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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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Vol-10, Issue-2, March - April 2025
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Impact of adding powdered Azolla (Azolla pinnata) leaves to the diet on the carcass and economic characteristics of turkey poults

Author(s): Keshram Meena, Lokesh Gupta, Bhavya Pal, Pradeep Nodal, Manoj Jat

DOI: 10.22161/ijeab.102.16

Page No: 153-156

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Morphometric Taxonomy of *Meloidogyne* spp. Infesting Vegetable Crops in Sri Lanka via Female Perineal Pattern Analysis

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Abstract— *Meloidogyne spp. are among the most significant plant-parasitic nematodes in agriculture, necessitating precise identification for effective management. This study aimed to identify and taxonomically characterize Meloidogyne spp. infesting vegetable crops in Sri Lanka using female perineal pattern analysis. A total of 54 root and soil samples were collected from 17 agricultural regions and analyzed at the Horticultural Crop Research and Development Institute (HORDI), Sri Lanka. Single egg-mass cultures were established using a nematode-susceptible tomato variety (KWR), and second-stage juveniles (J2) were used for precise inoculation. Perineal patterns of mature females were prepared following standard protocols and examined microscopically. A total of 101 female nematodes were identified, revealing the presence of M. arenaria (33%), M. javanica (30%), M. incognita (25%), and M. hapla (13%). M. arenaria exhibited forked lateral fields and a low dorsal arch, while M. javanica displayed distinct double lateral incisures. M. incognita was characterized by an angularly oval pattern with a high dorsal arch, and M. hapla showed a concentrated punctation between the anus and tail terminus. Host range analysis indicated that M. hapla had a narrow host range, whereas the other species were oligophagous, with M. incognita affecting a broad spectrum of crops, including tomato, spinach, brinjal, and okra. The study confirmed that M. incognita, M. javanica, and M. arenaria are the predominant Meloidogyne spp. in Sri Lanka's vegetable-growing regions, with M. arenaria being the most frequently detected. These findings align with previous research and highlight the necessity of targeted management strategies. The results provide a valuable foundation for developing species-specific nematode control measures in Sri Lanka's agricultural systems.*

Keywords— ***Female perineal pattern, Meloidogyne spp., KWR, Second stage juveniles***

I. INTRODUCTION

Meloidogyne spp. are among the most economically significant plant-parasitic nematodes in agricultural systems worldwide, requiring accurate identification and taxonomic characterization for effective management. Female perineal pattern analysis is a widely used and reliable method, particularly in the absence of molecular techniques, as it provides species-level identification essential for agricultural applications [3,8,12]. This technique involves examining the dorsal esophageal gland orifice and the surrounding perineal region of mature females, where distinct patterns, including the shape and arrangement of

striae and the presence of a dorsal arch, facilitate differentiation among *Meloidogyne* spp. Several studies [2,6,13] have demonstrated the effectiveness of this method for species identification. Given the variations in host preferences, life cycles, and resistance levels among *Meloidogyne* spp., species-specific management strategies are necessary for effective control. Therefore, this study aims to identify and taxonomically characterize *Meloidogyne* spp. present in Sri Lanka using female perineal pattern analysis to facilitate the development of targeted management strategies.

II. METHODOLOGY

2.1 Sample Collection and Nematode Culture Establishment

Fifty-four nematode-infested root samples were collected from 17 Agriculture Instructor (AI) ranges across Sri Lanka, covering various vegetable crops (Table 1.). Sampling was conducted from December 2016 to March 2017 at the Division of Entomology and Nematology, Horticultural Crop Research and

Development Institute (HORDI), Gannoruwa, Peradeniya, Sri Lanka.

Table 1: Details of the root samples collected from vegetables growing fields in seventeen AI rangers in Sri Lanka

Name of the AI range	Crop Type *	Name of the AI range	Crop Type *
Wendaruwa	Tomato	Eadanduwawa	Gotukola
Nugethenne	Carrot		Kankun
Kalunthenne *	Tomato	Galewela	Guava
	Beetroot	Dambagahapitiya	Tomato
Madamahanuwara	Tomato	Ukuwela	Brinjal
Mahawella	Tomato		Okra
Udispaththuwa	Tomato	Warallagama	Wing bean
	Chilli	Malabe	Wing bean
	Spinach	Kibissa	Thumba
Digana *	Knolkhol		
	Kankun		
	Spinach		
	Chinese kale		

* One sample from each location except three tomato samples from Kalunthenna and 3 spinach samples from Digana

Single egg-mass cultures of *Meloidogyne* spp. were established using a nematode-susceptible tomato variety (KWR) under controlled plant house conditions. The potting mixture (86% sand, 7% clay, 4% silt, 3% organic matter) was sterilized at 120°C for six hours. Sterilized clay pots (15 cm) were filled with 4 kg of the mixture, and basal fertilizers (Urea: 2.6 g/pot, TSP: 13.4 g/pot, MOP: 2.6 g/pot) were applied. Eighteen-day-old tomato seedlings were transplanted at a rate of one per pot.

Infested roots were washed, cut into 2–3 cm pieces, and egg masses were isolated and incubated in sterilized distilled water for second-stage juvenile (J2) hatching. One week after transplanting, seedlings were inoculated with J2s by introducing a suspension into four holes around each plant. Ten seedlings per sample were inoculated using J2s from ten separate egg masses to ensure consistency.

1.1. Perineal Pattern Preparation and Microscopy

One month after inoculation, plants were uprooted, and female nematodes were extracted from root galls. Perineal pattern preparation followed according to Hartman and Sasser [7]. Root galls were cut into 1–2 cm sections and placed in sterilized water. Individual females were transferred onto slides with 45% lactic acid drop, ruptured using a scalpel, and body contents were dissolved with forceps. The perineal region was carefully excised, and 10–15 slides per sample were prepared for microscopic examination at 10×100 magnification (Fig.1).

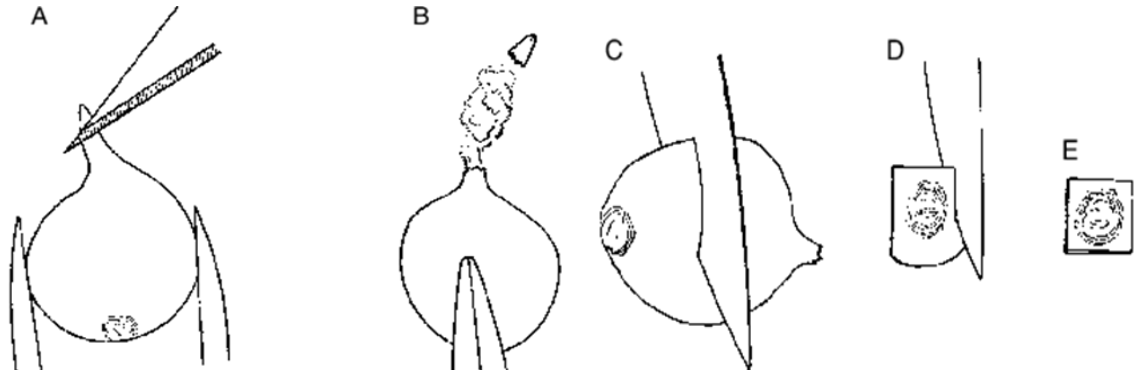


Fig.1: Procedure for microscope preparation of female perineal pattern for identification: A-B. Excised female being raptured and body tissues gently removed, C-E. Cuticle being trimmed from around the perineal pattern

2.2 Species Identification

Meloidogyne spp. were identified based on perineal pattern morphology using descriptions by Eisenback and standard plates from Dr. J. N. Sasser [5,7] (Figure 02, Plate 01). Identification focused on *Meloidogyne incognita, M. javanica, M. arenaria,* and *M. hapla*, considering striae patterns, lateral lines, and dorsal arch features (Table 2).

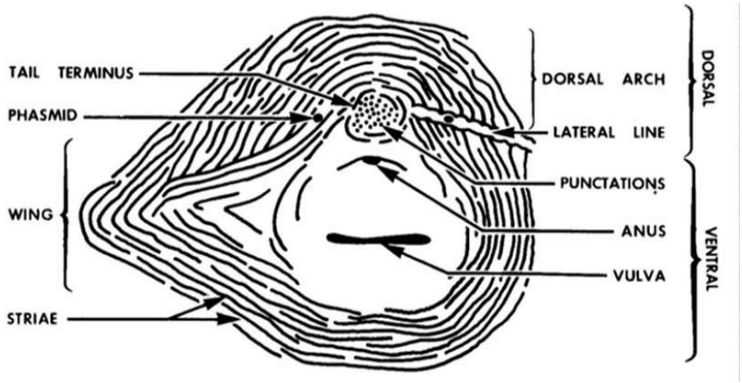
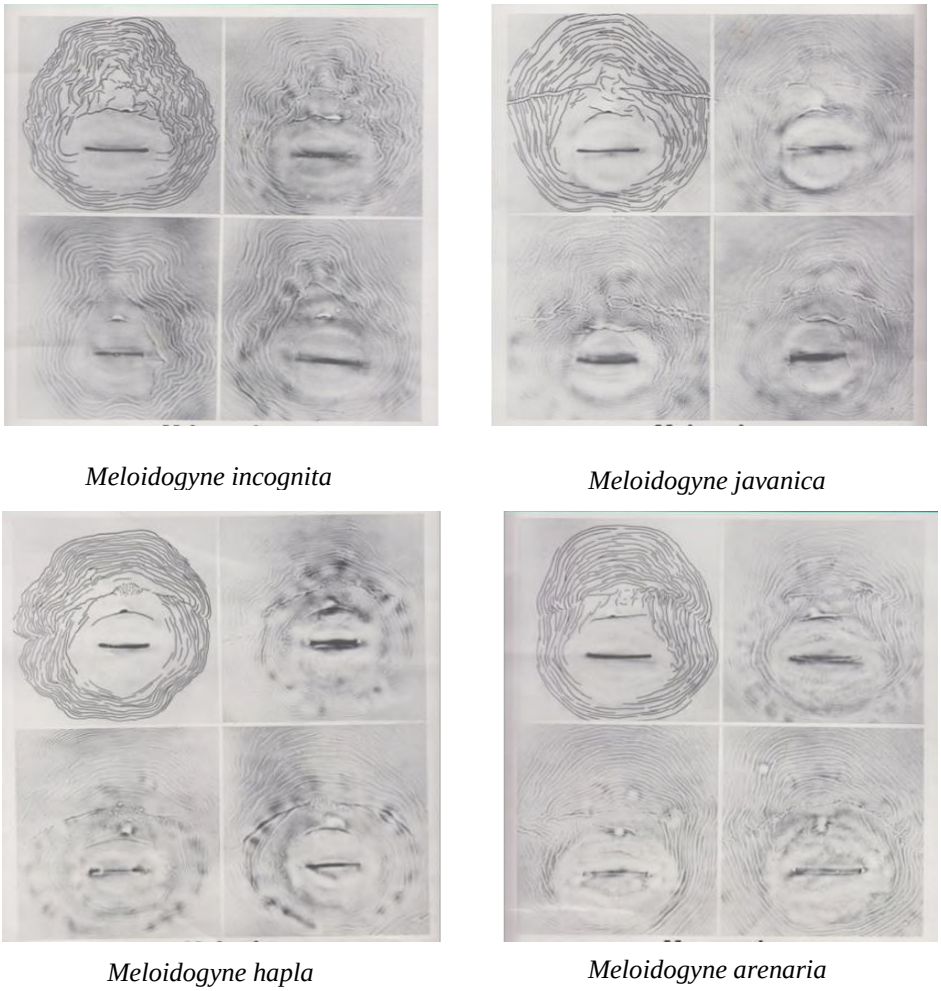


Fig.2: Diagrammatic representation of the perineal pattern of Meloidogyne spp.

Table 2: Comparison of Characteristics of female perineal pattern among the four main Meloidogyne spp.

Characteristic	*M. arenaria*	*M. javanica*	*M. incognita*	*M. hapla*
Striae (patterns of lines)	Smooth and slightly wavy, sometimes forming wings laterally	Smooth and somewhat wavy	Smooth and wavy, sometimes zigzag	Close, smooth, and wavy, sometimes forming wings
Lateral lines/ridges	Forked, irregular lateral fields	Unique, distinct lateral lines present	Absent	Lateral fields marked by irregularities in the striae
Dorsal arch characteristics	Low and indented near lateral fields, forming rounded shoulders	Low or rounded, sometimes with a whorl in tail terminus area	Squares and high, with a whorl around tail terminus	Usually low and rounded, but may be high and squarish



Meloidogyne incognita *Meloidogyne javanica*

Meloidogyne hapla *Meloidogyne arenaria*

Plate.1: Standard perineal pattern images for the most common Meloidogyne spp.

III. RESULTS AND DISCUSSION

Analysis of perineal patterns from 101 female nematode samples identified four *Meloidogyne* species: *M. arenaria* (n=33), *M. javanica* (n=30), *M. incognita* (n=25), and *M. hapla* (n=13). The perineal pattern characteristics observed were consistent with previous studies [1,10].

M. arenaria exhibited a forked lateral field, fractured striae in a winged form, and a low dorsal arch [1,10]. *M. javanica* showed distinct lateral fields with well-defined double incisures [5,10]. *M. incognita* displayed an angularly oval perineal pattern with a high dorsal arch and an Inverted-V shape [10]. *M. hapla* was distinguished by a concentrated punctation between the anus and tail terminus, consistent with previous descriptions [5,10].

