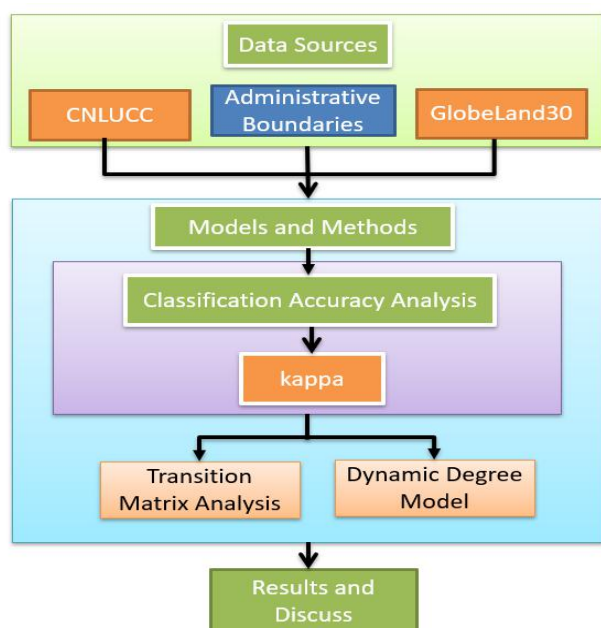


Fig.2 Technical Flowchart

3.1 Analysis of Land Use Dynamic Degree

3.1.1 Single Land Use Dynamic Degree

The single land use of dynamic degree refers to the rate of change in the area of a specific type of land use over a certain period. It reflects the dynamic changes of that type

of land use during the study period and is one of the important indicators for analyzing land use changes [4]. The formula is as follows:

$$K = \frac{A_m - A_n}{A_n} \times \frac{1}{T} \times 100\% \quad (1)$$

In Equation (1): K represents the single dynamic degree; A_n and A_m denote the area of a certain land use type at the beginning and end of the study period, respectively; T is the duration of the study period.

3.1.2 Comprehensive Land Use Dynamic Degree

The comprehensive land use dynamic degree refers to the overall changes in all land use types within a study area over a certain period. It reflects the general trend of land use change in the region [4]. The formula is as follows:

$$R = \frac{\sum_{i=1}^n |A_i - A_j|}{\sum_{i=1}^n A_i} \times \frac{1}{T} \times 100\% \quad (2)$$

In Equation (2): R is the comprehensive dynamic degree; A_i and A_j are the areas of the i-th land use type at the beginning and end of the study period, respectively; n is the number of land use types; and T is the duration of the study period.

3.2 Calculation Method of Land Use Transfer Matrix

The land use transfer matrix quantitatively describes the sources, destinations, areas, and rates of mutual conversion among various land use types during a study period by comparing land use data at two different time points (e.g., T1 and T2). Its core principle is based on the concept of Markov chains, where the future distribution of a system's state (land use types) depends only on its current state, and transitions between states can be described by a probability matrix (i.e., the transfer matrix). In summary, the land use transfer matrix is applied in system analysis to quantitatively describe system states and state transitions, encompassing both static data of land use types and dynamic data of mutual conversions among land categories within a specific region during a given period [5].

$$S_{ij} = \begin{bmatrix} S_{11} & S_{12} & \cdots & S_{1n} \\ S_{21} & S_{22} & \cdots & S_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ S_{n1} & S_{n2} & \cdots & S_{nn} \end{bmatrix} \quad (3)$$

In equation (3), where S is the area, n is the number of land use types before and after the transfer, i is the land use type before the transfer, and j is the land use type after the transfer, S_{ij} is the area of land type i that I converted to land type j , and each row element in the matrix represents the source information of each land type before the transfer for land type j .

Using the transfer matrix, the proportion of land use type i in period k converted to land use type j in period $k+1$ is calculated, as shown in formula (4):

$$A_{ij} = S_{ij} \times 100 / \sum_{j=1}^n S_{ij} \quad (4)$$

It can also calculate the proportion of land use type i in period k that transitions to land use type j in period $k+1$, as shown in formula (5):

$$B_{ij} = S_{ij} \times 100 / \sum_{j=1}^n S_{ij} \quad (5)$$

The application of land use transition matrices is extremely extensive, serving as a bridge connecting data with decision-making. Common research areas include (1) quantifying change processes and identifying characteristics (identifying dominant change types, revealing hotspot pathways of change, and analyzing the reversibility of changes); (2) analyzing driving mechanisms; (3) evaluating sustainable land use; (4) simulating and predicting future scenarios; and (5) assessing policies and testing their effectiveness.

3.3 Evaluation Metrics

To assess the stability and effectiveness of classification methods, scientific rigor, rationality, and accuracy serve as the core criteria for precision evaluation, providing indispensable reference value for method optimization and enhancement [6]. Research literature indicates that when analyzing experimental results, comparing the inherent differences between multi-class and

binary classification problems reveals that Overall Accuracy (OA) offers high precision in evaluating multi-class problems. In contrast, this metric struggles to comprehensively reflect the training efficacy of models for binary classification. Therefore, in land-use classification assessments, employing both OA and the Kappa coefficient for analysis can enhance reliability and credibility. Accordingly, this study adopts OA and the Kappa coefficient as key metrics to measure the precision of classification outcomes. The results of various classification models used in the experiments are compared with those of conventional models to validate the practical performance of the proposed method.

3.3.1 Overall Classification Accuracy

In classification methods, overall classification accuracy serves as an intuitive evaluation metric. It quantifies analysis by calculating the ratio of correctly predicted samples to the total number of samples (reflecting the proportion of samples that are accurately classified). The basic calculation formula is as follows:

$$OA = \text{Correct samples} / \text{Total samples} \quad (6)$$

Comprehensive classification accuracy is suitable for performing sample classification tasks involving multiple categories, during which the distribution of sample quantities across various categories should remain relatively balanced.

3.3.2 Kappa Coefficient

The Kappa coefficient is a crucial metric in land use classification, remote sensing image interpretation, and any scenario involving the evaluation of classification model accuracy. It is also an assessment indicator that integrates Producer's Accuracy and User's Accuracy. Widely applied in verifying the consistency between model-predicted data and actual monitoring data, it is frequently used in research to quantify the overall accuracy of simulated images. Its calculation is based on the confusion matrix, with the formula as follows:

$$Kappa = \frac{P_a - P_b}{1 - P} \quad (7)$$

In the formula, P_a represents the actual raster simulation accuracy, which is the overall accuracy (Total

Accuracy). As mentioned earlier, $TA = \text{number of correct samples} / \text{total number of samples}$; P_b refers to the expected raster simulation accuracy, while 1 symbolizes the simulation accuracy under ideal conditions. The Kappa coefficient ranges from [0, 1], and an increase in this value indicates an improvement in simulation accuracy. When Kappa is below 0.75, it suggests significant deviations in the simulation results; conversely, when Kappa is above 0.75, it implies a high consistency between the simulated image and the real landscape, indicating superior simulation quality.

IV. RESULTS AND ANALYSIS

4.1 Dataset Discrepancy Analysis

GlobeLand30 adopts a single-layer observational framework (10 parallel categories) (Table 2), directly mapping based on physical land cover characteristics to

ensure global consistency (e.g., uniform criteria for "built-up land 80"). However, this approach resulted in a 61.4% omission rate for wetlands in Dongguan. Essentially, the two datasets are products of distinct paradigms: "land resource management" (CNLUCC) and "surface process observation" (GlobeLand30), tailored respectively for territorial spatial planning (CNLUCC) and global change research (GlobeLand30) scenarios.

CNLUCC employs a three-tiered management framework (6 primary categories, 15 secondary categories, and 30 tertiary categories) (Table 3), hierarchically subdividing land use types based on regulatory logic. It supports refined governance through specialized classifications like "industrial/mining/warehouse land (203)," achieving a 92% industrial land detection rate in Dongguan. However, its cross-country comparability is limited.

Table 2 Classification System of GlobeLand30 Dataset

Code	Land Type	Content
10	Cropland	Land used for growing crops, including paddy fields, irrigated dry land, rain-fed dry land, vegetable fields, pastureland, greenhouse land, and cash cropland.
20	Forest land	Woodland with tree canopy coverage exceeding 30%, including deciduous broadleaf forests, evergreen broadleaf forests, deciduous coniferous forests, evergreen coniferous forests, mixed forests, as well as sparse woodlands with canopy coverage between 10% and 30%.
30	Grassland	Land naturally covered by herbaceous vegetation with a coverage rate of over 10%, including grasslands, meadows, savannas, desert steppes, and urban artificial lawns.
40	Shrubland	Shrub-covered land with a shrub coverage of more than 30%, including mountain shrubs, deciduous and evergreen shrubs, as well as desert shrubs with a coverage of more than 10% in desert areas.
50	Wetland	Located at the interface between land and water, wetlands are areas with shallow accumulations of water or waterlogged soil, typically supporting the growth of marsh or aquatic plants. .
60	Water body	Land areas covered by liquid water, including rivers, lakes, reservoirs, ponds, etc.
70	Tundra	Land covered by lichens, mosses, perennial cold-resistant herbs, and shrub vegetation in frigid and alpine environments, including shrub tundra, grass tundra, wet tundra, alpine tundra, and bare tundra.
80	Construction land	A surface formed by human construction activities, including various types of settlements such as towns, industrial and mining areas, transportation facilities, etc., excluding contiguous green spaces and water bodies within construction sites.
90	Bare land	Natural land with vegetation coverage below 10%, including deserts, sandy areas, gravel lands, bare rocks, saline-alkali lands, etc.
100	Glaciers and permanent snow cover	Land covered by permanent snow, glaciers, and ice sheets, including permanent snow and glaciers in alpine regions, as well as polar ice sheets.