Host range analysis (Table 3) confirmed that *M. hapla* had a more restricted host range compared to the other three species, which exhibited an oligophagous nature. The most frequently infested crops included Centella, *Alternanthera sessilis*, Knolkhol, Water Spinach, Spinach, Chinese Kale, Brinjal, Beetroot, Okra, Tomato, Guava, Carrot, Chilli, and Spine gourd.

The study found *M. incognita*, *M. javanica*, and *M. arenaria* to be the dominant *Meloidogyne* spp. in vegetable-growing areas of Sri Lanka, with *M. arenaria* being the most prevalent (32.67%). These findings align with previous reports on *Meloidogyne* spp. distribution in Sri Lanka [15] and globally [10].

Effective management strategies, including resistant cultivars, crop rotation, soil amendments, and strict quarantine measures, are essential to mitigate the impact of root-knot nematodes in vegetable production systems in Sri Lanka.

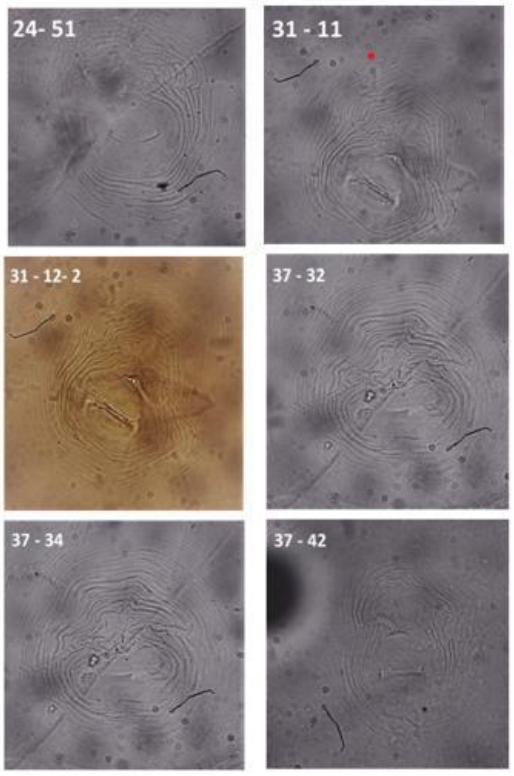


Plate.1: Perineal patterns of Meloidogyne arenaria isolates collected from the Central region of Sri Lanka

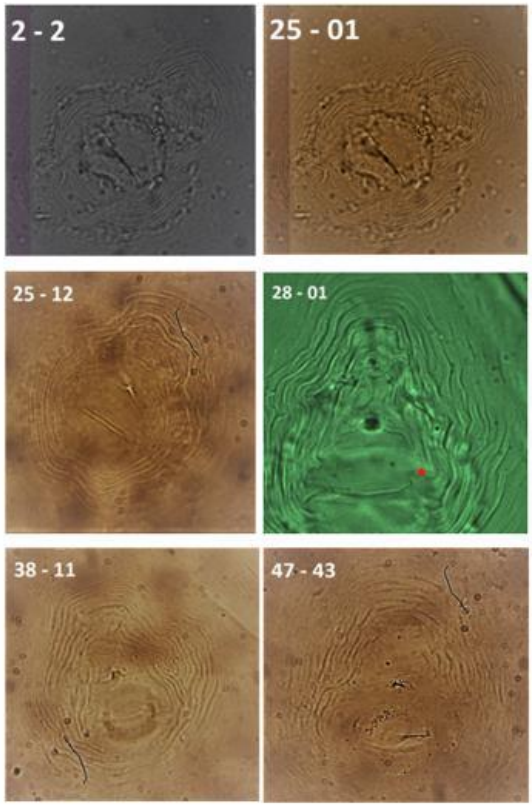


Plate.3: Perineal patterns of Meloidogyne incognita isolates collected from the Central region of Sri Lanka

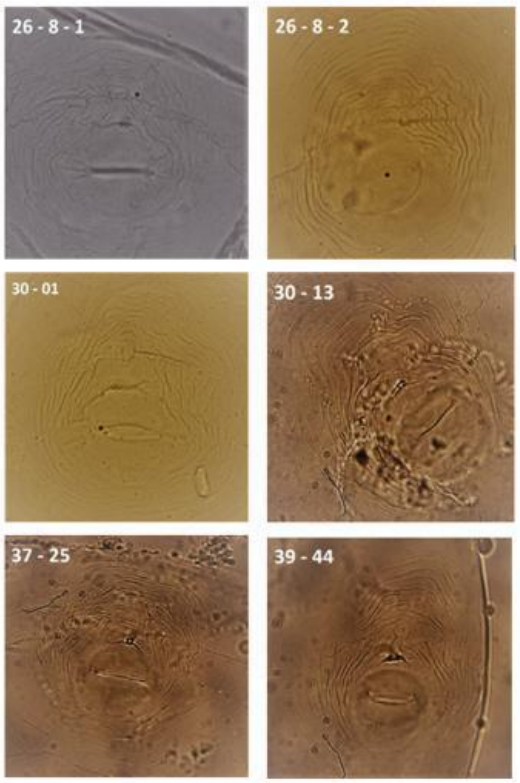


Plate.2: Perineal patterns of Meloidogyne javanica isolates collected from the Central region of Sri Lanka

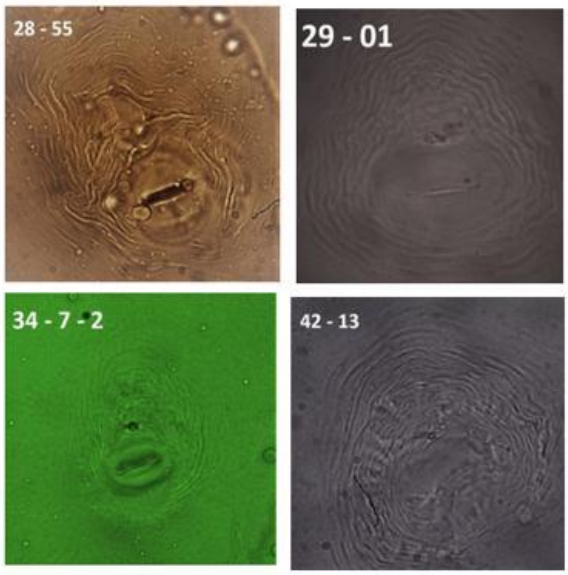


Plate.4: Perineal patterns of Meloidogyne hapla isolates collected from the Central region of Sri Lanka

Table 3: Main crops attacked by the Meloidogyne spp. in Sri Lanka

***Meloidogyne* spp.**	**Host**
Meloidogyne arenaria	Guava (*Psidium guajava*), Gotukola (*Centella asiatica*), Mukunuwenna (*Alternanthera sessilis*), Tomato (*Solanum lycopersicum*), Carrot (*Daucus carota*), Spinach (*Spinacia oleracea*), Wing bean (*Psophocarpus tetragonolobus*), Okra (*Abelmoschus esculentus*)
Meloidogyne javanica	Mukunuwanna, Carrot, Tomato, Brinjal (*Solanum melongena*), Beetroot (*Beta vulgaris*), Gotukola, Guava
Meloidogyne incognita	Gotukola, Mukunuwanna, Knolkhol (*Brassica oleracea*), Water Spinash (*Ipomoea aquatica*), Spinash, Chinese Kale (*Brassica oleracea var. alboglabra*), Brinjal, Beetroot, Okra, Tomato, Spinach, Guava
Meloidogyne hapla	Mukunuwanna, KnolKhol, Chilli (*Capsicum annum*), Spine guard (Thumba) (*Momordica dioica)*

IV. CONCLUSION

Female perennial patterns based on taxonomic characterization identified four common species of *Meloidogyne* spp., namely *M. incognita*, *M. javanica*, *M. Arenaria*, and *M. hapla* on wide range of crops grown in Sri Lanka. The occurrence of each species varied in numbers demonstrating a wide to narrow host range. *M. arenaria* was detected at the highest frequency but *M. incognita* shows a broad host range indicating a high impact on crops. *M. hapla* was detected at low frequency with a narrow host range showing its minimum contribution on crop losses. These findings are useful for decision making on affective management of nematodes.

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Efficiency of Use Chicken Manure in Optimizing the use of KNO_3 Supply on Sweet Corn (*Zea mays saccharata* Sturt L.)

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Abstract— *Sweet corn (Zea mays saccharata* Sturt L.*) is one of the horticultural commodities that has increasing market demand but declining production levels. This is because most of the fertilization that is done is continuous and excessive chemical fertilization. The use of chemical fertilizers can have a negative impact on the soil, one of which can cause damage to soil structure and function, so that it will slowly reduce soil quality. This study aims to assess the effectiveness of using chicken manure in optimizing KNO_3 fertilizer so as to increase the growth and yield of sweet corn plants (Zea mays saccharata* Sturt L.*). The research was conducted in Jemekan Village, Ringinrejo Subdistrict, Kediri District, using a Spli Plots Design using doses of chicken manure (0, 4, 8, and 12 tons.ha^{-1}) and KNO_3 fertilizer (0, 25, 50, and 75 kg.ha^{-1}). The results showed that the combination of chicken manure dose of 12 ton.ha^{-1} with KNO_3 25 kg.ha^{-1} produced higher cob fresh weight than other treatments. Chicken manure contributed to the improvement of soil structure and increased nutrient availability, while KNO_3 supported vegetative growth and yield. The interaction between the two fertilizers showed an increase in macronutrient uptake (N, P, K) by plants, with nitrogen and potassium uptake increasing significantly in the late vegetative to early generative phase. This study concludes that the use of chicken manure and KNO_3 is an effective and environmentally friendly solution to increase sweet corn productivity.*

Keywords— ***Sweet Corn, Chicken Manure, KNO_3 Fertilizer.***

I. INTRODUCTION

Sweet corn (*Zea mays saccharata* Sturt L.) is a high economic value horticultural commodity with significant development potential. However, the high demand has not been met optimally, as seen from the increase in import volume by 42.46% from 517.5 thousand tons in 2020 to 737.2 thousand tons in 2021 (Badan Pusat Statistik Republik Indonesia, 2022). Therefore, optimizing fertilization is the main strategy in increasing sweet corn productivity to support national food security. Fertilization has a central role in supporting plant growth and yield. Applying large amounts of inorganic fertilizers, such as 300 kg ha^{-1} compound NPK and 200 kg ha^{-1} urea, has been shown to increase yields (Murzani & Nurhayati, 2011). However, excessive use of chemical fertilizers can cause a decrease in soil organic matter because microorganisms that rely on organic matter lose their food source, leading to a decrease in the activity of soil microorganisms and degradation of soil structure. The Directorate General of Food Crops also confirms that excessive use of chemical fertilizers causes soil to become compacted due to lack of aggregation in soil particles (Kementerian Pertanian, 2022).

The use of organic fertilizers, such as chicken manure, is a strategic solution to reduce the negative impact of chemical fertilizers and increase the efficiency of nutrient uptake. Chicken manure is considered more effective than goat and cow manure in increasing the growth and yield of sweet corn (Melese Damtew, 2022). Chicken manure has high nitrogen, phosphorus, and potassium content as well as microorganisms that support soil fertility (Hartatik *et al.*, 2015; Manogaran *et al.*, 2022). Nitrogen plays a role in chlorophyll formation and vegetative growth, phosphorus supports root development and seed formation, while potassium increases photosynthetic efficiency and plant resistance to environmental stress (Fathi, 2022; Taufiq & Yetti, 2016). The combination of chicken manure with KNO_3 fertilizer

has the potential to increase fertilizer efficiency and yield. KNO_3 fertilizer which contains 46% K_2O and 13% nitrogen plays a role in photosynthesis, carbohydrate translocation, and increases plant resistance to environmental stress (Pangaribuan *et al*., 2017).

The optimal dose of KNO_3 fertilizer of 100 kg ha^{-1} can increase yields up to 24 tons ha^{-1}, while the application of 150 kg ha^{-1} increases the sweetness level of corn (Dewanda *et al*., 2021). This study aims to assess the effectiveness of a combination of chicken manure in optimizing the use of KNO_3 fertilizer in increasing the growth and yield of sweet corn and determining the optimal dose that is efficient. The results of this study are expected to be a reference for farmers in developing a fertilization system that is more productive, sustainable, and supports the improvement of the quality of former sweet corn land.