Table 3 CNLUCC Dataset Classification System

Code	Land Type	Content
1	Cropland	Land used for growing crops, including cropland, newly reclaimed wasteland, fallow land, rotational fallow land, and land used for crop rotation with grass; land primarily used for growing crops such as fruit trees, mulberry trees, forests; and tidal flats and coastal land that have been cultivated for more than three years. (Paddy fields, dry land)
2	Forest land	Referring to forestry land where trees, shrubs, bamboo, and coastal mangroves grow. (Includes forested land, shrubland, sparse woodland, and other forested areas)
3	Grassland	It refers to various types of grasslands dominated by herbaceous plants with a coverage of more than 5%, including shrub grasslands primarily used for grazing and sparse forest grasslands with a canopy density of less than 10%. (High-coverage grassland, medium-coverage grassland, low-coverage grassland)
4	Water body	Refers to natural land water areas and water conservancy facility land. (Rivers and canals, lakes, reservoirs and ponds, permanent glaciers and snowfields, tidal flats, beaches)
5	Construction land	Urban and rural residential areas and land used for industrial, mining, transportation, and other purposes (urban land, rural residential land, other construction land).
6	Bare land	Currently unused land, including difficult-to-utilize land. (Sand land, Gobi, saline-alkali land, marshland, bare land, bare rocky land, others)
9	Ocean	Ocean

4.2 Dataset Accuracy and Sensitivity Differences

4.2.1 Analysis of Accuracy Differences

1. The three main versions of GlobeLand30 (2000, 2010, 2020) do not share identical accuracy levels, and the accuracy assessment of each version itself is an ongoing process of optimization. It is important to note that accuracy validation requires significant human and material resources; therefore, for earlier versions, the official accuracy assessment may not be the final or most comprehensive. Typically, the latest version (2020) undergoes the most rigorous and reliable accuracy evaluation.

According to the accuracy validation report released by the official GlobeLand30 team (based on extensive sample points): GlobeLand30 V2000: The global overall validation accuracy ranges between 80.00% and 83.00%, with a Kappa coefficient of 0.76. GlobeLand30 V2010: The global overall validation accuracy is 85.72%, with a Kappa coefficient of 0.78. GlobeLand30 V2020: The global overall validation accuracy is 85.72%, with a Kappa coefficient of 0.82 [6].

Overall, GlobeLand30's accuracy is internationally recognized as high, with officially reported overall accuracy exceeding 85%. Moreover, the accuracy has progressively improved with each version update. However, it is important to note that this "overall accuracy" is a macro-average, and actual accuracy can vary depending on factors such as region, land cover type, and year. Accuracy trend: From the 2000 version to the 2010 version and then to the 2020 version, the overall accuracy shows a clear upward trend. This improvement is primarily attributed to: (1) Enhanced satellite data quality: Transitioning from Landsat 5/7 to Landsat 8/9, which provide higher spectral and geometric quality in imagery. (2) Advances in classification algorithms: Evolving from more pixel-based classification to smarter methods integrating remote sensing, geographic information, and artificial intelligence. (3) Optimized validation methods: Increased and more balanced validation samples, along with easier access to reference data (e.g., high-resolution imagery).

2. CNLUCC30 (30-meter resolution): In the validation conducted in the Qinghai-Tibet Plateau region, the

overall accuracy was 76.41% (considering only the suitable, moderately suitable, and generally suitable categories) [7]. The accuracy of the CNLUCC dataset improves with higher spatial resolution but is generally slightly lower than that of 30-meter resolution products such as GlobeLand30.

Based on the comparative analysis above, GlobeLand30 stands out for its globally consistent high

accuracy (>80%) at 30-meter resolution, making it suitable for land-use change comparisons. CNLUCC, characterized by its "China-specific + long time series" approach, has slightly lower accuracy ($\approx 72\text{--}76\%$) but is more suitable for research on national land planning needs. If the study area is within China and spans a long period, CNLUCC can first be used for trend analysis, followed by GlobeLand30 for accuracy correction or result validation (Table 4).

Table 4 Comparison of GlobeLand30 and CNLUCC Datasets

Dataset	GlobeLand30	CNLUCC
Spatial resolution	30 m	30 m
Classification system	10 first-level categories (cultivated land, forest, etc.)	6 first-level categories (cultivated land, forest land, grassland, water area, urban, industrial, mining and residential land, unused land)
Overall accuracy	80.33 %	76.41%
Kappa coefficient	0.76–0.82	0.64 around
Advantages	(1) 30 m globally consistent; (2) Accuracy improves significantly with version upgrades; (3) Good comparability with international counterparts	(1) Covering long-term time series in China (since the 1980s); (2) Providing dual scales of 30m and 1000m; (3) Facilitating long-term time series change detection.

4.2.2 Analysis of Sensitivity Differences

Analysis of Dynamic Responsiveness: A comparison of two datasets on the dynamic degree of land use in Dongguan City shows that the CNLUCC annual update (with a 3-month lag) has a 92% sensitivity to the fragmented

expansion of industrial land, with an annual fluctuation of wetlands at $\pm 12.3\%$ [8]. In contrast, the GlobeLand30 fixed-phase update (with a 2–3-year lag) exhibits only $\pm 3.1\%$ fluctuation in wetlands, but its sensitivity to the annual expansion of industrial land is merely 1.9% [8] (Table 5).

Table 5 Comparison of Land Use Dynamics between Two Datasets in Dongguan City

Dynamic Degree	Land Use Type	CNLUCC		Land Use Type	GlobeLand30	
		2020-2010	2010-2000		2020-2010	2010-2000
Single Land Use	Cropland	-2.01%	-5.19%	Cropland	-4.19%	-2.00%
	Forest land	-0.90%	-2.24%	Forest land	-1.20%	0.92%
	Grassland	4.85%	-3.02%	Grassland	-3.83%	-2.02%
	Water body	-1.21%	-0.02%	Water body	-0.91%	-1.95%
	Construction	1.04%	8.85%	Construction	4.61%	2.54%
	Bare land	0.11%	-9.56%	Shrubland	-3.16%	2.26%
	--	--	--	Wetland	42.31%	-7.87%
Comprehensive Land Use		0.62%	2.28%		1.57%	0.94%

4.2.3 Analysis of Dataset Discrepancy Causes

The differences in results between the two datasets stem from fundamentally distinct design philosophies and technical approaches. CNLUCC's classification system originates from land management needs, assigning independent codes to artificial land types like "industrial and mining storage land," making it highly sensitive to rapid industrialization and fragmented expansion (e.g., 92% recognition rate for industrial land). However, its classification of "water bodies" and "unused land" emphasizes administrative attributes rather than pure land cover, leading to compatibility challenges when aligning with global standards.

In contrast, GlobeLand30 strictly adheres to physical land cover characteristics, using spectral features and texture information to directly map land types, ensuring global consistency. Yet, this approach limits its ability to identify land classes with mixed spectra or seasonal variations (e.g., certain wetlands in Dongguan and scattered green spaces within built-up areas), resulting in 61.4% of wetlands being misclassified in early versions.

Additionally, differences in update mechanisms (CNLUCC's near-annual updates vs. GlobeLand30's fixed-phase updates) directly affect their ability to capture change rates. CNLUCC responds more quickly to policy-driven annual changes (e.g., dynamics of unused land), while GlobeLand30 excels at reflecting stable long-term trends. Therefore, when selecting a dataset, it is essential to consider how well its inherent logic aligns with research objectives.

4.2.4 Dataset Analysis Result

The differences between the two datasets stem from fundamental divergences in design objectives and technical approaches: CNLUCC prioritizes precision for land management optimization (e.g., unused land at -9.56%

reflects ecological restoration), while GlobeLand30 sacrifices granularity for cross-border comparability in global research (e.g., forestland change rate at -1.20%).
Application Recommendations:

- (1) Land Management Scenarios: Prioritize CNLUCC data (e.g., unused land at -9.56% reflects ecological restoration outcomes).
- (2) Cross-Border Research Scenarios: Opt for GlobeLand30 (e.g., forestland change rate at -1.20% ensures global comparability).
- (3) Hybrid Validation Method: For critical land types like construction land, cross-validate both datasets (CNLUCC +1.04% vs. GlobeLand30 +4.61%).

4.3 Analysis of Land Use in Dongguan City

4.3.1 Land Use Transfer Matrix and Change Characteristics

Through the comparative analysis of the two datasets mentioned earlier, it was found that the GlobeLand30 data is more suitable for the study area. Subsequently, land use transfer matrices for the periods 2000–2010 and 2010–2020 (Tables 6–9) were constructed to identify the transformation pathways and intensities among different land types (Figures 3–6).

Ultimately, the GlobeLand30 analysis data revealed that during 2000–2010, the most significant land use change was the conversion of cropland, primarily to construction land (168.78 km²) and water bodies (36.99 km²). Between 2010 and 2020, construction land continued to absorb large areas of cropland (293.09 km²), forest land (103.61 km²), and grassland (45.49 km²). The GlobeLand30 data further indicated that during 2010–2020, construction land was mainly converted from cropland, grassland, and forest land, with cropland conversion reaching 77.78 km² and grassland conversion amounting to 17.61 km².

Table 6 Land Use Transfer Matrix from 2000 to 2010 (GlobeLand30) (Unit:km²)

2000/2010	Grassland	Cropland	Shrubland	Forest Land	Construction	Wetland	Water Body	Total
Grassland	44.39367	2.85389	7.461344	33.65264	26.70493	0	1.807861	116.8743
Cropland	9.925816	586.2058	2.412814	39.28281	168.7827	0	36.9889	843.5988
Shrubland	1.362783	0.81006	23.91611	9.389227	1.389379	0	0.820459	37.68802
Forest land	16.40199	6.969456	7.646919	645.6413	13.27807	0.004133	10.53001	700.4719

Construction	9.321681	19.27319	2.780501	13.05403	738.9536	0	4.310993	787.694
Wetland	0.003093	0.074399	0	0.002062	0.005155	0	0.041326	0.126035
Water body	11.81802	58.79769	1.993317	23.5818	38.59871	0.022713	276.8359	411.6481
Total	93.22705	674.9844	46.21101	764.6039	987.7126	0.026846	331.3354	2898.101

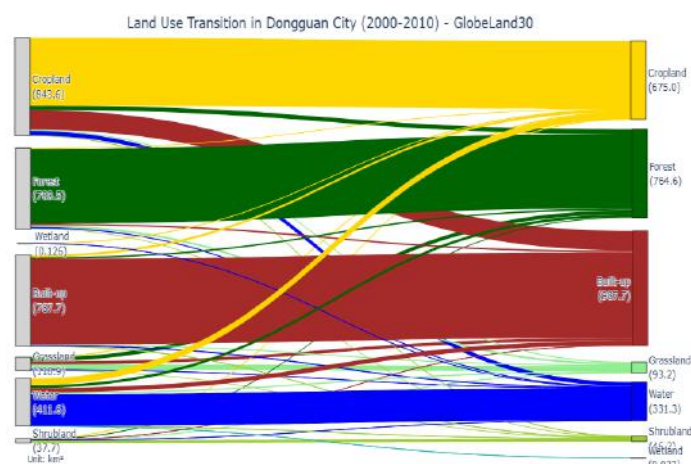


Fig.3 Land Use Transition Matrix from 2000 to 2010 (GlobeLand30)

Table 7 Land Use Transfer Matrix (GlobeLand30) for 2010-2020 (Unit:km²)

2010/2020	Grassland	Cropland	Shrubland	Forest land	Construction	Wetland	Water body	Total
Grassland	28.81426	3.109169	1.697769	10.78734	45.49444	0	3.324071	93.22705
Cropland	1.60707	344.4549	0.481748	6.971231	293.0932	0	28.37629	674.9844
Shrubland	1.850512	0.731993	19.76364	9.758944	13.12766	0	0.978261	46.21101
Forest land	17.20142	7.533227	7.787002	619.6369	103.6147	0	8.830632	764.6039
Construction	4.881305	8.881937	1.389571	17.59749	949.1989	0	5.763318	987.7126
Wetland	0.001033	0.007221	0	0	0	0.008265	0.010327	0.026846
Water body	3.108684	27.44837	0.465146	7.89268	38.53564	0.132287	253.7547	331.3375
Total	57.46428	392.1668	31.58487	672.6446	1443.065	0.140553	301.0376	2898.103

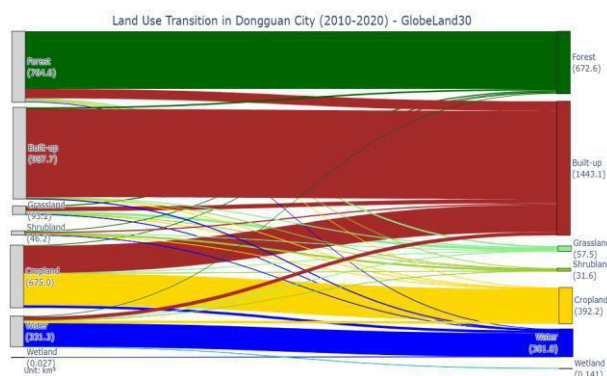


Figure 4 Land Use Transition Matrix from 2010 to 2020 (GlobeLand30)

Table 8 Land Use Transition Matrix (CNLUCC, 2000-2010) (Unit:km²)

2000/2010	Grassland	Construction	Cropland	Forest land	Water body	Bare land	Total
Grassland	54.126	19.1547	3.7863	4.5864	2.043	0	83.6964
Construction	1.3734	577.1097	18.5787	23.0562	13.0491	0.0531	633.2202
Cropland	1.7181	341.1207	264.2022	39.2643	43.0155	0.0297	689.3505
Forest land	1.0233	196.1433	35.4438	514.9251	10.881	0	758.4165
Water body	0.1656	59.4252	9.5832	6.9345	199.8999	0.0009	276.0093
Bare land	0	0	0	0.0036	1.9188	0	1.9224
Total	58.4064	1192.9536	331.5942	588.7701	270.8073	0.0837	2442.615

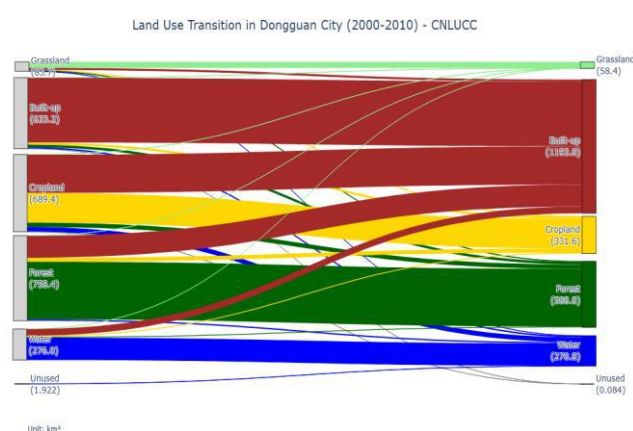


Fig.5 Land Use Transfer Matrix Map (CNLUCC) for 2000-2010

Table 9 Land Use Transfer Matrix Table (CNLUCC, 2010-2020) (Unit:km²)

2010/2020	Grassland	Construction	Cropland	Forest land	Water body	Bare land	Total
Grassland	52.4574	3.24	0.666	1.5669	0.4761	0	58.4064
Construction	17.6076	1120.4514	13.8978	22.3092	19.2762	0	1193.542
Cropland	1.3392	77.7798	236.5749	6.084	9.783	0	331.5609
Forest land	9.8136	65.7558	7.0065	499.6035	6.4458	0	588.6252
Water body	5.5035	50.3487	6.6996	6.2271	206.3043	0.0144	275.0976
Bare land	0	0	0	0	0.0135	0.0702	0.0837
Total	86.7213	1317.8187	264.8448	535.7907	242.2989	0.0846	2447.559

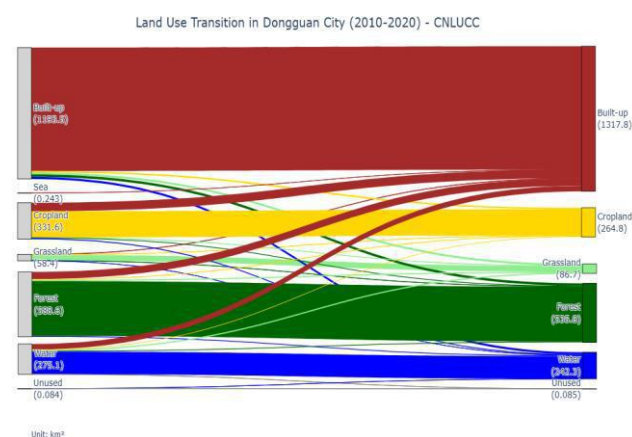


Fig.6 Land Use Transition Matrix Map (CNLUCC) for 2010-2020

4.3.2 Changes in Land Use Types and Area

Analysis based on the CNLUCC and GlobeLand30 datasets indicates significant changes in Dongguan's land use structure between 2000 and 2020. According to CNLUCC data, from 2000 to 2010, Dongguan experienced a 5.19% reduction in arable land, with forest and grassland decreasing by 2.24% and 3.02% respectively, while construction land increased by 8.85%. Between 2010 and 2020, the decline in arable land narrowed to 2.01%, forest land decreased by 0.90%, grassland transitioned to a growth of 4.85%, and the growth rate of construction land slowed to 1.04%.

GlobeLand30 data corroborates this trend: arable land decreased by 2.00% from 2000 to 2010, and the decline

expanded to 4.19% from 2010 to 2020. Construction land continued to grow in both periods, with growth rates of 2.54% and 4.61% respectively. Notably, wetlands saw a significant increase of 42.31% between 2010 and 2020, indicating initial success in ecological restoration measures.

4.3.3 Analysis of Land Use Type Changes and Their Causes

By comprehensively comparing the land use changes in Dongguan from 2000 to 2020, including the spatiotemporal transitions of land use and the variations in area (Figures 7 and 8), we can effectively understand the development trajectory and related implications over the past two decades. The findings are summarized as follows:

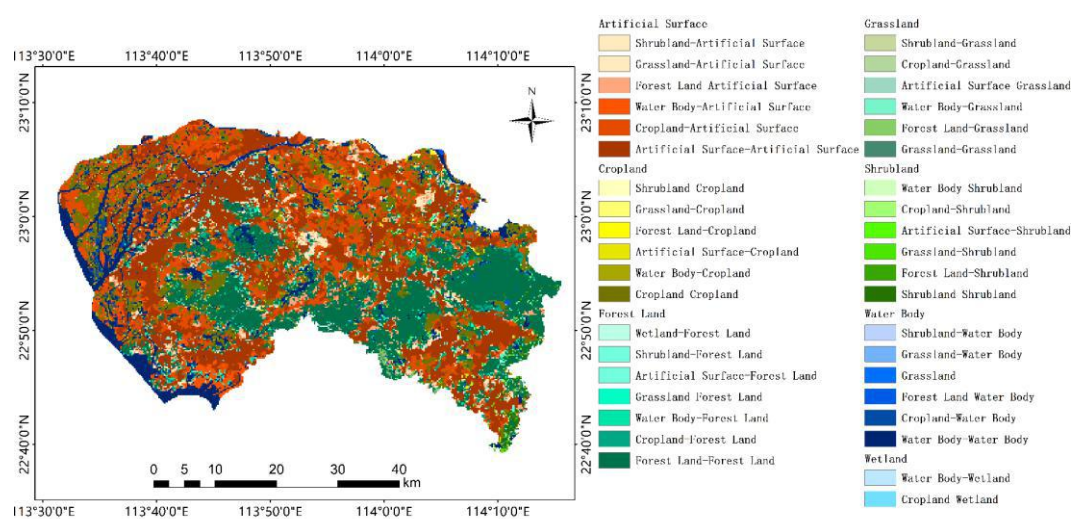


Fig.7 Land Use Type Map of Dongguan City (2000-2020, Globeland30)