II. MATERIALS AND METHOD

This research was conducted from September to November 2024 in Jemekan Village, Ringinrejo District, Kediri Regency, with a gray brown regosol soil type at an altitude of 67 meters above sea level. The experimental design used was the Split Plots Design, with chicken manure doses as the main plot (0, 4, 8, and 12 tons.ha^{-1}) and KNO_3 fertilizer doses as subplots (0, 25, 50, and 75 kg.ha^{-1}), so there were 16 treatments with 3 replications in a total of 48 experimental plots. The research stages included land preparation, planting, fertilization, maintenance, and harvesting. Land preparation was carried out by clearing weeds, tillage, and soil analysis in the laboratory before and after fertilizer application. Planting was done using the tugal method, by planting one seed per planting hole. The

The parameters observed included plant growth and yield. Growth parameters included plant height, number of leaves, stem diameter, chlorophyll index, leaf area, wet and dry weight, crop growth rate, and number and condition of leaf stomata. Yield parameters included length and diameter of the cob, fresh weight of the cob with and without kelobot, brix content. In addition, farming business analysis was conducted through the calculation of Revenue Cost Ratio (R/C Ratio) to evaluate economic aspects. As supporting parameters, soil analysis was conducted for C-organic content, N, P, K, and Cation Exchange Capacity (CEC) before and after treatment. Nutrient accumulation of N, P, and K were analyzed using wet deconstruction, spectrophotometry, and atomic absorption spectrophotometry (AAS) methods. The data obtained were analyzed using analysis of variance (F test) at the 5% level, followed by the Honestly Significance Difference (HSD) test if there were significant differences.

III. RESULT AND DISCUSSION

The application of chicken manure and KNO_3 fertilizer showed a relationship between the uptake of macronutrients (nitrogen, phosphorus, and potassium) by sweet corn plants at 35 DAP (Days After Planting) and 63 DAP with changes in nutrient content in the soil after planting. Based on the results of N, P and K uptake, it can be seen that the uptake of nitrogen (N), phosphorus (P), and potassium (K) by plants increased significantly from 35 DAP to 63 DAP. Meanwhile, nitrogen, phosphorus and potassium contents in the soil decreased after planting. This pattern shows a close relationship between the physiological needs of plants at various growth phases and the dynamics of nutrients in the soil due to the influence of the fertilizer used, namely the combination of chicken manure and KNO_3. This is evidenced by the results of the analysis presented in diagram.

Based on Figure 1 to Figure 5, the application of chicken manure and KNO_3 had a significant effect on the uptake of macronutrients (N, P, K) by sweet corn at 35 DAP and 63 DAP and the changes in nutrient content in the soil after planting. The uptake of nitrogen, phosphorus, and potassium increased significantly from 35 DAP to 63 DAP, while the content of nitrogen, phosphorus and potassium in the soil also decreased. This indicates that plant nutrient requirements increase during the late vegetative to early generative phase. The higher nitrogen uptake at 63 DAP reflects its role in protein synthesis, chlorophyll formation and photosynthesis. In the early phase (35 DAP), nitrogen demand is lower because the plant is still in the stage of root and leaf formation, but it increases sharply at 63 DAP when the plant enters the rapid growth phase (Wang *et al*., 2021). Chicken manure releases nitrogen gradually through microbial mineralization, while KNO_3 provides nitrogen in the form of nitrate that is quickly absorbed by plants. However, the high uptake of nitrogen leads to a decrease in soil nitrogen content, as the release from chicken manure is not able to fully replace what the plants have absorbed. Phosphorus uptake increases from 35 DAP to 63 DAP, due to its role in ATP synthesis, cell division and root development. Phosphorus in chicken manure is released gradually through soil microbial activity, causing the accumulation of phosphorus that is not directly utilized by plants (Zhang *et al*., 2014). Meanwhile, potassium uptake increases with plant requirements in osmotic regulation and

photosynthate transport, but soil potassium content decreases due to high uptake and leaching (Li *et al*., 2022).

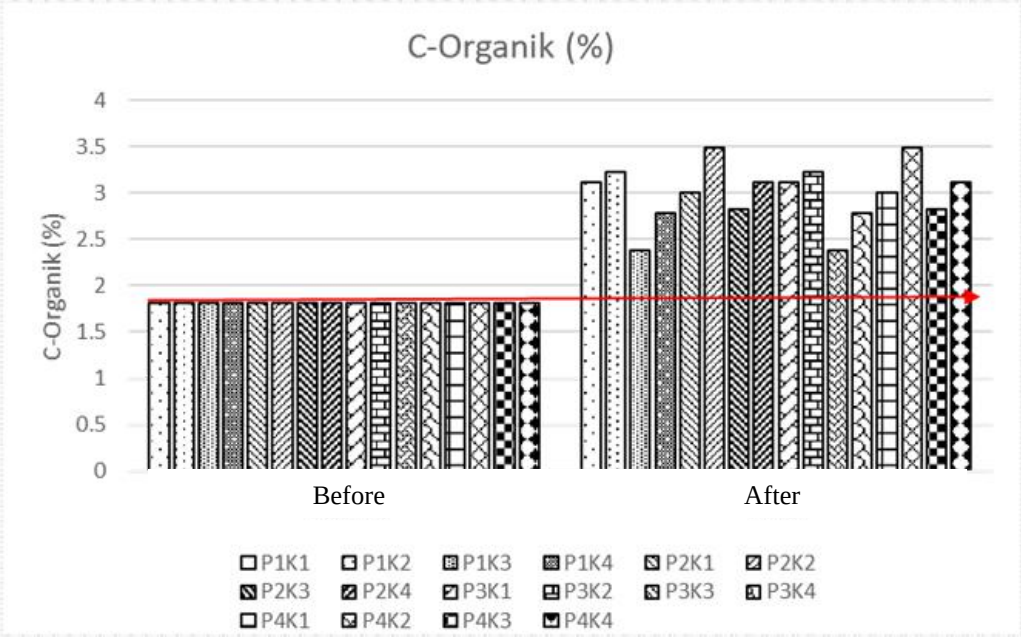

Fig. 1. Diagram of Changes in Soil Nutrient C-Organic Elements

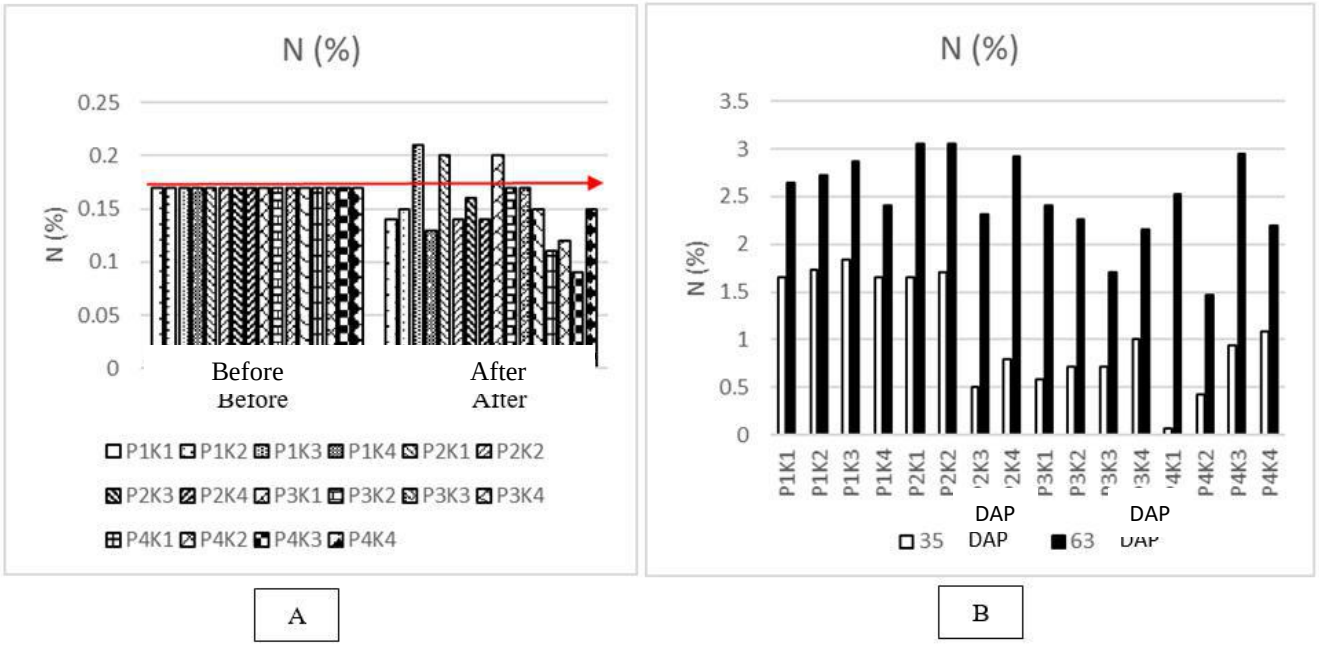

Fig. 2. Diagram of Nitrogen Change in Soil Nutrients (A) and Plant Nutrient Uptake (B)

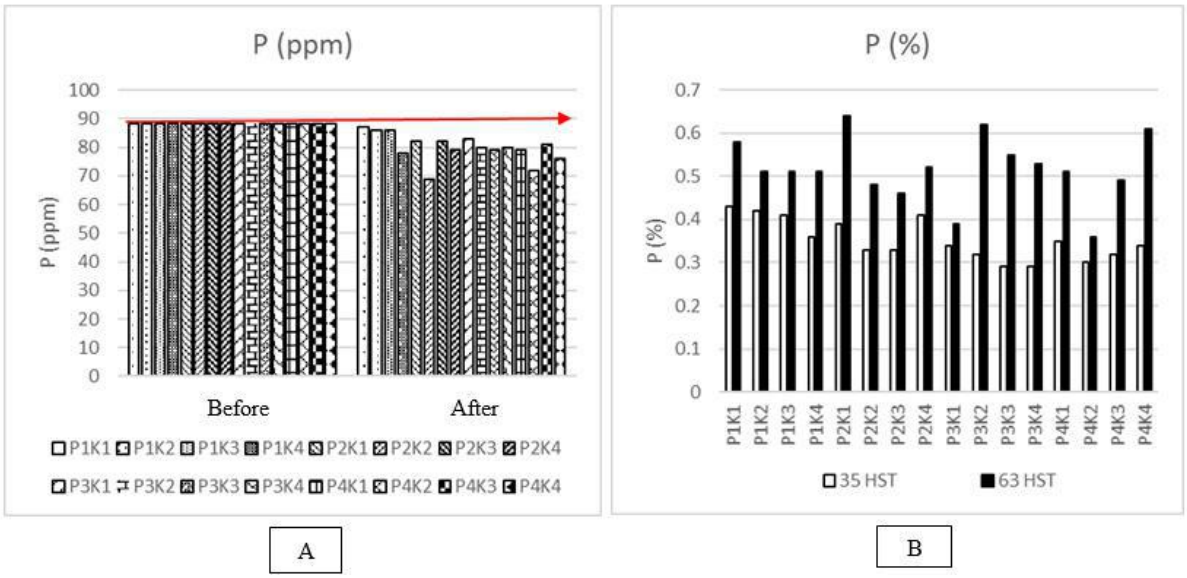

Fig. 3. Diagram of Phosphorus Changes in Soil Nutrients (A) and Plant Nutrient Uptake (B)

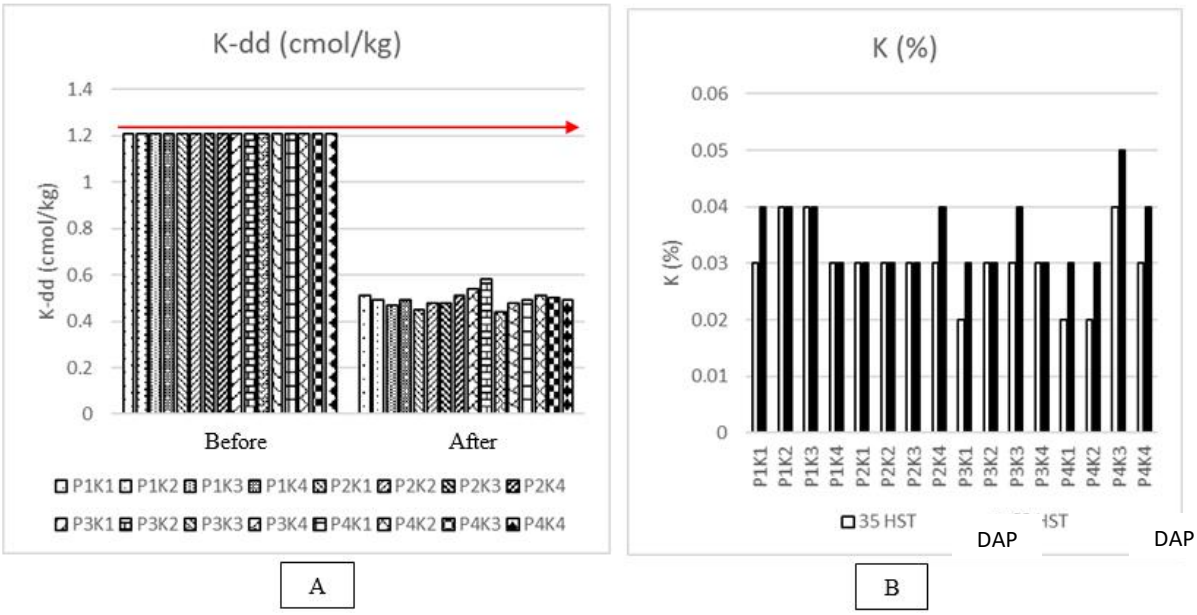

Fig. 4. Diagram of Potassium Changes in Soil Nutrients (A) and Plant Nutrient Uptake (B)

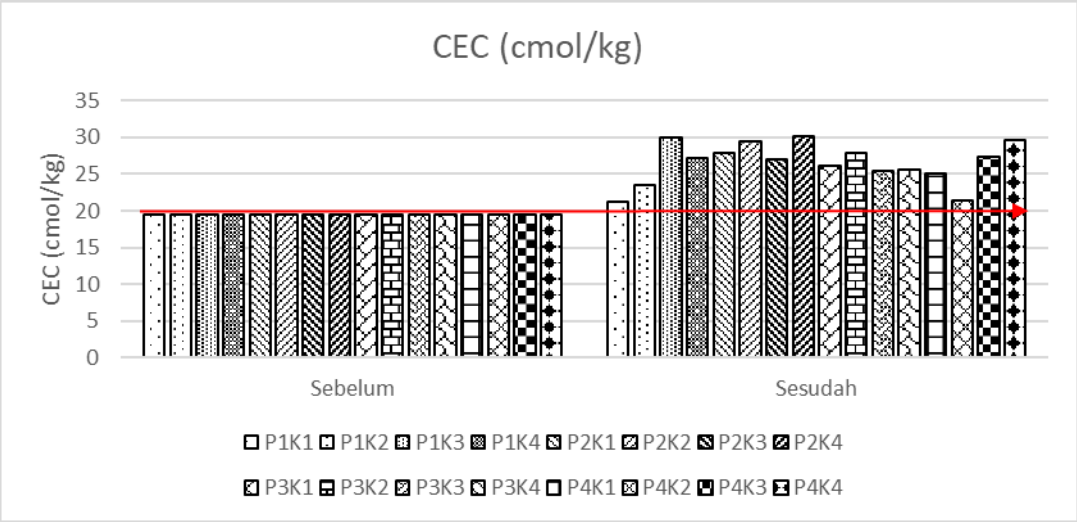

Fig. 5. Diagram of Changes in Soil Nutrients Cation Exchange Capacity

Overall, the relationship between nutrient uptake by plants and nutrient content in soil can be explained through the interaction between the physiological needs of plants, the chemical properties of nutrients, and the effect of fertilizers applied. Increased uptake of nitrogen, phosphorus and potassium by plants leads to a decrease in nitrogen and potassium content in the soil, indicating that plants utilize these nutrients intensively to support their growth and development. This phenomenon confirms the importance of a combination of organic and inorganic fertilizers in supporting plant growth while maintaining soil fertility. The use of chicken manure in this study contributed to the initial availability of N, P and K in the soil. Zhang *et al*. (2020) explained that organic fertilizers such as manure can increase nutrient availability in the short term through nutrient mineralization by soil microbes. However, after one crop growth cycle, the nutrient content in the soil decreases due to uptake by plants and losses through leaching and fixation. Therefore,a sustainable fertilization strategy is needed to maintain soil nutrient balance and support optimal growth of sweet corn plants in the next growing season.

Then, the increase in plant nutrient uptake at 35 DAP to 63 DAP shows the importance of nutrient availability during the active growth phase of sweet corn. Chicken manure plays a role in increasing the organic matter content of the soil, which supports nutrient retention, while KNO_3 fertilizer provides nitrogen in a form that is easily absorbed by plants to support vegetative and generative development. Both contribute to an increase in leaf area, which maximizes the photosynthesis process, and supports biomass accumulation, as seen from the increase in the dry weight and fresh weight of the plants. The decrease in nutrients in the soil during this period shows that the plants are actively utilizing the available nutrients to support their optimal growth. This is supported by several growth data that will be presented in the table below.

Leaf Area ($cm^2.tan^{-1}$)

Based on the data in Table 1, at the observation age of 63 DAP, the treatment of 12 $tons.ha^{-1}$ chicken manure with the use of 50 $kg.ha^{-1}$ KNO_3 fertilizer is considered to produce a higher leaf area among others with a leaf area value of 8255.78. According to Fitriyah *et al*. (2024), this is due to increased nutrient availability and improved soil physical properties by chicken manure, as well as the supply of potassium from KNO_3 which supports leaf cell division and elongation. Potassium also plays a role in stomatal

regulation, increasing water use efficiency and photosynthesis, which contributes to the increase in leaf area. In addition, this combination can increase the activity of soil microorganisms, accelerate the decomposition of organic matter, and increase nutrient availability to plants.The increase in leaf area has implications for increasing photosynthetic area, which in turn can increase corn yield.

Table 1. The Value of Leaf Area Due to Interaction in the Treatment of Chicken Manure and KNO_3 Fertilizer at the Age of Observation 63 DAP

Leaf Area ($cm^2.tan^{-1}$)						
Chicken Manure	**KNO_3 fertilizer ($kg.ha^{-1}$)**				**HSD AP (5%)**	**CV AP (5%)**
($tons.ha^{-1}$)	**0**	**25**	**50**	**75**		
0	7221,46 a A	7560,04 a A	8071,92 a B	7453,41 a A		
4	7883,22 a AB	7242,24 a A	6984,93 a A	7387,02 a A		
8	8328,37 a AB	8055,69 a A	7929,67 a AB	7643,47 a AB	1279,15	5,29
12	7980,23 a AB	7943,27 a A	8255,78 a B	8539,69 a B		
HSD PU (%)				992,08		
CV PU (%)				11,33		

Description:

Numbers accompanied by the same lowercase letter indicate not significantly different in rows, while numbers followed by the same uppercase letter indicate not significantly different in columns, based on the 5% HSD test, DAP = Days after Planting, CV = Coefficient of Variance.

Dry Weight ($g.tan^{-1}$)

Table 2. Dry Weight Value Due to Interaction in the Treatment of Chicken Manure and KNO_3 Fertilizer at 63 DAP Observation Age

Dry Weight ($g.tan^{-1}$)						
Chicken Manure	**KNO_3 fertilizer ($kg.ha^{-1}$)**				**HSD AP (5%)**	**CV AP (5%)**
($tons.ha^{-1}$)	**0**	**25**	**50**	**75**		
0	62,45 a A	60,89 a AB	61,68 a A	60,59 a A		
4	60,79 a A	62,98 a B	61,00 a A	61,34 a A	4,57	1,84
8	60,74 a A	61,29 a AB	63,38 a A	63,02 a B		
12	62.16 ab A	58,30 a A	63,10 b A	62.33 ab A		
HSD PU (%)				3,95		
CV PU (%)				3,40		

Description:

Numbers accompanied by the same lowercase letter indicate not significantly different in rows, while numbers followed by the same uppercase letter indicate not significantly different in columns, based on the 5% HSD test, DAP = Days after Planting, CV = Coefficient of Variance.

Based on the data in Table 2, at the observation age of 63 DAP, the treatment of 12 tons.ha^{-1} chicken manure with 50 kg.ha^{-1} KNO_3 fertilizer treatment was considered to produce higher dry weight compared to the other treatments. This was proven by the dry weight value of 63.10 g.tan^{-1}. According to Prasetya *et al*. (2021), higher plant dry weight indicates better nutrient absorption efficiency, especially nitrogen (N) and potassium (K), which play an important role in protein synthesis and regulation of plant cell osmotic pressure. Chicken manure can increase the availability of macro and micro nutrients in the soil, thus supporting plant biomass growth. In addition, potassium in KNO_3 plays a role in strengthening cell walls and increasing photosynthate transport efficiency, which has an impact on increasing plant dry weight (Cendana *et al*., 2021). The interaction between chicken manure and KNO_3 can also increase the activity of soil microorganisms, which helps in the mineralization of nutrients (Pangaribuan *et al.*, 2017).

Fresh Weight of Cob with Husk (tons.ha^{-1})

The results of the analysis of variance showed that there was an interaction at the observation age of 71 DAP between the treatment of chicken manure and KNO_3 fertilizer on the observation of the fresh weight of the cob with the cob of sweet corn plants. Separately, the treatment of chicken manure and KNO_3 fertilizer treatment gave a significant effect at the observation age of 71 DAP. The value of Fresh Weight of Cobs with Cobs due to the interaction on the treatment of manure and KNO_3 fertilizer at the age of 71 DAP is presented in Table 3.

Table 3. Value of Fresh Weight of Cob with Lint as a Result of Interaction on the Treatment of Chicken Manure and KNO_3 Fertilizer at 71 DAP Observation Age (ton.ha^{-1})

Fresh Weight of Cob with Husk (tons.ha^{-1})						
Chicken Manure (tons.ha^{-1})	**KNO_3 fertilizer (kg.ha^{-1})**				**HSD AP (5%)**	**CV AP (5%)**
	0	**25**	**50**	**75**		
0	12,25 a A	13.82 bc A	14,42 c A	13,45 b A		
4	13,29 a B	14,52 b B	15,18 b B	13,57 a A	0,70	2,10
8	14,02 a C	15,04 b BC	13,96 a A	14,34 a B		
12	15,12 a D	15.55 ab C	15,94 b C	15.34 ab C		
HSD PU (%)				0,67		
CV PU (%)				2,36		

Description:

Numbers accompanied by the same lowercase letter indicate not significantly different in rows, while numbers followed by the same uppercase letter indicate not significantly different in columns, based on the 5% HSD test, DAP = Days after Planting, CV = Coefficient of Variance.

Based on the data in Table 3, at the observation age of 71 DAP, the Fresh Weight of Cobs with cob both in grams and tons, 12 tons.ha^{-1} chicken manure treatment with 25 kg.ha^{-1} and 50 kg.ha^{-1} KNO_3 fertilizer treatment is considered to produce higher fresh weight on the cob. This is evidenced by the higher yields of sweet corn at 15.55 and 15.94 kg.ha^{-1} with more efficient use of KNO_3 fertilizer. The increase in fresh weight of cob with kelobot in sweet corn is influenced by the optimal availability of nutrients during the generative phase. Chicken manure contributes to improving soil fertility by providing nitrogen (N), phosphorus (P), and potassium (K) in a form that is easily absorbed by plants. The results showed that the combination of 12 tons.ha^{-1} of chicken manure with 25-50 kg.ha^{-1} of KNO_3 produced the highest cob fresh weight. Potassium in KNO_3 supports the

translocation of photosynthates from leaves to cobs, which increases the size and weight of sweet corn cobs (Fitriyah *et al*., 2024). In addition, according to Agustina *et al*. (2020), chicken manure can increase the activity of soil microorganisms, improve soil aeration, and increase nutrient availability, which contributes to the formation of larger cobs. This interaction between organic and inorganic fertilizers increases the efficiency of nutrient uptake, resulting in increased yields (Pangaribuan *et al*., 2017).

From supporting data the research results, it is proven that there is interaction between the application of chicken manure and KNO_3 fertilizer. The application of chicken manure and KNO_3 fertilizer shows a relationship between the absorption of macro nutrients (nitrogen, phosphorus, and potassium) by sweet corn plants at 35 days after planting (DAP) and 63 DAP with changes in nutrient content in the soil after planting. Based on the results of N, P and K absorption, it can be seen that the absorption of nitrogen (N), phosphorus (P), and potassium (K) by plants increased significantly from 35 DAP to 63 DAP. Meanwhile, the nitrogen, phosphorus and potassium content in the soil decreased after planting. This pattern shows a close relationship between the physiological needs of plants in various growth phases and the dynamics of nutrients in the soil due to the influence of the fertilizer used, namely a combination of chicken manure and KNO3. This is evidenced by the results of the analysis presented in the following explanation of interaction and efficiency

Response of Interaction Effect of Chicken Manure and KNO_3

Based on the data in tables 1,2,3 the application of chicken manure and KNO_3 showed a significant interaction on the growth and yield of sweet corn. This combination increases nutrient availability, improves soil structure, and optimizes plant growth. Chicken manure increases cation exchange capacity (CEC) and water retention, while KNO_3 provides nitrogen (NO_3) and potassium (K) that support photosynthesis and growth (Singh *et al*., 2020). The results showed that a dose of 12 ton.ha^{-1} chicken manure with 25 and 50 kg.ha^{-1} KNO_3 produced optimal yields of about 15.55 ton.ha^{-1} and 15.94 ton.ha^{-1}. In contrast, too high doses (>12 tons.ha^{-1}) and high KNO_3 (75 kg.ha^{-1}) caused nutrient saturation, which inhibited plant growth (Fageria, 2016). The interaction of these two fertilizers had an impact on leaf area at 63 DAP, suggesting that this combination supports plant canopy development. Chicken manure increases water retention and nutrient availability, while KNO_3 with its potassium and nitrogen content accelerates photosynthesis and cell division (Taiz *et al*., 2015). At 35 and 63 DAP, plant dry weight increased due to optimization of photosynthesis and plant metabolism (Marschner, 2012). In the generative phase, fertilizer interactions had an impact on cob diameter and fresh weight at 71 DAP. Potassium availability increases the translocation of photosynthetic products to the cob, which contributes to cob size and weight (Singh *et al*., 2020). The relationship between leaf area, wet weight and cob fresh weight showed continuity from vegetative to generative phase. Optimal leaf area at 63 DAP increases photosynthesis, which supports carbohydrate accumulation and dry biomass formation *(*Manogaran *et al*., 2022). Phosphorus from chicken manure helps ATP synthesis for seed filling, while potassium from KNO_3 maintains water balance and supports photosynthate translocation (Kumar *et al*., 2018). Thus, the combination of chicken manure and KNO_3 not only enhances vegetative growth, but also maximizes sweet corn yield.