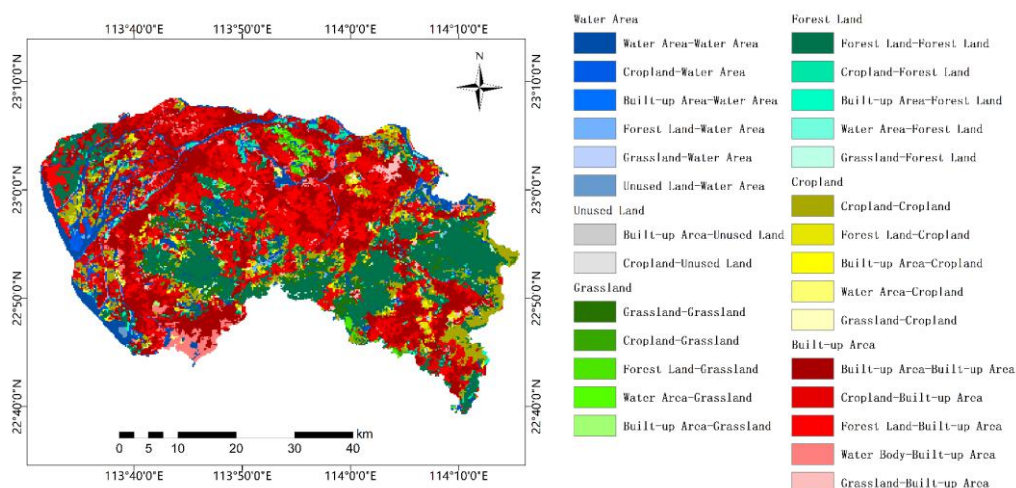


Fig.8 Land Use Type Map of Dongguan City (2000-2020, CNLUCC)

(1) Expansion of Construction Land

The continuous expansion of construction land in Dongguan is a core feature of its rapid urbanization, primarily driven by policy-led industrial parks and transportation infrastructure, economic growth and population agglomeration, advantageous geographical location, and the vitality of town-level development under the city-administered-town system. Its spatial pattern has evolved from scattered points to contiguous clusters and from edge expansion to infill development. While this process has supported economic growth, it has also led to the large-scale occupation and fragmentation of ecological land. Currently, Dongguan is promoting a shift toward sustainable development by improving land-use efficiency and fostering harmony between human activities and the environment through measures such as territorial spatial planning and ecological restoration.

(2) Reduction and Protection of Cropland

The area of cropland showed a decreasing trend in both decades, primarily converted to construction land and water bodies. Despite the government's implementation of cultivated land protection policies, urban expansion still makes it difficult to completely avoid occupying farmland. The narrowing of the reduction in the later period indicates that the farmland red line policy has begun to take effect.

(3) Changes in Water Bodies and Wetlands

The area of water bodies showed a slight decrease in the first decade but recovered in the following decade,

which is related to water resource management policies and water environment governance. Wetland area significantly increased in the later period of GlobeLand30 data, reflecting the implementation effects of ecological restoration measures.

(4) Changes in Forestland and Grassland

The overall area of forestland and grassland has decreased, but grassland showed a rebound in later periods, which is related to projects such as returning farmland to grassland and ecological restoration. The reduction in forestland is mainly influenced by urban expansion and agricultural restructuring.

5. Conclusion

5.1 Analysis and Summary

This study systematically compares the applicability differences between two mainstream land-use datasets, GlobeLand30 and CNLUCC, in Dongguan, a high-density urban area of the PRD, and conducts an in-depth analysis of the spatiotemporal dynamics of land use in Dongguan from 2000 to 2020 based on the more accurate GlobeLand30 dataset. The main conclusions are as follows:

(1) Clear distinctions in dataset applicability, with selection depending on research objectives

CNLUCC, with its management-oriented three-tier classification system, is more suitable for localized fine management scenarios such as territorial spatial planning and land law enforcement monitoring. In contrast, GlobeLand30, with its observation-oriented global unified

standards, holds irreplaceable advantages in global environmental change research and cross-border ecological comparisons.

(2) Significant differences in data accuracy and dynamic responsiveness

GlobeLand30 exhibits higher overall accuracy (80.33%–85.72%) and consistency (Kappa: 0.76–0.82) compared to CNLUCC, with continuous improvements across versions. Meanwhile, CNLUCC, benefiting from its higher update frequency, responds more quickly to intense human activities such as construction land expansion.

(3) Land-use changes in Dongguan reveal typical urbanization drivers and transformation characteristics

Based on the more accurate GlobeLand30 data analysis, Dongguan underwent a complete urbanization process from 2000 to 2020, characterized by "spatial polarization—boundary sprawl—functional restructuring." The early phase featured the polarized concentration of construction land in central areas and rapid sprawl at the edges, significantly encroaching on farmland and ecological spaces. The later phase shifted toward functional restructuring marked by stock renewal, infill development, and ecological restoration (e.g., significant recovery of wetland areas), reflecting a profound transformation in urban development patterns.

In summary, this study provides clear scientific guidance for dataset selection in land-use research within highly dynamic urban regions: CNLUCC excels in serving localized management, while GlobeLand30 is superior for supporting international research. The insights into Dongguan's evolution also offer valuable references for the sustainable development and territorial spatial optimization of similar high-density cities.

5.2 Recommendations

Based on the above findings, the following targeted recommendations are proposed:

- (1) For territorial spatial control and policy evaluation, it is recommended to prioritize the use of CNLUCC data to leverage its high-precision monitoring capabilities for key managed land types such as construction land, cultivated land, and unused land.
- (2) For cross-border ecological research or global model

inputs, GlobeLand30 data is recommended to ensure the comparability and consistency of results at the international level.

- (3) For comprehensive studies, a hybrid validation strategy can be adopted, especially for key and easily confused land types such as construction land and wetlands, to cross-validate the results of the two datasets, thereby complementing each other and reducing uncertainty.

5.3 Research Prospects

Future research could delve into the following aspects: First, explore data fusion methods between CNLUCC and GlobeLand30 to generate new land-use products that combine management details with global comparability. Second, introduce socio-economic driving factors (such as industrial upgrading and population mobility) and use quantitative methods like geographic detectors and regression models to more deeply uncover the intrinsic mechanisms of land-use change. Third, apply the evaluation framework established in this study to other types of cities (such as mountainous or coastal cities) to test its universality and refine the methodological system for remote sensing monitoring of highly dynamic urban areas.

ACKNOWLEDGEMENTS

The author is grateful for the research grants given to Ruei-Yuan Wang from GDUP T Talents Recruitment (No.2019rc098), Peoples R China under Grant No.702-519208, and Academic Affairs in GDUP T for Goal Problem-Oriented Teaching Innovation and Practice Project Grant No.701-234660.

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Selection and Breeding Methodology of Orchids

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Received: 03 Apr 2024; Received in revised form: 12 Oct 2024; Accepted: 17 Oct 2025; Available online: 25 Oct 2025

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Abstract— For evolution of ecosystem, conservation of advanced organism is important. Orchids are one of the advanced plants. But most of the orchid species are endangered because of lack of endosperm, self-pollination inhibiting nature. Also, insects destroy orchid seeds unconscious for food. And so orchid breeding and conservation is important. Orchids are very much known for medicine, ornamentation along with devotional believes. In this chapter we have discussed about cross breeding, mutation breeding, selection breeding, molecular marker assisted breeding, polyploidy breeding and transgenic breeding.

Keywords— Orchid Breeding, Orchid Cultivation, Orchid Selection and breeding Methodology, Dendrobium breeding



I. INTRODUCTION

Orchid is one of the biggest family of angiosperms (monocotyledons). Flowering plants in the worldwide distribution, consisting of a 28000 species, subspecies 800 [1]. They are present throughout the world. Orchids plants have attractive flowers. They come in different colors and sizes. Orchids flowers have 3 petals. Most orchids blooms once a year. Flower usually remains 6 to 10 weeks. The flowers have secreted nectar. Orchids are believed to bring good luck, weath and prosperity. Orchids are suitable for indoor growth. Botanical and economical role of orchids in modern cultivation structures take part in use of horticulture and environments which increase with control of proper weather, especially temperature which permits the induction of flowering regardless of the time of year, specially aiming scheduled on the delivery of potted flower market. Seed plants of species 6-11%. The biggest genera are *Bulbophyllum* (2000), *Epidendrum* (1500), *Dendrobium* (1,000). The genera *Dendrobium*, *Gastrodia* and *Bletilla* used for medicinal and Chinese medicine purposes [2]. Additionally includes *vanilla* (The genus of vanilla plant)utilization of food purposes [3]. Because the creation of tropical species into cultivation in the 19th century, horticulturists produced greater than 100,000 hybrids and cultivars. Those are bilateral symmetry of the zygomorphism flower, many resupinate flowers, almost

usually exceptionally modified petal, fused stamens and carpels and extremely small seeds. Commercial classification for orchids individually from botanical classification . In order to produce the most flowers from a given genus, interspecific crosses must be made. These crosses must involve species from the same genus as well as species from other genres (intergeneric hybrids). As an illustration, *Doritis* in crossings with *Phalaenopsis* result in the hybrid genus known as *Doritaenopsis* [4,5]. *Phalaenopsis* is another name for commercial hybrids. In plant families, it is feasible to produce such a large number of fertile progenies from very different morphologically distinct species and genera. Breeders incorporate many traits from single plants, the modern aspect of flower production as the advancement in breeding, the use of those identical commonly fertile hybrids, the development of generation of crosses, and the creation of new hybrids[6]. The hybridization process of early protocorm improvement and embryogenic growth in orchids. It has been hypothesised that in other species, loss of hybridization and hybrid seed absorption are linked to appropriate endosperm development or an imbalance between endosperm and embryo development. Moreover, embryo development occurs in the absence of the endosperm in the family of orchidaceae during zygotic embryogenesis [7]. One of an orchid's most valuable components, the zygomorphic

flower has three sepals, two petals, a specific labellum, an appendage or a basal spur or nectary or not, and a gynostemium united by style and at the very least a portion of the androecium [8]. With an increase in trade volume, orchids, which are potted plants that don't produce as many flowers, are moving more widely throughout the market [9]. Orchids are employed in the pharmaceutical, food, and beverage industries for their polysaccharides, alkaloids, and other chemical components [10].