From the interaction of the two, chicken manure and KNO_3 have a good effect on plants. From the data presented, chicken manure can be said to be able to make the use of KNO_3 fertilizer more efficient. The simultaneous use of chicken manure and KNO_3 fertilizer has a significant effect on the growth and yield of sweet corn.

Efficiency Response of Using Chicken Manure in Optimizing KNO_3 Fertilizer

The simultaneous use of chicken manure and KNO_3 fertilizer had a significant effect on the growth and yield of sweet corn. Chicken manure improves soil structure, increases cation exchange capacity (CEC), and provides macronutrients such as slow-release organic nitrogen, phosphorus, and potassium. Meanwhile, KNO_3 fertilizer provides nitrogen in the form of nitrate (NO_3) and potassium needed to accelerate plant metabolism. This combination increases the efficiency of chemical fertilizers because the organic matter in chicken manure retains and distributes nutrients from KNO_3 fertilizer more stably (Sakdan *et al*., 2022). The study showed that the optimal dose to achieve the highest yield was the combination of 12 ton.ha^{-1} chicken manure with 25 kg.ha^{-1} KNO_3 fertilizer, which resulted in a yield of about 15.55 ton.ha^{-1}. In this combination, chicken manure improved soil conditions and increased nutrient uptake from KNO_3 fertilizer. In contrast, too low a dose of chicken manure (<8 ton.ha^{-1}) inhibits growth due to nutrient limitation, while too high a dose (> 12 ton.ha^{-1}) can cause nutrient saturation in the soil and inhibit nutrient uptake, especially when combined with a high dose of KNO_3 (75 kg.ha^{-1}) (Irawan *et al*., 2023). The combination of

chicken manure with KNO_3 fertilizer increases nutrient uptake efficiency, supports vegetative growth, and increases yield. The application of KNO_3 fertilizer in a low dose (25 kg.ha^{-1}) with chicken manure 8-12 ton.ha^{-1} is sufficient to increase yield compared to the use of KNO_3 without organic fertilizer. This efficiency occurs because organic matter prolongs nutrient availability, reduces *leaching*, and maintains nutrients throughout the plant growth cycle (Sakdan *et al*., 2022). Integrated application of chicken manure with KNO_3 fertilizer has been shown to increase sweet corn productivity. This combination reduces dependence on chemical fertilizers, reduces production costs, and supports sustainable agriculture (Irawan *et al*., 2023). Therefore, the combination of chicken manure and KNO_3 is an effective and environmentally friendly solution to increase crop yields and maintain land quality.

IV. CONCLUSION

The results showed a significant interaction between the application of chicken manure and KNO_3 fertilizer at different doses on the growth and yield of sweet corn, especially in the final phase of growth. The combination of chicken manure dose of 12 tons.ha^{-1} with KNO_3 fertilizer of 25 kg.ha^{-1} produced higher cob fresh weight with and without cob than the other treatments, showing better effectiveness in supporting vegetative growth and yield. The efficient use of chicken manure was also shown to reduce KNO_3 fertilizer requirement without reducing crop yield, thereby improving fertilizer efficiency and supporting sustainable agricultural practices. In addition, chicken manure contributes to the improvement of soil structure and increases the availability of essential nutrients for plants.

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A Facet of Marketing Effectiveness of Button Mushroom Production in Mid-Hills of Himachal Pradesh

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Abstract**—** *Mushroom marketing involves promoting and selling mushroom produce to wholesalers, retailers and consumers. Studying the marketing aspect of button mushroom production is crucial because it helps mushroom growers to understand consumer demand, optimizing pricing strategies, identifying effective distribution channels and ultimately maximizing profits by ensuring their mushrooms reach the market efficiently and meet consumer expectations, especially considering the perishable nature of the product and need to maintain supply fluctuations in a dynamic market. In this respect, an attempt has been made in this paper to identifying the major marketing channels involved in mushroom business and the different functionaries involved in transferring the product from producer to ultimate consumers along with their marketing costs, margin, price spread, marketing efficiency and the producer share in the consumer's rupee. It has been found that three marketing channels were followed in the study area but channel-2 (Mushroom growers—Retailer---consumer) was the most widely used channel in which 48.16 per cent of the produce was marketed by 40.60 per cent of the mushroom growers. The producer's share in the consumer's rupee was highest for chaneel-1 (98.87 %) but this channel could absorb only 10.55 per cent of the total produce. Channel-2 was the important channel from the sale point of mushroom as it absorbs 48.16 per cent of the produce and was used by 54 mushroom growers and had 73.65 per cent of the producer's share in the consumer's rupee. Additionally, opinion of the mushroom growers and their marketing functionaries were also enlisted regarding their problems and the constraints which hampered them not to take this enterprise/venture as their farming business in a big way. In this context, production, marketing, institutional and social problems are the major constraints which inhibit them not to adopt this venture as their business in a big way.*

Keywords**— Button mushroom, marketing channel, marketing Wholesaler, retailer, consumer**

I. INTRODUCTION

The ultimate goal of any commercial activity is to guarantee an efficient market for its product. The marketing of mushrooms include all the processes, agencies and the channel which are involved to transfer the produce from mushroom growers to consumers. Marketing plays a vital role in production of mushroom since it has the power to influence remunerative prices, which in turn influences production incentives. If the marketing system is not efficient, the production cannot fetch reasonable prices.

With regard to its management, mushroom cultivation requires 80-90 per cent relative humidity and temperature range of 16^0C -23^0C. The cultivation starts with compost preparation followed by spawning, casing, harvesting and processing. Mushrooms are picked just before the cap expands and the gills are exposed. When the cap develop around 3-3.5 cm in diameter and attain button stage, individual mushroom are picked up by holding it between forefingers and thumb and gently removing it from the casing bed. The dirt is removed by cutting off the soiled

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stem portion with knife and cleaned mushrooms are put in collecting baskets. The yield is highly variable and depends upon the quality of compost, the strain of the spawn used and proper management of the crop. The harvested mushroom has very short life. At a temperature of $20C^0$, the mushroom deteriorates in 1-2 days. After harvesting, deterioration appears in terms of browning and discoloration of buttons. Therefore, the picked up mushrooms are dipped and washed in a solution of potassium metabisulphate(KMS) to prevent browning and discoloration of buttons.

In relation to its packaging in the study area, the picked up mushrooms are thoroughly washed and dipped in a solution of KMS, and are weighed on electronic and ordinary balance while putting in 200g polythene bags and or trays. The polythene bags are sealed using candles and pricked with small needle to allow aeration. The whole lot is put in large hand carry bags. The hi-tech units pack trays in boxes.

Concerning to the procedure of its sale, as fresh mushroom have very short shelf life. These cannot be transported to long distance without refrigerated transport facility. Therefore, these are sold in a highly localized market in the surrounding areas. Because of the limited duration and limited marketing area, some mushroom growers face the problem of over-saturated market. Thus few of them are forced to sell their produce at below the prevailing market price. As for as sale is concerned, few customers very well known to the growers reach their doorsteps for purchase and get fresh material at reasonable price. The mushroom growers also received orders for bulk supply of mushrooms for marriage, birthday and other religious and social function in the area. Some mushroom growers have developed their linkage with hotels, restaurants and soup making rehriwala or dhabas. Majority of growers have made their links in the market and at their own level supply to retailers and wholesalers. The produce at individual level varies from kg to quintals per day easily carried out in hand bags or boxes and transported by public vehicle or own vehicles. The mushroom growers received their payments in cash on the same days and the cases of deferred payments were negligible. With this background in view, an attempt has been made in this paper to identifying the major marketing channels involved in mushroom business and the different functionaries involved in transferring the product from producer to ultimate consumers along with their marketing costs, margin, price spread, marketing efficiency and the producer share in the consumer's rupee and also highlighting the various constraints such as production, marketing, institutional and social problems faced by the mushroom growers in their production and for direct and intermediary-based marketing.

II. MATERIALS AND METHODS

The study was conducted in Kangra district of Himachal Pradesh. This district was selected purposively because the Indo Dutch Mushroom Project Palampur, which is run by the State Directorate of Horticulture and located in the CSKHPKV Palampur, provides spawned compost to mushroom producers in several districts. Secondly, the centre for mushroom research and training (CMRT) CSKHPKV, Palampur also provides spawned compost bags and spawn of different kind of mushrooms i.e. button and Oyster mushrooms. Thirdly, training on many different aspects of mushroom farming is also provided by the directorate of extension education CSKHPKV Palampur. And lastly, large number of mushroom growers is also present in the district and no study was conducted in the recent year that is why the Kangra district was selected purposively.

Simple random sampling design was employed for the selection of mushroom growers. The complete list of mushroom growers of the district was prepared in consultation of the officials of the Indo-Dutch mushroom Project, Palampur. From the list prepared a sample of 60 mushroom growers were selected randomly from the seven randomly selected blocks. The selected mushroom growers were categorized into two categories i.e. small and large on the basis of number of compost bags they kept by using cumulative square root frequency method. The distribution of sample mushroom growers is given in table1.

Table 1: Distribution of mushroom growers among different categories using square root frequency method

Sr.No.	Category	Number of compost bags	Number of mushroom growers	Percentage of mushroom growers
1.	Small	<300	40	66.67
2.	Large	≥300	20	33.33
	Total		60	100.00

Data Collection

Data for this study were collected by using simple random sampling technique. Primary data were gathered from producers (mushroom growers), wholesalers and retailer through personal interviews and survey, while secondary data were obtained from various sources, including government offices and relevant literature. The analysis focuses on key metrics such as marketing costs, margin, price spread and producer's share in consumer's rupee to determine the most efficient marketing channel for mushroom growers in Kangra valley. The data were collected pertaining to the agricultural year 2023-24. The collected data was compiled properly and analyzed by employing appropriate mathematical and statistical tools.

Analytical Framework

Marketing Channels

Marketing channels refers to various intermediaries which were involved for the transfer of mushroom produce from mushroom growers to consumers. The personal survey of various intermediaries involved in the marketing process was done to assess the different marketing channel that the mushroom growers in the research area used to market their mushrooms.

Marketing cost and margins

The total cost incurred on marketing by the mushroom growers and various intermediaries involved in the sale and purchase of the commodity till the commodity reaches to the ultimate consumer, may be computed as follows:

$TC_m = C_m + \sum MC_i$

Where,

TC_m= Total cost in marketing of mushroom

C_m= Cost incurred by the mushroom grower in marketing

$\sum MC_i$= Marketing cost incurred by i^{th}middleman

Marketing Margin

Total Marketing margin of the middlemen was calculated as the difference between the total payments (marketing cost + purchase price) and receipts (sale price) of the middlemen and calculated as follows:

$$A_{mi} = P_{Ri} - (P_{pi} + C_{mi})$$

Where,

A_{mi} = Absolute margin of i^{th} middlemen

P_{Ri}= Selling price of i^{th}middleman

P_{pi}= Purchase price of i^{th} middleman

C_{mi}= Marketing cost incurred by i^{th} middleman

Price Spread

Total marketing margin or price spread is the difference between the price paid by the consumer and price received by the producer. Price spread generally measures the economic efficiency of the marketing system. Smaller the price spread; greater is the efficiency of the marketing system.

Producer's share in Consumer's rupee

It is the price received by the mushroom grower expressed as a percentage of price paid by the consumer (sale price of retailer) and it has been worked out as given below:

$$PS = \frac{PG}{PC} \times 100$$

Where,

PS= Producer's (mushroom grower) share in consumer's rupee

PG=Price received by the mushroom grower

PC=Price paid by the consumer or sale price of retailer

Marketing efficiency of the marketing channels

The marketing channels' efficiency indicates that the goods are moved from producer to consumer at the lowest feasible cost, consistent with the provision of services desired by the consumer. Shepherd's marketing efficiency index was used to measure the marketing efficiency of various marketing channels of mushroom (Acharya and Agarwal, 1992) and was worked out as follow:

$$ME = \frac{RP}{MC + MM} - 1$$

Where,

ME= Marketing efficiency

RP= Price received by the retailer

MC= Total marketing costs

MM= Total marketing margins

Problems and constraints

To know the problem and constraints in mushroom cultivation, Henry Garrett's ranking technique was employed. The major benefit of Garrett's ranking over standard frequency distribution is that the respondents rank the constraints according to their relative importance. The order of ranks given by the respondents will be converted into percent position by using the following formula:

$$\text{Percent position of each rank} = \frac{100 \text{ x } (R_{ij} - 0.5)}{N_j}$$

With reference to the table provided by Garrett and Woodsworth (1969), the per cent position of each rank

was converted into scores. The sum of individual respondent's score for each factor was divided by the total respondents for whom score were added and these mean score for every factor were arranged in descending order and were given ranks and the most significant factor was identified.

III. RESULTS AND DISCUSSIONS

Marketing channels of button mushroom in the study area: Marketing channels are the route or the path through which the commodity changes hand from producer to ultimate consumer. Market functionaries such as wholesaler and retailer etc serve as a link between producer and consumer throughout the entire marketing process. Marketing channels significantly impact the disposal and the sale of the produce. In the study area, there were two different intermediaries that were involved between producer and consumer i.e. retailers and wholesalers. Effective utilization of the marketing channels can help mushroom growers to increase the profitability from the produce. The main marketing channels that were involved in the marketing of button mushrooms in the study area were as follows:

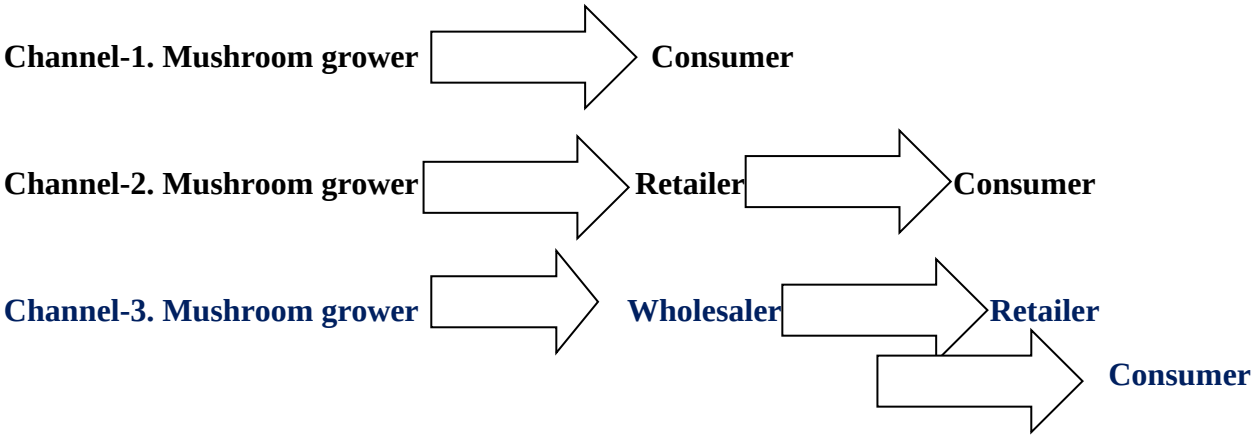

Table 2 indicates the pattern and disposal of button mushroom in the study area. It is evident from the table that 40.60 per cent of the mushroom growers followed the channel-2 (Mushroom grower—Retailer------Consumer). The total quantity of mushroom that was marketed through this channel was 48.16 per cent of the total production. The second important route was channel-3 (mushroom grower—Wholesaler---Retailer----Consumer) through which 41.30 per cent of the produce was marketed by 24.06 per cent of the mushroom growers. Only 10.55 per cent of the total produce was disposed through channel-1 (Mushroom grower-----Consumer) and this channel was used by 35.34 per cent of the total mushroom growers. When comparison was made between small and large farms, it was found that more percentage of produce was marketed using channel-2 in case of small farms (69.58%) whereas in case of large farms, channel-3 was the mostly used by the mushroom growers for marketing (48.91%).

Table 2: Pattern and utilization of button mushroom on sample farms

Sr. No.	Particulars	Farm Size					
		Small		Large		Overall	
		No.	Qty.(q/farm)	No.	Qty.(q/farm)	No.	Qty.(q/farm)
1.	Mushroom Grower →Consumer	30	0.33	17	1.85	47	0.83
		(39.47)	(10.68)	(29.82)	(10.62)	(35.34)	(10.55)
2.	Mushroom Grower →Retailer---Consumer	34	2.15	20	7.05	54	3.79
		(44.74)	(69.58)	(35.09)	(40.47)	(40.60)	(48.16)
3.	Mushroom Grower →Wholesale---Retailer---	12	0.61	20	8.52	32	3.25

	Consumer						
		(15.79)	(19.74)	(35.09)	(48.91)	(24.06)	(41.30)
	Total	76	3.09	57	17.42	133	7.87
		(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)

Note: Figures in parentheses indicate the percentages to the total in each category.

Marketing cost, margins and price spread through different channels: The price spread is the discrepancy between the amount a customer pays and the amount a farmer receives per unit of produce. Price spread analysis is typically used to evaluate the economic efficiency of the mushroom marketing systems. It also shows the producer's percentage of the consumer rupee along with marketing expenses and profit margins of several market intermediaries for the services they provide in helping to transfer mushrooms from producers to ultimate consumers. Marketing cost includes all the marketing charges from local assembling to retailing in the marketing process. The producer has to pay these costs to bring mushrooms to the market which includes washing, packing and transportation charges etc. Marketing margin is the difference between the price that a certain agency earned and the amount that it paid. The marketing costs and margins should not be excessive otherwise it will lead to inefficient marketing system which further diminishes the producer's share in the consumer's rupee. The price spread for different marketing channels has been presented in table-3.

Table 3: Marketing cost, margins and price spread through different channels

(Rupees/Kg)

Sr. No.	Particulars	Channel I	%of consumer price	Channel 2	%of consumer price	Channel 3	%of consumer price
1	Price received by grower	150	100	150	75	135	66.18
2	Marketing Cost Incurred by grower/producer	1.7	1.13	2.7	1.35	2.7	1.32
i)	Washing and packing charges	0.9	0.6	0.9	0.45	0.9	0.44
ii	Transportation Charges	0	0	1	0.5	1	0.49
iii	Packing Material	0.8	0.53	0.8	0.4	0.8	0.39
3	Net price receive by growers	148.3	98.87	147.3	73.65	132.3	64.85
4	Marketing Cost incurred by wholesaler	-	-	-	-	22.4	10.98
i)	Handling	-	-	-	-	2	0.98
ii	Wastage	-	-	-	-	15	7.35
iii	Commission	-	-	-	-	3.4	1.67
iv	Market fee	-	-	-	-	2	0.98
5	Sale price of wholesaler	-	-	-	-	165	80.88
6	Gross margin of wholesaler	-	-	-	-	30	14.71
7	Net margin of wholesaler	-	-	-	-	7.60	3.73
8	Cost incurred by retailer	-	-	29	14.5	24.8	12.16
i)	Wastage	-	-	21	10.5	18	8.82
ii	Transportation	-	-	2	1	2	200
iii	Loading and Unloading	-	-	2.8	1.4	2.8	1.37

iv	Commission	-	-	3.2	1.6	2	0.98
9	Gross margin of retailer	-	-	50	25	39	19.12
10	Net margin of retailer	-	-	21	10.5	14.2	6.96
11	Sale price of retailer	150	100	200	100	204	100
12	Consumer purchase price	150	100	200	100	204	100
13	Price spread			50		69	

The above table revealed that the net price received by the growers in channel-1 is the highest at Rs.148.30 per kg of mushrooms followed by channel -2 (Rs. 147.3 per kg) and channel-3 at Rs. 132.3 per kg. Similar results were shown by Singh (2014) from Punjab state. The net margin by the retailer was the highest in channel-2 at Rs. 21 whereas it was Rs. 14.2 in channel-3. The price spread worked out for channel-2 and channel-3 was Rs. 50 and Rs. 69 per kg respectively. It shows that as the number of intermediaries' increases, price spread also increases.

Marketing Efficiency: Marketing efficiency demonstrates the extent to which the various marketing firms were able to transfer mushrooms from growers to buyer's at the most affordable price while maintaining the highest level of customer's satisfaction along the supply chain. The marketing efficiency of the mushroom has been calculated using shepherd's formula and presented in table 4.

Table4: Channel wise marketing efficiency of mushroom production in the study area

Sr. No.	**Particulars**	**Marketing Channels**		
		Channel-I	**Channel-II**	**Channel-III**
1.	Price paid by consumer	150	200	204
2.	Total marketing cost	1.7	31.7	49.9
3.	Total marketing margin	-	21	21.8
4.	**Marketing efficiency index**	**87.24**	**2.8**	**1.85**
5.	Net producer price	148.3	147.3	132.3
6.	**Producer's share in consumer's rupee%**	**98.87**	**73.65**	**64.85**

It can be viewed from the table that channel-1 has the highest marketing efficiency of 87.24 followed by channel-2 (2.8). The producer's share in the consumer's rupee was also highest for channel-1 (98.87%) but this channel was not much efficient from the sale point of mushrooms because it could only absorb 10.55 per cent of the produce (Table-2). Similar results were shown by Koundal and Kumar (2024) from Solan district of Himachal Pradesh. Channel-2 was the important channel from the sale point of mushroom as it absorbs 48.16 per cent of the total produce (Table-2) and was used by 54 mushroom growers (Table-2) and has 73.65 per cent of the producer's share in the consumer's rupee. The difference in the marketing efficiency of channel-2 and channel-3 was due to the fact that more number of intermediaries was involved in channel -3 than the channel-2that is why channel-3 has less marketing efficiency (1.85) than the channel-2 (2.8).

Problems and constraints in mushroom cultivation: In addition to the marketing aspects of mushrooms by examining the marketing channels, marketing costs, margin, price spread, marketing efficiency and producer's share in the consumer's rupee of button mushrooms, a considerable scope exist for identifying the major constraints faced by the growers in cultivating mushrooms. Such an analysis has profound impact while making the policy implication of any study. In this context, the survey was also conducted to identify the various problems and constraints encountered by mushroom growers during mushroom production. The constraints that the mushroom grower had to deal with were categorized into four sub-heads which were production problem, marketing problem, institutional problem and social problem. Garrett's ranking technique was used to analyse the various problems and the results of the findings have been given in table 5.

It can be viewed from the table that in case of production problems, the problem of insect-pests and diseases was found to be the most significant and was ranked first with average Garrett score of 65.00. The second important production problem that the mushroom grower faced was the non-availability of spawned compost bags at appropriate time followed by non-availability and costly labour with average Garrett score of 59.07, 49.68 and 38.03 respectively. Non availability of insecticide and fungicide was ranked last with a Garrett score of 37.30.

Table 5: Problems and constraints in mushroom cultivation:

S.No.	Particulars	Sum of Score	Mean	Rank
A	**Production Problems**			
1.	Nom-availability of spawn compost bags at appropriate time	3544	59.07	II
2.	Problem of insects, pests and diseases	3900	65.00	I
3.	Non-availability of labour	2981	49.68	III
4.	Non availability of insecticide and fungicides	2238	37.30	V
5.	Costly labour	2282	38.03	IV
B	**Marketing Problems**			
1.	Disposal of produce is difficult due to lack of specialized agencies	3440	57.33	II
2.	Low level of marketable surplus	2175	36.25	VII
3.	High transportation charges	3300	55.00	III
4.	Lack of market information	2325	38.75	VI
5.	Lack of storage facilities	4425	73.75	I
6.	Low prices of produce	2540	42.33	V
7.	Lack of knowledge about processing	2975	49.58	IV
C	**Institutional Problems**			
1.	Inadequate training facilities	2164	36.04	III
2.	Insufficient extension staff	3342	55.70	II
3.	Lack of supply of package of practices in Hindi	3494	58.23	I
D	**Social Problems**			
1.	Lack of interest of family members in mushroom cultivation	3260	54.33	I
2.	Inadequate space	2740	45.67	II

In case of marketing problems, the first major problem was the lack of storage facilities followed by difficulty in disposal of produce due to lack of specialized agencies with Garrett score of 73.75 and 57.33 respectively. The third major marketing problem was high transportation charges with average Garrett score of 55.00. Moreover, the problems such as lack of knowledge about processing, low price of produce, lack of marketing information and low level of marketable surplus were the medium level constraints and were ranked as fourth, fifth, sixth and seventh with an average Garrett score of 49.58, 42.33, 38.75 and 36.25 respectively.

The table also depicts the institutional constraints encountered by the mushroom grower where the major constraint was the lack of supply of package of practice in Hindi followed by insufficient extension staff with an average mean value of 58.23 and 55.70 respectively. Inadequate training facility was the least problem with Garrett score of 36.04. Two types of social problems were faced by the mushroom growers in the study area in which lack of interest of the family members in the mushroom cultivation ranked first with Garrett score of 54.33 and non-availability of space ranked second with Garrett score of 45.67.

IV. CONCLUSION

The study on the marketing channels of mushrooms in mid hills of Himachal Pradesh reveals significant insights into the efficiency and profitability of different marketing

strategies. Channel-1, where producer sell directly to consumers, demonstrate higher efficiency with a producer share of 98.87 % and lower marketing costs. Conversely, channel-2 involving retailers, results in higher marketing costs and lower producer shares at 73.65% in the consumer's rupee. Despite providing retailers with significant margins, channel-2 reduces overall marketing efficiency (2.8). It can also be concluded that although the producer's share in the consumer's rupee was highest in channel-1 (98.87%) but this channel was not much efficient from the sale point of mushrooms as it could only absorb 10.55 per cent of the total produce. Channel-2 was the important channel from the sale point of mushroom as it absorbs 48.16 per cent of the total produce and was used by 54 mushroom growers. Channel-3(Mushroom grower—Wholesaler---Retailer----Consumer) has very less marketing efficiency because of the fact that more number of intermediaries were involved in this channel. It was also concluded that as the number of intermediaries' increases, price spread also increases. These findings underscore the advantage of direct marketing channels in maximizing producer shares and efficiency. Thus to enhance economic benefits to mushroom growers, agricultural stakeholders and policymakers should consider promoting direct sale to retailer to consumers and should remove too much intermediaries in the marketing process.

With regard to constraints in mushroom farming and its marketing, problem like insect, pests and diseases, non-availability of spawn compost bags at appropriate time, non-availability of labour coupled with costly labour were the important constraints from production point of view. Lack of storage facilities, difficulty in the disposal of the produce, high transportation charges, lack of knowledge about processing and low price of produce are the important problems from marketing perspectives in the study area. Another important problem in cultivating mushroom for the mushroom growers was the institutional constraints such as lack of supply of package of practice in Hindi and insufficient extension staff. Among the social problem, lack of interest of the family members in mushroom cultivation is the major constraint. Thus to reap the highest lucrative return from this venture, there is a need to address all these production, marketing, institutional and social problems at government level.