II. CROSS BREEDING

Among flowering plants, Orchidaceae family shows the majority of diversity. The family include more than 28000 Species, which shows numerous breeding strategy and attribute. *Acianthera aphthosa* has a character of self pollinating flowers, has fewer seeds missing embryos than cross pollinated flowers [11]. Each natural and artificial cross breed process combine the exceptional traits of the two parent in cross breed offspring. One of the earliest artificial orchids, *Calanthe*, was created from the cross-breeding of *Calanthe masuca* and *Calanthe furcata* and was first documented by Dominy in 1856. *Phalaenopsis inrermedia*, a hybrid of *P. aphrodite* and *P. rosea* that was originally described in 1853, is one of the natural hybrids. Cross breeding is easy cultivating method of orchids. While doing cross breeding, a number of factors, including the hybrid combination's fertility, the evaluation of the goal features, and the selection of better hybrid progeny, should be taken into account [13]. The F1 offspring of two parents often exhibits phenotypic variations, with one parent having long blooming time and tiny flowers in size, and the other having large flowers in size but short flowering time. For instance, lonmesa popcorn Flowers produced by Haruri are distinct from those of its parents [14,15]. A suitable nurturing strategy is needed after obtaining hybrid seeds to keep the population expanding. The in-vitro method of propagation is a crucial part of orchid breeding since orchid seeds are difficult to grow in their natural habitat. Seed age, culture conditions, and culture media all have an impact on how effective in-vitro propagation is. The genera *Cymbidium*, *Phalaenopsis*, *Dendrobium*, *Oncidium*, *Dactylorhiza*, and *Calanthe* alliance contain a large number of orchid species [16,17]. Breeding cycle, improvements of the hybrid grex and a shorting, the principle targets of in-vitro propagation, and considerable development has been made in achieving those objects.

III. MUTATION BREEDING

Many species of ornamental plant breeding process propagated easily, with the natural and artificial process. Several breeding process exhibit seeds to

chemicals, radiations and enzymes [18,19]. Benefits of mutation individual traits [20]. Phenotypic trait of used the breeding process, content material and medicinal instruments. The common technique of mutation breeding is polyploidization. Ploidy breeding in this breeding process plants include two paired sets of chromosome are increased. Increases in cell size features that organs are vegetative and reproductive. *Cymbium*, *Dendrobium*, and *Oncidium* are only a few of the polyploidy breeding orchid species. Colchicine hybrids produce tetraploid plants with deeper stem colours, slower charge increases, thick leaves, roots, and rhizomes [21]. To increase the alkaloid concentration of medicinal *Dendrobium* generated by tissue culture, nitric oxide provider sodium nitroprusside was added to the protocorm of the hybrid *Dendrobium huoshanensi* plant. *D. catenatum* seedlings were more recently exposed to UV light, which led to an increase in the total amount of polysaccharides, flavonoids, alkaloids, and many significant secondary metabolisms [22]. Increase in orchid heterozygosity raises the apparent genetic variation and causes a rapid cycle of extraordinary mutation types. Nevertheless, random mutations in the genome might result in harmful changes, which are frequently acquired by the smallest single adjustments [23].

IV. SELECTION BREEDING

As the only source of material for the selective breeding process, natural variants of existing kinds are used [25]. Heritability, genetic correlations between phenotypes, and interactions between genotype and environment as well as by genetic material are the three basic genetic parameters. Hybridization, selection, and in vitro propagation were used to create the new *Phalaenopsis* cultivar "SM 333." Plant somaclonal mutation determined on lines screening, genetic characterization, tissue culture, and multifactor testing were used to create the new *Oncidium* variety "jinhui." Moreover, three lines of *Calanthe nipponica* with pure yellow sepals and petals were found during the area survey; nonetheless, the plant's typical colour is purple-brown. Since mutation is heritable, it would provide for excellent stock for selective breeding.

V. MARKER ASSISTED BREEDING

Using genotype-based selection rather than visible qualities, a plant is chosen using molecular markers. Use of molecular biotechnology in practical breeding and selection, advantages of speed and accuracy, and impact of environmental factors [28]. The following are relevant in terms of prevalence and potential among the numerous kinds of molecular markers available to scientists and breeders. Simple sequence repeats employed in breeding

outcomes, RELP, AFLP, and SNP [29]. *Cymbidium encifolium* may be used with many orchid species for mapping investigations and genetic connection assessments. These marker types will aid in locating candidate genes with distinct functions in combination with the helpful annotations provided by unigenes. *Papilionopodium concolor* root transcriptome sequencing has shed light on the process and the genes involved in root secondary metabolism [30,31]. SSR genes are critical for genetic modification breeding because they are connected to floral colour, shape, and resistance in *Phalaenopsis*. In order to assess the efficacy of predicting flower colour and aid in the breeding of the most recent *phalaenopsis* variants, the genetic diversity of distinct species of *phalaenopsis* was examined using gene specific single nucleotide amplified polymorphism markers [32]. In addition to providing an exceptional resource for improving the breeding performance of horticultural orchids, integrating the *phalaenopsis* genome with an SNP-based genetic linkage map and validating it through optical mapping has contributed significantly to studies on the difference genomics of epiphytes for future reference.

VI. POLYPLOIDY BREEDING

Polyploidy is defined as two or more sets of chromosomes and may occur naturally. The fundamental characteristic of plants, polyploidy is what allows for species adaptability, diversity, evolution, and development. [33]. Their evolutionary history all through polyploidy approximately 70% of angiosperms [34]. Duplication of genetic material the maximum frequency have been particularly discovered in domesticated plants in place of wild plants [64]. Chromosome duplication the angiosperm genome as minimum [35]. According to their origin polyploidy classified into autopolyploidy (increase in basic number of chromosome), allopolyploidy (The presence of more than two basic sets of distinct chromosomes indicates that two separate species have hybridised) [36,37]. Assumed that almost all of flowering plants are allopolyploidy [38,39]. Unique species variant genome presents and possibilities novel diversity, with the introduced benefit that gene excess may mask recessive deleterious alleles by dominant one [40]. In addition, the expression of genes essential for chromatid cohesion and meiosis can be enhanced, as discovered in the *Arabidopsis suecica* allopolyploidy [41]. More than three chromosome pairs used as cytological element to differentiate auto and allopolyploids. For example, multivalent pairing at metaphase can also additionally factor to homology between chromosome set and consequently (42). Although dissimilarity, bivalent formation is high at diakinesis from

pairing between non-homologous parental chromosome sets, which may suggest allopolyploidy. *Phalaenopsis micholitzii* (with more than one little spike) and *Phalaenopsis tetraspis* are two species that are crossed during orchid breeding to improve cultivars with more than one spike (lengthy spikes). Tetralitz P.Tzu-Chiang grows five spikes. Tissue culture frequently causes hybrids to spontaneously become polyploid, although this process is tedious and requires repeated crossings to keep the progeny's developmental balance. Early artificial allopolyploid generations involved extensive restructuring of the combined genomes, including chromosomal rearrangements and changes to chromosome diversity, as well as epigenetic changes such as transposon activation, chromatin alterations, and altered methylation patterns. Actually, micronuclei in tetrads and chromosomal rearrangement are commonly seen in meiocytes of presumed orchid allopolyploids. These micronuclei human cancer cells and arise from hypomethylation in pericentromeric DNA, poor organization of the spindle.

VII. TRANSGENIC BREEDING

A breeding method Genetically engineered transgenic plants to make plant a new characteristics, identified as a type of genetically modified organisms (GMO). Transgenic breeding method is time consuming, characters of plants and more useful traits at common sexual hybridization technique almost unsuitable. Molecular genetic technique to transform orchid biotechnology. Gene transformation systems associated with rapid selection and regeneration and technique for the production advanced diversity of orchids with proper characters. Experiment/study of transgenic orchids formation and developing of gene transformation procedure, with specific significance use of different selectable marker. A selectable marker gene present into cell, specially a bacterium cell in culture that characteristic appropriate for artificial selection. Some selectable marker used in transgenic breeding-aminoglycoside antibiotic resistance, herbicide resistance and other antibiotics, pathogen resistance, visual selection. Agrobacterium-mediated transformation, particle bombardment, pollen tube route, electrophoresis, and polyethylene glycol are examples of genetic transformation techniques. Technique of Agrobacterium-mediated and particle bombardment used in orchid breeding [29]. Orchid variations first reported in *Vanda* [30] and *dendrobium* [31,32] mediated via particle. Powerful transformation structures have been established for a few commercial orchids, such as *Vanda* [33], *Cymbidium* [34], *Dendrobium* [35,36], *Cattleya* [37] and *Erycina pusilla* [38]. Using electrophoresis, Griesbach and Hammond (1993) inserted an anthocyanin synthesis gene into the powders of *Doritis*

pulcherrima and acquired fleeting expression in flowers, which served as an alternative in the colour of the petals [39]. To create transgenic plants resistant to kanamycin, the bombardment approach was employed to introduce a plastid containing the NPTII and GUS marker genes into *Cymbidium* orchids [40] RAPD was then utilised to identify genes related to smell. With regard to floral characteristics, plant architecture, and biotic and abiotic tolerance, transgenic breeding technology is a crucial way of orchid cultivation [41,42]. For many years, Orchidaceae family transgenic studies in first development and specially transgenic plants to the ornamental orchid genera such as *Cymbidium*, *Phalalaenopsis*, *Dendrobium* and *oncidium*. *Agrobacterium* mediated and particle bounded technique used whereas the ovary injection and pollen tube method pathway generally used.