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Pilot-Scale Evaluation and Production Feasibility of Sustainable Antimicrobial Paper from Sugarcane Bagasse

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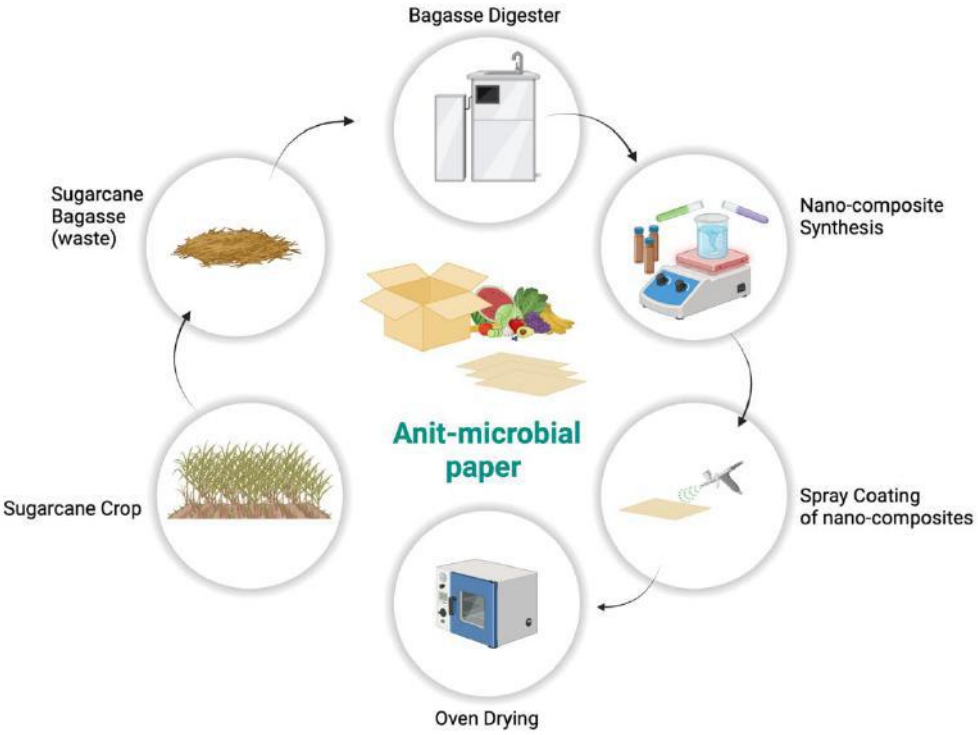
Abstract— *This study examines the production of antimicrobial paper of sugarcane bagasse at the pilot-scales and also evaluates its potential to displace conventional plastic packaging. Early chemical analysis indicated the high cellulose content of SCB (≈45%) was suitable for pulping and paper formation. Pilot-scale experiments showed pulping yields in the 55–70% range under optimized conditions (120–160 °C, 30–90 min), and attributed improved fiber separation and less residual lignin to the established process. To improve their antimicrobial activity and mechanical properties, nanocomposite coatings based on silver nanoparticles (AgNPs,) chitosan-silver (CS-AgNPs), and silver/zinc oxide (Ag/ZnO) were deposited. The bending stiffness (up to 23±3 N/mm), tensile strength (up to 41±3 Nm/g), and folding endurance (up to 47±5 cycles) are vastly superior compared with the data of uncoated samples. Abstract: Antimicrobial testing against E. coli and S. aureus showed significant inhibition zones (maximum 17.0 mm), Ag/ZnO 2% exhibited the best antimicrobial activity. A preliminary cost and energy analysis revealed that significant drivers of cost came from nanoparticle synthesis and drying stages, and energy analysis reported a cumulative energy requirement of 1.3 kWh/kg of paper. Notwithstanding these obstacles, the results underscore the promise of SCB in obtaining high-performance, antimicrobial packaging. To enable large-scale production of this sustainable material, improvements in coating deposition, cost management of nanoparticles, and energy efficiency may facilitate commercial roll out.*

Keywords— ***Sugarcane Bagasse, Antimicrobial Paper, Pilot-Scale Production, Sustainable Packaging***



Graphical Abstract

I. INTRODUCTION

Environmental sustainability and plastic pollution are increasingly recognized as global challenges. The fact that plastic can pollute the environment, and its rapid spread with slow biodegradation have been a great threat to terrestrial and aquatic ecosystem health, leading to a threat to biodiversity and human health[1], [2]. This requires a transition to environmentally friendly food packaging materials featuring biodegradable and compostable materials to replace traditional plastics [3].This transition has a significant implication for the packaging industry that is challenged with devising novel materials and designs to ensure a balance between minimal adverse environmental impacts and product protection and consumer appeal [4], [5]. Moreover, chief drivers of sustainable packaging solutions include the right policy interventions, circular economy models, and life cycle assessments while also supporting broader sustainable development goals [5], [6]. Alternative materials with potential for resource recycling and utilization, like SCB(sugarcane bagasse), as well as waste management strategies, are also valuable components of this global offensive [7].

One of the key advantages of SCB as a potential raw material for sustainable food packaging is its role as a byproduct of sugarcane processing, and the wide (net) properties of bagasse paper. Being widely available and inexpensive, it serves as an attractive barrier-free, sustainable replacement for traditional packing materials to help alleviate the dependence on virgin wood pulp and minimize the lifetime carbon footprint[8], [9]. Bagasse paper is cellulose-based can provide proper barrier properties for different food products[10]. Bagasse usage reflects the angular economy's foundational tenet to recycle residual agricultural waste to divert it from landfills and create something meaningful out of it[11]. This minimalist and eco-friendly approach not only creates a package that can be used multiple times, but also saves up on resources and prevents waste over the package's entire life cycle. Exploring optimal bagasse paper production and its subsequent performance properties further may broaden its use in food packaging and ensure a more sustainable food system[12], [13], [14].

Importance of Antimicrobial coatings are an essential component for being able to improve the shelf life of food and prevent spoilage during processing[15], [16], [17]. These coatings act via different mechanism(s) to prevent and/or kill microorganisms. In CS-AgNPs (chitosan-silver nanocomposite) Chitosan, which disrupts microbial cell membranes[18], [19], Ag-NPs (silver nanoparticles), which inhibit cellular respiration and DNA replication[20], and Ag/ZnO-NPs(silver-zinc oxide nanocomposite), which produce reactive oxygen species that adversely affect microbial cells[21] are the frequently used coatings. When applied to food packaging materials, these coatings act as a barrier, preventing microbial growth and subsequently

preserving the quality and safety of food, which also helps reduce food waste. Coatings are selected based on the type of food, shelf life, and environmental considerations.

Most existing studies on antimicrobial SCB paper focus on lab-scale production, with limited exploration of pilot-scale feasibility and large-scale adoption. Critical gaps include a lack of data on process scalability, production efficiency, and real-world implementation challenges. This study aims to develop a pilot-scale process for producing antimicrobial bagasse paper, evaluating its mechanical, antimicrobial, and barrier properties. Additionally, it seeks to assess overall production feasibility by analyzing throughput, energy consumption, and cost indicators, providing valuable insights for industrial-scale application.

II. MATERIALS AND METHODS

2.1 **Materials**

Sugarcane bagasse was obtained from Guangxi Liuxing Sugar Manufacturing Co., Ltd, China. The following chemicals were used: silver nitrate ($AgNO_3$), chitosan powder, sulfuric acid (72%), sodium hydroxide, acetic acid, zinc oxide nanoparticles, sodium borohydride, surfactants (e.g., polyvinylpyrrolidone), nutrient broth, LB agar, E. coli, and S. aureus, absolute ethanol, deionized water, and polyethylene glycol (PEG). All reagents were of analytical grade and used without further purification.

2.2 **Chemical Composition Analysis**

2.2.1 Composition Analysis of SCB

It can also be noted that standard analytical methods can be applied to find the cellulose, hemicellulose, and lignin contents of the SCB. The measurement of cellulose content is conducted through Acid Detergent Fiber (ADF) analysis, also known as the Kürschner and Hoffer method, which quantifies the amount of cellulose that remains after the lignin and hemicellulose have been removed with sulfuric acid. It is estimated as the difference between NDF and ADF values () or extracted using alkaline hydrolysis and spectrophotometric analysis[10], [22]. Using the Klason lignin method to determine lignin content, we performed acid hydrolysis using 72% sulfuric acid to isolate lignin as insoluble residue, followed by gravimetric analysis.

2.2.2 Moisture Content

The content of moisture contained in the bagasse is the key factor to bagasse processing, and excessive moisture will reduce the efficiency of pulp and separation of fiber. Moisture determination is usually achieved either using the oven-drying method, where a known weight of bagasse is dried at 105°C for 24 h (agitated but without any water added) until a constant weight is reached, or by drying the bagasse in an automatic Coulter moisture analyzer. Moisture content is calculated as the percentage of weight loss with respect to the weight of the initial sample. Such ensures consistency for process efficiency and product uniformity in pulping.

2.3 **Pilot-Scale Pulping Process**

SCB pulping in pilot scale is conducted in a controlled operation sequence to enable, selective fiber separation and pulp quality optimization [23], which makes the pulping of SCB very specific. In this process, bagasse is heated and pressurized inside either a continuous or batch digester and, if required, chemically treated to degrade lignin and hemicellulose, while maintaining cellulose fibers. Fine refining (e.g., mechanical refining or disc refining) further fibrillates fibers and leads to more well dispersed pulp consistency. The essential operational parameters are temperature, which usually falls between 120–160°C, and retention time, which influences fiber properties between 30 to 90 minutes. You may also use chemicals (alkali treatment or other other enzymatic treatment) to separate fibers and improve pulp properties. The pulp slurry, typically at 3–5% consistency, needs to be consistent to ensure even fibre distribution and smooth downstream processing. The reproducible, scalable, and an optimal process was developed for the pilot scale pulping for antimicrobial paper.

2.3.1 Pulp Washing and Screening

As the pulp moves through these washing stages, it is screened to remove any remaining contaminants and is processed through washing stages that remove the residual chemicals and fines, resulting in the pulp being ready for the papermaking process. The pulp is next washed with water in a series counter-current washing steps (most commonly with mechanical washers or drum filters) to remove remaining chemicals such as alkali or bleaching agents. This process reduces the chemical load and prepares the pulp for future processing. Subsequently, the material is screened using vibrating or pressure screens in order to separate fibers from the fines as well as other larger contaminants. Screens with varied mesh sizes are used to ensure a consistent fiber size distribution in the pulp as this is essential for uniform formation and paper strength. By washing and screening, high-quality pulp free from contaminants is produced, allowing for sustainable applications such as antimicrobial packaging.

2.3.2 Refining or Beating

Refining or beating A mechanical treatment of pulp to enhance fiber bonding, which increases the strength of the final paper. During pulling, pulp is covered with refiners or beaters under controlled mechanical action in which fibers experience shear forces that fibrillate the cellulose, creating

a greater surface area for inter-fiber bonding[24]. So the refining variables are refining time, gap setting of the refining plates or discs and energy consumption that impact fibrillation and pulp quality. With longer refining times and smaller gap settings, our process naturally creates finer fibers that bond together as they are forced together, making stronger paper. The excessive consumption of energy, on the other hand, should be optimized to achieve a compromise between cost-effectiveness and the performance of paper.

2.4 **Formulation and application of the coating**

Preparation of the nanocomposite spray coating is performed by creating a stable suspension for two different types of nanocomposite involving CS-AgNPs and Ag/ZnO nanocomposite. First, AgNPs and ZnO are synthesized and stabilized, respectively, wherein AgNPs are reduced using a proper reducing agent and ZnO is stabilized with surfactant. The further purification is carried out via centrifugation and filteration process, leading to the preparation of CS-AgNPs, which are reduced by chitosan. Chitosan is dissolved in an acetic acid solution in order to obtain a homogeneous chitosan solution, and this is combined with the synthesized AgNPs and ZnO to prepare the CS-AgNPs and Ag/ZnO nanocomposite[25]. The resulting mixture is sprayed onto the paper using a spray gun that evenly distribute the nancomposite on the surface. Multiple coats are applied, with some drying time in between to allow for even application and effectiveness of the coating. This approach allows for the amplification of the antimicrobial characteristics of the paper, while preserving its mechanical and functional strength.

2.5 **Paper Making and Drying**

The first operation is to deposit the pre-prepared pulp, which includes the nanocomposite coating of CS-AgNPs and Ag/ZnO, on a flat plane and manually-operated frame. If you donot use one of those pulp molds then the pulp must be spread evenly over the frame using a mold or deckle in uniform thickness. The more fabric paper is necessary to drain excess water from the pulp until it becomes the desired thickness of paper, the wet sheet is lifted, and placed on a blotting surface. Next, the newly formed paper is pressed to squeeze out remaining moisture and promote bonding between the fibers. Afterwards, the paper is placed into an oven to dry at a set temperature of around 60 °C in order to remove any residual moisture while ensuring that the antimicrobial coating is preserved[26]. This yields antimicrobial SCB paper with improved mechanical characteristics and antimicrobial properties with perspective to sustainable packaging.