VIII. CONCLUSION

Orchid breeding and production advance things to be a— manufacture of high variety clones by micropropagation. Breeding new varieties by application of biotechnology is important for conservation of those species. Essential gene development To produce extraordinary offspring with objective developments, substratum forward as well as reversed genetic, other traditional breeding, or molecular breeding are used. Each strategy has advantages and limitations, and if used alone will not speed up the breeding process. The use of polyploidization in orchid breeding is crucial to its success. As a result, it is necessary to combine a number of approaches and research concepts to enable the development of orchids with distinctive flower morphologies, innovative colours, and powerful floral aromas.

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Smart Extremozymes: The Next-Generation Biocatalysts for Sustainable Industrial Wastewater Management

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Received: 12 Sep 2025; Received in revised form: 10 Oct 2025; Accepted: 16 Oct 2025; Available online: 25 Oct 2025

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Abstract— Industrial wastewater treatment face various challenges like high pollutant load, extreme environmental conditions and the need for cost-effective and eco-friendly solutions. These challenges could be sustainably managed using biocatalysts like enzymes. Although enzymes are sustainable approach towards this, but they often act futile due to the extreme conditions present in the wastewater. Extremozymes are specialized enzymes with the ability to function under extreme conditions and offer a promising approach to address these challenges. The types of extremozymes explored in this study include thermozymes, psychrozymes, halozymes, alkalozymes and acidozymes. Their mechanism of action enables the efficient breakdown of pollutants making them highly suitable for industrial applications. Extremozymes have proven effective in treating effluents from industries such as textiles, pulp and paper, pharmaceuticals and food. These enzymes provide high stability and efficiency in harsh wastewater environments. Moreover, these enzymes also offer advantages such as reduced operational costs and minimized environmental footprints. Recent advancements such as AI-driven enzyme design, synthetic biology and nano-immobilization strategies have further enhanced extremozyme performance which leads to the development of intelligent and reusable “bionanoextremozymes” capable of functioning efficiently under fluctuating industrial conditions. By leveraging extremozymes, industries can adopt a more sustainable and efficient approach to wastewater treatment. This review highlights their potential to revolutionize industrial effluent management, paving the way for sustainable, environmentally friendly and economically viable wastewater treatment solutions.



Keywords— Biocatalyst, Extremozymes, Industry, Sustainability, Wastewater

I. INTRODUCTION

Industrial wastewater poses significant challenges due to its diverse composition and environmental impacts. It contains a mix of organic pollutants, heavy metals, toxic chemicals and nutrients that can harm ecosystems and human health [1]. This content in wastewater varies depending on the type of industry. The high volumes of discharge from industries such as textiles, pharmaceuticals and food processing intensify the problem. Untreated wastewater contributes to water pollution, soil pollution and eutrophication that enhances the disruption of ecosystems and agricultural productivity [2]. Meeting stringent regulatory requirements demands advanced treatment methods. Whereas conventional processes like coagulation and activated

sludge systems are often energy-intensive and costly. The demand for sustainability has added pressure to recover resources such as water, nutrients and energy from wastewater. Climate change further complicates wastewater management by impacting water availability and quality. Hence, these challenges need to be addressed. Furthermore, certain pollutants like synthetic dyes and heavy metals resist traditional treatments. This necessitates the development of innovative approaches such as membrane filtration, advanced oxidation processes and bioremediation utilizing metabolites like enzymes [3]. Enzymes represent a sustainable and environmentally friendly solution for wastewater treatment, as they operate without requiring external energy input [4]. However, conventional enzymes

often lose functionality under extreme environmental conditions like high pH or temperature [5]. This necessitates the need for alternative strategies that are effective in such challenging extreme conditions. Extremozymes derived from extremophiles exhibit remarkable stability and activity under harsh industrial conditions. These specialized enzymes can enhance wastewater treatment processes by efficiently utilizing recalcitrant pollutants as substrate. This can build up an enzyme-substrate (ES) complex enhancing the velocity of ES reaction to maximum [6]. Hence, this can be effectively used in wastewater treatment without any effect of harsh environmental conditions [7].

Extremozymes are specialized enzymes produced by extremophilic microorganisms that thrive in extreme conditions such as high temperature, salinity and acidity. These enzymes are highly stable and maintain their catalytic activity under harsh industrial environments and hence they remain a sustainable approach for wastewater treatment [8]. Extremozymes effectively degrade recalcitrant pollutants like synthetic dyes, hydrocarbons and heavy metal complexes [9]. These pollutants are resistant to conventional methods. Furthermore, extremozymes support sustainability by enabling the biodegradation of pollutants without producing harmful byproducts aligning with eco-friendly practices [10]. They can also be incorporated into advanced treatment technologies like bioreactors or enzymatic membranes.

II. ROLE OF EXTREMOZYMES IN SUSTAINABLE WASTEWATER TREATMENT

Extremozymes play a pivotal role in sustainable wastewater treatment by enhancing the efficiency and eco-friendliness

of the process. Derived from extremophiles, these enzymes remain active under harsh industrial conditions where conventional enzymes fail. This resilience allows extremozymes to degrade recalcitrant pollutants including synthetic dyes, hydrocarbons and toxic chemicals that are resistant to traditional methods [7]. Conventional enzymes need extensive energy inputs to adjust optimum pH and temperature in their production and purification process to maintain the performance [11]. However, the unique ability to operate without any temperature or pH adjustments makes the use of extremozymes cost-effective and energy efficient [12]. By reducing the reliance on chemical-intensive processes and aligning with circular economy principles, extremozymes contribute significantly to resource recovery that includes clean water and reusable byproducts. Their application represents a shift towards greener and more sustainable industrial wastewater management practices. This addresses the dual challenges of pollution control and resource conservation.

Types of Extremozymes used in Wastewater Management

The unique properties of extremozymes make them valuable in wastewater management, as they efficiently degrade a wide range of pollutants even in harsh environments. Different types of extremozymes include thermostable, halotolerant, acidophilic and alkaliphilic enzymes [13]. These enzymes target various contaminants based on the specific conditions of industrial wastewater. Understanding these types and their applications enables tailored solutions for efficient and sustainable wastewater treatment. The major types of extremozymes used in wastewater treatment are discussed in the following Fig. 1.

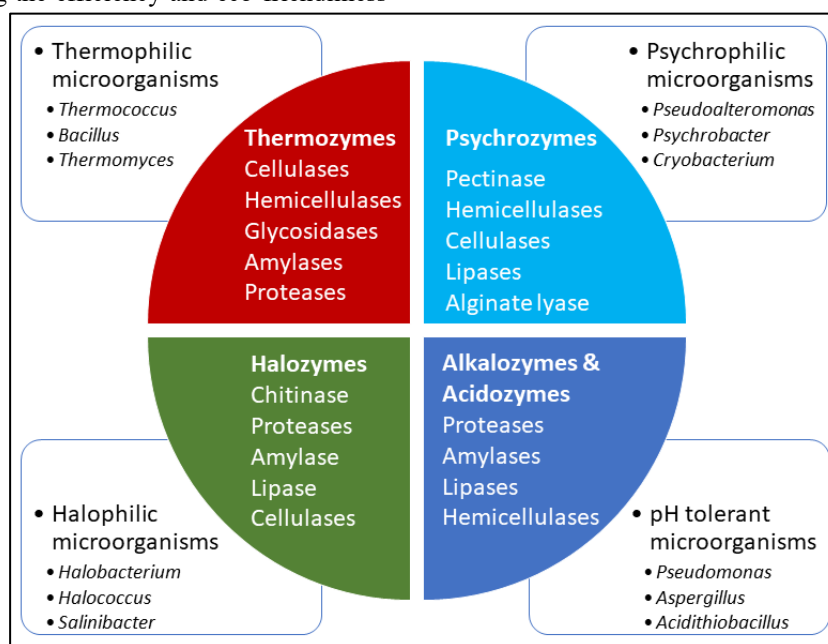


Fig. 1. Types of extremozymes used in wastewater treatment

2.1 Thermozyymes

Thermozyymes are enzymes derived from thermophilic microorganisms that thrive at elevated temperatures typically ranging from 50°C to 80°C or higher. These enzymes exhibit remarkable thermal stability by retaining their catalytic activity under high-temperature conditions commonly found in industrial wastewater treatment processes. Their ability to function efficiently at high temperatures eliminates the need for extensive cooling systems and hence reduces energy costs and enhancing process efficiency. Thermozyymes also accelerate the biodegradation of recalcitrant pollutants like dyes and phenolic compounds that are resistant to conventional methods [14]. Moreover, high-temperature operations facilitated by thermozyymes minimize the risk of microbial contamination and enhance reaction rates that make these processes faster and more effective. They are particularly useful in industries like textiles, pulp and paper and food processing in which hot effluents are common. Many of the thermozyymes are cellulases, hemicellulases, glycosidases, pullulanases, proteases etc. Examples of thermophilic microorganisms producing these enzymes include *Thermococcus*, *Bacillus* and *Thermomyces* etc. [13].

2.2 Psychozyymes

Psychozyymes are enzymes produced by psychrophilic microorganisms that thrive in cold environments typically at temperatures below 15°C. These enzymes retain their catalytic activity and stability at low temperatures that makes them highly effective for wastewater treatment in cold climates or processes involving low-temperature effluents. In wastewater management, psychozyymes are particularly valuable for industries where heating is impractical or unaffordable [15]. They efficiently degrade organic pollutants such as proteins, fats and carbohydrates in cold conditions, ensuring effective treatment without the need for energy-intensive temperature adjustments. Their functionality at low temperatures also minimizes the risk of thermal denaturation that ensures prolonged enzyme activity and reduces operational costs. Psychozyymes are instrumental in the treatment of low-temperature effluents from industries like dairy, food processing and breweries where conventional enzymes often lose effectiveness [16]. They are also advantageous in municipal wastewater systems in cold regions in which ambient temperatures can hinder biological treatment processes. Their eco-friendly operation aligns with the goals of reducing carbon footprints and operational costs that make them an essential tool for industries and regions aiming to optimize the process in challenging cold environments. Some psychozyymes include cold-active polygalacturonase, α -amylase, phosphatase, proteases etc. Examples of psychrophilic

microorganisms producing these enzymes include *Pseudoalteromonas*, *Psychrobacter* and *Cryobacterium* [17].

2.3 Halozyymes

Halozyymes are specialized enzymes produced by halophilic microorganisms that thrive in high-salt environments. These enzymes retain their catalytic activity and stability under saline conditions that make them invaluable for treating wastewater with elevated salt concentrations. Effluents from industries like textiles, tanning, oil and gas and seafood processing releases wastewater that is high in salt concentration. Haloenzymes can be an effective approach to treat such kinds of wastewater effluents. Conventional enzymes and microbial systems often lose efficiency in saline wastewater due to osmotic stress and denaturation [18]. In contrast, halozyymes are naturally adapted to such environments that enable efficient degradation of organic pollutants like proteins, lipids and hydrocarbons in saline effluents. Their functionality helps reduce the biological oxygen demand (BOD) and chemical oxygen demand (COD) of wastewater. Halozyymes also support advanced treatment methods like enzymatic bioreactors and membrane technologies by maintaining activity under saline conditions [19]. This adaptability reduces the need for costly desalination or chemical adjustments that makes the process more cost-effective and sustainable. Furthermore, the use of halozyymes aligns with eco-friendly practices that minimize the need for harsh chemicals in saline wastewater treatment. By leveraging the unique properties of halozyymes, industries can effectively manage saline effluents. Some common halozyymes include Halophilic proteases, lipases, amylases, esterases, dehydrogenases, etc. These halozyymes are typically derived from halophilic microorganisms such as *Halobacterium*, *Halococcus* and *Salinibacter* [20].

2.4 Alkalozymes

Alkalozymes are particularly effective in degrading pollutants in alkaline wastewater from textile, paper, detergent and tannery industries. They catalyze the breakdown of recalcitrant compounds like synthetic dyes, lignin derivatives and industrial chemicals, ensuring efficient detoxification [21]. Their ability to withstand and operate in high pH conditions eliminates the need for chemical neutralization. Some major alkalozymes include alkaline proteases, lipases and amylases that are widely used for breaking down proteins, fats and starch present in alkaline wastewater [22]. Several microorganisms produce alkalozymes. Some of the prominent examples of alkalozymes producing microorganisms include *Bacillus*, *Pseudomonas*, *Aspergillus* etc. [13].

2.5 Acidozymes

Acidozymes play a crucial role in treating acidic effluents from industries like mining, battery manufacturing and food processing. These enzymes degrade pollutants such as organic acids, heavy metals and sulfates that are prevalent in acidic wastewater. By functioning directly in low pH environments, acidozymes eliminate the need for pH adjustments by reforming the treatment process. Examples of acidozymes include acidic proteases, amylases and lipases that facilitate the breakdown of proteins, starch and fats respectively in acidic effluents. Acidophilic microorganisms such as *Acidithiobacillus*, *Aspergillus* and *Fusarium* are common producers of acidozymes [23].

III. MECHANISM OF ACTION OF EXTREMOZYMES

Extremozymes operate under extreme environmental conditions such as high or low temperatures, pH or salinity. Their mechanism of action in wastewater treatment involves breaking down complex pollutants into simpler and non-toxic compounds to enhance the efficiency of the treatment process. The extremozymes have a specific mode of action by which they act against the pollutants present in

wastewater. Like every enzyme, extremozymes also have specialized active sites that bind to specific pollutants present in wastewater. This step is called substrate binding. Extremozymes interact with these substrates through non-covalent forces that form enzyme-substrate complex which is followed by catalysis. Once bound, extremozymes catalyze the breakdown of complex pollutants into simpler and biodegradable products. In the case of industrial pollutants like dyes or phenolic compounds, extremozymes like laccases and peroxidases initiate oxidation reactions [18]. This leads to the decolorization and detoxification of these pollutants. The extremophilic ability of extremozymes is achieved through structural adaptations like increased hydrophobic interactions and stronger enzyme-substrate binding. This prevents denaturation and ensures prolonged activity even in harsh wastewater environments. Through their catalytic action, extremozymes effectively degrade toxic and recalcitrant pollutants into less harmful substances (Fig. 2). This action often converts them into water-soluble and biodegradable compounds. This reduces the toxicity of the effluent and hence makes it safer for discharge or further treatment [24]. Extremozymes contribute to more sustainable wastewater management by reducing the need for harmful chemicals and energy-intensive processes like heating or pH adjustment.

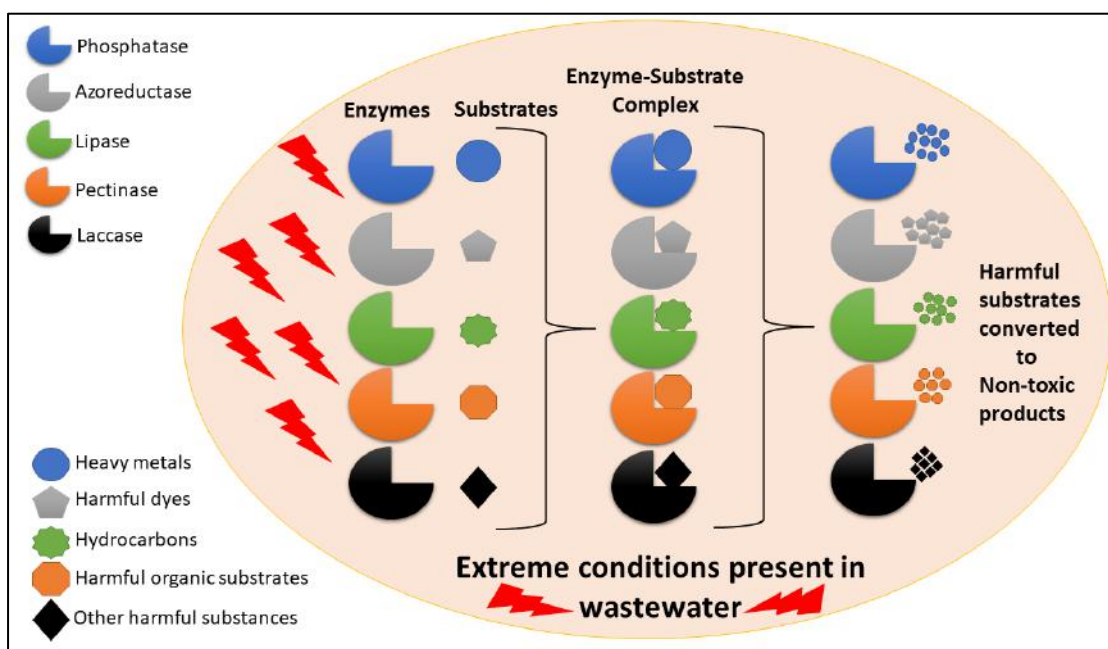


Fig. 2. Mechanism of action of extremozymes in wastewater treatment

IV. APPLICATIONS OF EXTREMOZYMES IN INDUSTRIAL WASTEWATER TREATMENT

4.1 Textile Industry Effluents

The textile industry generates effluents containing high levels of synthetic dyes, organic pollutants, salts and heavy

metals. These effluents are often resistant to biodegradation due to the presence of azo bonds and aromatic rings in dyes. Extremozymes like laccases and peroxidases (manganese peroxidase and lignin peroxidase) play a pivotal role in treating such effluents (Fig. 3). Laccases catalyze the oxidation of phenolic and non-phenolic compounds by

breaking down dye molecules into less toxic fragments [9]. While peroxidases use hydrogen peroxide to cleave azo bonds by transforming colored compounds into colorless by-products. Moreover, their efficiency is enhanced by redox mediators that enables the degradation of complex dyes.

4.2 Paper and Pulp Industry Effluents

Effluents from the paper and pulp industry are characterized by high chemical oxygen demand (COD). It contains high amounts of lignin, cellulose, hemicellulose and chlorinated organic compounds that pose significant environmental concerns [25] (Fig. 3). Extremozymes such as xylanases, cellulases and ligninases play a crucial role in addressing these issues. Ligninases like lignin peroxidase and manganese peroxidase degrade lignin into smaller phenolic compounds, reducing toxicity. Xylanases and cellulases hydrolyze cellulose and hemicellulose into simpler sugars and enhance their biodegradability [26]. Additionally, xylanases minimize the use of harsh chemicals like chlorine in bleaching thereby reducing chlorinated by-products [27]. These enzymes are integrated into pulping and effluent treatment processes to promote sustainable practices by reducing COD and improving effluent quality.

4.3 Pharmaceutical Industry Effluents

The pharmaceutical industry effluents are characterized by high concentrations of antibiotics, active pharmaceutical

ingredients (APIs), organic solvents and heavy metals. Along with this, persistent bioactive compounds that pose significant risks to ecosystems are also present in these effluents. Extremozymes such as P450 monooxygenases, lipases and nitrilases play a crucial role in addressing these pollutants (Fig. 3). P450 monooxygenases catalyze oxidation reactions by breaking down complex APIs into inactive or biodegradable metabolites. Lipases hydrolyze fatty and ester-based pollutants thereby mitigating their bioactivity. While nitrilases detoxify nitrile groups by converting them into non-toxic carboxylic acids and ammonia [19].

4.4 Food Industry Effluents

Effluents from the food industry are characterized by high levels of organic matter like fats-oils-grease (FOG), starch, proteins and variable pH that may be acidic or alkaline. Extremozymes such as proteases, amylases, lipases and pectinases are effectively used to treat these effluents. Amylases hydrolyze starch into simpler sugars (Fig. 3). This reduces biological oxygen demand (BOD) and chemical oxygen demand (COD). Proteases break down proteins into peptides and amino acids. While lipases convert fats and oils into glycerol and free fatty acids and reduce FOG accumulation. These extremozymes operate efficiently under the challenging pH conditions of food waste that helps in enhancing biodegradability in effluent treatment plants.

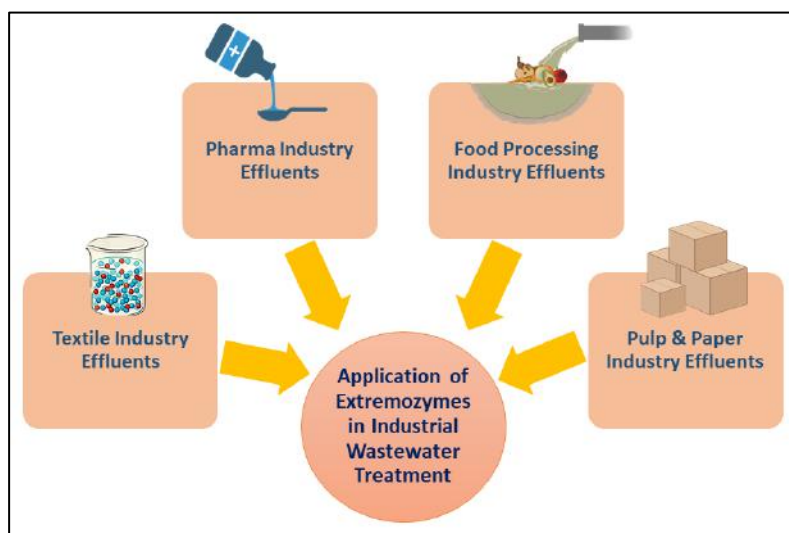


Fig. 3. Applications of Extremozymes in Industrial Wastewater Treatment

V. ADVANTAGES OF USING EXTREMOZYMES

Extremozymes are exceptionally stable and active under extreme conditions like high temperatures, extreme pH levels and high salinity that are often encountered in industrial effluents. Unlike conventional enzymes, extremozymes maintain their catalytic efficiency under

harsh conditions that reduce the need for stringent control measures during treatment processes. This stability allows for prolonged operational cycles, decreasing enzyme replacement frequency and lowering costs [28]. Moreover, extremozymes facilitate the breakdown of complex and recalcitrant pollutants that help in enhancing the

degradation of industrial waste. Their ability to function in diverse environments makes them ideal for bioremediation strategies [29]. This contributes to sustainable and eco-friendly wastewater management.

VI. FUTURE PROSPECTS

The next frontier in wastewater bioremediation lies in the integration of computational enzyme design, synthetic biology and nanotechnology to create intelligent and highly efficient extremozyme systems. Artificial intelligence (AI) and machine learning algorithms have revolutionized protein engineering by enabling the prediction of enzyme structures, stability and catalytic efficiency under diverse conditions. AI-based platforms such as AlphaFold and Rosetta can be employed to model the active sites of extremozymes and predict mutations that enhance their thermostability, halotolerance and pH resilience. This rational enzyme design approach can lead to the development of smart extremozymes capable of dynamically adapting to the fluctuating physicochemical properties of industrial wastewater. Synthetic biology further strengthens this concept by facilitating the construction of hybrid or chimeric extremozymes that combine multiple stress-tolerance traits, expanding their applications across different industrial effluent systems.

Parallelly, the immobilization of extremozymes on nanomaterials introduces another promising strategy for improving their stability, reusability and catalytic efficiency. Nano carriers like magnetic nanoparticles, graphene oxide, mesoporous silica and metal organic frameworks provide a high surface area and functional groups that promote stable enzyme binding. These nano-immobilized systems exhibit enhanced resistance to denaturation and can be easily recovered magnetically which reduces operational costs. Furthermore, nanocarrier-assisted immobilization minimizes enzyme leaching and allows continuous treatment in bioreactors. The combination of extremozymes with nanotechnology not only prolongs their functional lifespan but also aligns with circular bioeconomy principles by enabling enzyme reuse and waste valorization.

VII. CONCLUSION

The use of extremozymes in industrial wastewater treatment presents a sustainable and cost-effective solution for addressing the challenges posed by harsh environmental conditions and complex pollutants. Their exceptional stability, efficiency and adaptability under extreme conditions make them superior to conventional enzymes. By integrating extremozymes into wastewater treatment processes, industries can achieve efficient remediation

while reducing environmental impact and operational costs and thereby promoting a greener and more sustainable approach to waste management. Furthermore, the emergence of AI-driven enzyme design, synthetic biology and nano-immobilization strategies has opened new horizons in extremozyme research and application. These cutting-edge approaches enable the development of “smart extremozymes” and “bionanoextremozymes” with enhanced catalytic resilience, reusability and adaptability to dynamic wastewater conditions. The convergence of these technologies will likely revolutionize the field of biocatalytic wastewater management, driving the transition from conventional treatment systems toward intelligent, self-optimizing and sustainable biorefinery-based solutions for a cleaner environment.

ACKNOWLEDGEMENTS

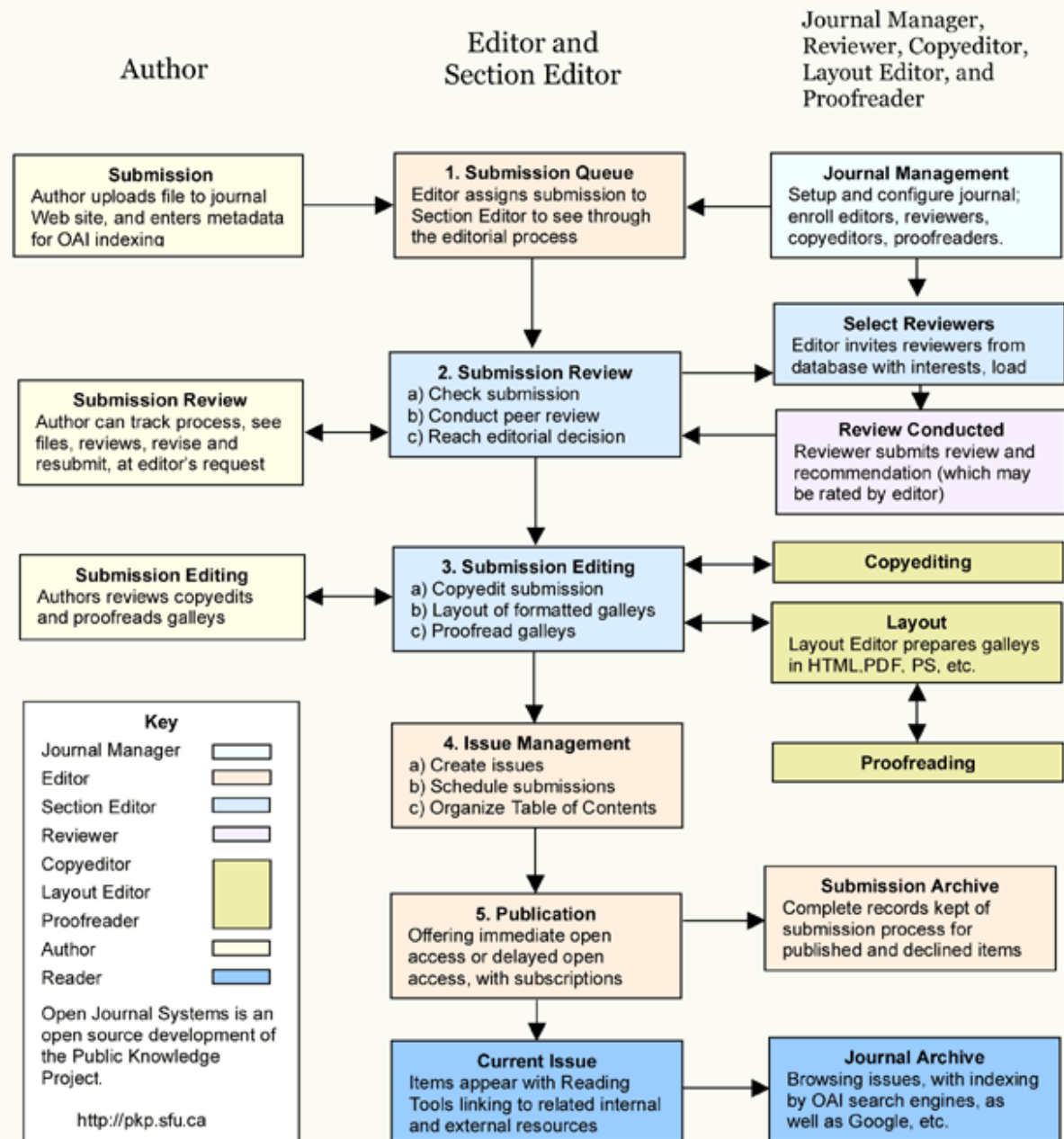
The authors acknowledge the management and Principal of R. C. Patel Arts, Commerce and Science College, Shirpur for providing research infrastructure.

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