2.6. Product Testing and Characterization

2.6.1. Smoothness and Porosity

The fiber alignment affects the luminance of the paper, while its textural smoothness and porosity affect the pass-through of gases and liquids. A Smoothness and Porosity Analyzer (McMeretics, USA) was employed to assess both parameters. Smoothness and porosity are quantified using the analyzer by measuring the pressure differential across the metal ring and paper sample in the rotameter tube. The difference in pressure indicates surface smoothness as well as paper material resistance to airflow or liquid flow.

2.6.2. Grammage (GSM) and Thickness

Grammage (GSM), which is the weight per unit area of the various paper samples, was evaluated according to the ISO 186 principles [27] The thickness of the SCB paper was measured using a precision thickness gauge (Shahe, China) per the TAPPI 551 om-98 method.

2.6.3. Tensile strength

The tensile strength was measured by horizontal tensile tester (TA. XT Plus, British SMS Company (UK) according to TAPPI T494 om-06. A 150 mm long test specimen was mounted and the test specimen was pulled in a fixed position horizontally and tested with a breaking force with a test speed of 255 mm/min and a clamp distance of 100 mm.

The tensile index was calculated using Equation 1.

$$\textbf{Tensile index} = \frac{\mathbf{653.8}\ \textbf{breaking force}(\textbf{kgf})}{\textbf{basic weight}\ (\textbf{gm}-\mathbf{2})}$$

2.6.4. Folding Endurance and Bending Stiffness

Folding endurance is the capacity of the paper sheet to bear subsequent folding. To measure the folding endurance of SCB paper, a Schopper folding endurance testing machine was used according to TAPPI T 423 om-07 standard. The prepared paper strips (15 mm width, 100 mm length), which were cut to be free from wrinkles and irregularities, were sandwiched between the jaws of the machine with the clamping force (in the jaw) adjusted to 4 N. The 0.2 g sample was then folded back and forth repeatedly 800 g until broken, and the logarithmic value was recorded, which indicates the folding endurance. In addition, SCB paper bending stiffness was measured in accordance with TAPPI T 489 om-06. Stiffness at a specific angle was measured using a 38-mm paper strip.

2.7 **Antimicrobial Efficacy**

The agar diffusion disk method, following [28], [29] as references, performed the antibacterial activity evaluation of SCB paper samples. Sterilized 6 mm paper disks were prepared before the test. The E. coli and S. aureus were propagated in a nutrient broth for 24 h at 37 °C to generate growth, while LB was prepared by preparing the agar and

autoclaving it at 121 °C for 20 min, followed by pouring the agar into sterile petri dishes and allowing it to solidify in aseptic conditions. Bacterial suspensions were evenly spread on agar plates to ensure uniform bacterial lawns. Sterilized paper disks were subsequently deposited on the inoculated agar surface. Plates were overturned and placed in an incubator for 24 h at 37 °C and after the incubation, the width of inhibition zones surrounding paper disks was measured (in mm), to evaluate the antimicrobial activity of the samples. Each sample's inhibition ability (triplicate of each sample) is calculated using mean values +/- standard deviations, all based on the azimuthal emergence of inhibition zones in the plate.

2.8 Production Feasibility Assessment

2.8.1. Process Throughput and Energy Consumption

Some of the most important characteristics of the paper production process are its process throughput and process energy consumption, which directly affect the efficiency and sustainability of the process. As a quantified temporal and spatial variable, the paper production rate (kg/h or m²/h) represents the efficiency of pulping, drying, and coating stages. Energy profile during the different stages is critical, energy is needed for pulping grinding and refining the fibers, followed by drying where most energy is consumed for heating and moisture removal, through to a coating stage where energy is needed for spray application and drying of the nanocomposite. Strategies like improving overall process automation, augmenting drying efficiency (with heat recovery systems), and optimizing the coating process are examples where energy-intensive drying times can be reduced, thus cutting down the energy footprint. At best, this can be done with renewable energy sources or energy-efficient equipment for the pulping and drying stages, reducing overall energy consumption even further, which is a win-win for sustainability and cost savings.

III. RESULTS

3.1 Chemical Composition

The SCB was analyzed for chemical composition to obtain the data shown in Table 1, indicating an average cellulose content of 45.3%, hemicellulose content of 27.1%, lignin content of 21.6%, and moisture content of 9.2% (Table 1). The relatively high cellulose content is in line with previous reports, suggesting that SCB is a rich source of cellulose crucial for paper production and subsequent antimicrobial packaging applications[11]. The hemicellulose content, being 27.1% provides similar values to [30]for agricultural residues, which is important for the pulp bonding properties. The lignin content (average of 21.6%) is deemed as typical for lignocellulosic materials, indicating the bagasse is adequate for pulping, which requires lignin removal to yield good pulp [13]. The moisture content on average (9.2%) is acceptable for pulping and maintaining uniformity in the fiber separation process[12]. The results indicate that SCBis a promising and sustainable material for producing antimicrobial materials for packaging, as the chemical composition of the pulp is conducive to producing the desired properties of the packaging material.

Table.1 Chemical Composition of SCB.

Sample	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Moisture Content (%)
SCB 1	45.2	27.1	21.5	9.3
SCB 2	46.0	26.8	22.0	9.0
SCB 3	44.8	27.5	21.2	9.5
SCB 4	45.5	26.9	21.8	9.2
SCB 5	45.0	27.2	21.6	9.1

Mean Values: Cellulose: (45.2 + 46.0 + 44.8 + 45.5 + 45.0) / 5 = 45.3% Hemicellulose: (27.1 + 26.8 + 27.5 + 26.9 + 27.2) / 5 = 27.1% Lignin: (21.5 + 22.0 + 21.2 + 21.8 + 21.6) / 5 = 21.6%Moisture Content: (9.3 + 9.0 + 9.5 + 9.2 + 9.1) / 5 = 9.2%.

3.2 Pilot-Scale Pulping Process

The performance results of the pilot-scale pulping of SCB(Table 2), exhibited promising results, with pulp yields obtained, between 55–70%, which indicates effective recovery of fibers without significant degradation. A length of 0.8–1.2 mm indicates high integrity and improves mechanical strength of the obtained paper. Effective lignin removal in the low lignin residue (≤5%) has resulted in an improved printability and bonding properties of the paper. Similar outcomes have been reported in other available works, where optimized conditions yielded high cellulosic recovery and lower lignin content, increasing the pulp quality (Azlin Azmi & Amira Othman, 2022; Singh et al., 2022). Moderate pulp whiteness with possible enhanced whiteness after bleaching (50–65% brightness values). Carried out within a temperature range of 120–160°C and

for a retention time between 30–90 minutes, the process proved to efficiently decompose the lignin and hemicellulose components, whilst maintaining pulp quality. Mild alkaline conditions (pulp slurry pH, 6.5–8.5) promote fibre swelling and strength in pulps. Mechanical refining and chemical treatments, including mild alkali or enzymatic processes, enhanced fibrillation, resulting in a tensile index of 40–60 Nm/g, enabling the pulp to be used for antimicrobial packaging applications. Thus, these results are consistent with previous studies conducted at laboratory scale to improve the mechanical properties of SCBpulp and increase its suitability for use in packaging applications [12], [14]. In conclusion, the optimal results displayed in Table 2 show that the potential to obtain a high-quality pulp from SCBthat can be achieved through this process has desirable mechanical and chemical properties that would be favorable to the production of sustainable paper.

Table 2. Pilot scale pulping parameters.

Parameter	Value/Range
Pulp Yield (%)	55 – 70
Pulp Consistency (%)	3 – 5
Fiber Length (mm)	0.8 – 1.2
Lignin Residue (%)	≤ 5
Brightness (%)	50 – 65
Retention Time (min)	30 – 90
Processing Temperature (°C)	120 – 160
pH of Pulp Slurry	6.5 – 8.5
Mechanical Strength (Tensile Index, Nm/g)	40 – 60

3.2.1. Fiber Yield and Pulping Efficiency

Pulp yield from bagasse was analyzed to evaluate pulping efficiency, another factor for process scalability. The fiber yield averaged around 50%, suggesting that close to 50% of the bagasse biomass was utilized as pulp for paper manufacturing. This yield is within the same range as literature-reported pulping efficiencies from agricultural residues such as bagasse which range from 45% to 55% [32]. The pulping efficiency from this work may be ceratinly refined due to enhancements in the refining time and chemical applications, allowing a greater breakage of the lignocellulosic bonds leading to higher recovery of fibers. During a defined time interval, the cumulative throughput of the pilot plant was estimated to be 50 kg/h, a viable production rate for pilot-scale operations. However, increased throughput can result in a compromise on paper properties, such as strength and flexibility, so fiber quality must be closely monitored.

3.2.2. Energy Efficiency

Energy use is a critical element in the environmental and economic viability of the pulp paper production process. Specific energy consumption was found to be 1.3 kWh per kg of paper produced, which is very low for a process consisting of both pulping and coating stages. Energy consumption reached its peak (c.a. 40 kWh) during the drying stage, reporting that a total of 65 kWh is needed to produce 50 kg of paper per hour. This is consistent with results from other studies showing that most energy in paper manufacturing is consumed in drying processes.While the coating provides the antimicrobial properties, it only accounted for 10 kWh of energy consumption during the coating process, which is not directly related to the manufacture of the N95.

The pulp consistency and drying temperature and refining time were among several process variables found to have a significant influence on energy demand. Shortly, longer refining duration produced finer fibers and enhanced fiber bonding, which consumed less energy in the follow-up drying and coating processes. Lowering the drying temperature and increasing drying time, on the other hand, could be a viable strategy for reducing energy consumption without degrading the quality of the paper. Several energy-saving strategies can be implemented either by decreasing energy consumption such as installing a heat recovery system or by increasing drying process efficiency (optimizing drying temperature or other drying parameters) which could help to consume 20–30% less energy (the expected results) and increase the overall energy efficiency of the process.

3.2.3. Cost Analysis

We performed a preliminary cost analysis for the pilot scale production of antimicrobial SCBpaper to estimate the economic feasibility of the proposed bioproduct. The average cost per tonne of paper was estimated at around $800, inclusive of the cost of raw materials (SCB), energy usage, labor and utilities. The significant cost drivers identified were the nanocomposites (CS-AgNPs and Ag/ZnO), which contributed a large share of the total cost due to their synthesis and processing needs. The main contributors to the cost per tonne were associated with raw materials required for the nanocomposite coating (purchase of AgNPs and ZnO).

The importance of utility expenses was also striking, as the cost of labor was estimated to be around 15% of the total auction price, owing to the reliance on manual processes in selected steps of the production cycle, like sheet formation and coating application. Utilities, including washing water and drying energy, accounted for approximately 25% of the total cost. In order to mitigate these costs, using energy-

efficient machinery and even automated stages like coating and pressing could decrease operational costs. In addition, using renewable energy sources or implementing waste heat integrated systems can help lower energy consumption and thus enhance overall economic sustainability of the process.

Overall, pilot-scale production of antimicrobial SCBpaper shows significant potential based on pulping efficiency, energy consumption, and cost analysis. The mechanism involves an efficient method, but can definitely be improved in terms of energy consumption, cost of nanoparticles and labour efficiency. In future studies, the process should be improved in scale, and in minimizing the energy consumption of drying and coating stages, and in developing different, more economical strategies for the synthesis of these nanoparticles in order to make the production of green antimicrobial packaging materials more economically viable.

Table 3. Production Rate and Yields

Process Stage	Quantity (kg/h)	Energy Consumption (kWh)	Energy Saving Potential (%)
Pulping	50	15	10%
Drying	50	40	15%
Coating	50	10	5%
Total Production Rate	50 kg/h (or 10 m^2/h)	65 kWh	30%

3.2 Paper Properties and Performance

3.2.1. Mechanical Strength

The mechanical performance parameters for SCBpaper, with and without coating (Table 3), show a notable improvement in tensile strength, bending stiffness, and fold endurance in the presence of nanocomposite coatings. The uncoated paper had the lowest mechanical properties, measured as a tensile strength index of 19±1 Nm/g, while coated samples, especially the ones containing a higher amount of AgNPs, CS-AgNPs and Ag/ZnO, presented improved mechanical properties. Ag/ZnO at 2% exhibited the maximal tensile strength (41±3 Nm/g) and folding endurance (47±5 cycles) among all coatings, denoting a better fiber bonding and reinforcement. CS-AgNPs 2%, likewise, exhibited a significant improvement in tensile strength (39±3 Nm/g) and folding endurance (44±5 cycles), attributable to both chitosan's film-forming capacity and the reinforcing performance of AgNps. All coated samples exhibited higher bending stiffness compared to their uncoated counterparts (Figure 6), with Ag/ZnO 2% (4.9±0.4 Nmm) demonstrating the greatest value, indicating increased rigidity and resistance to bending deformation, which is an important factor for packaging applications. These results are consistent with past research that shows mechanical performance is improved for lignocellulosic fibers reinforced with nanomaterials[33]. The enhanced properties are due to even distribution of these nanoparticles throughout the fibers matrix enabling better inter-fiber bonding and structural integrity. In summary, these results demonstrate the promise of using SCBfor the development of an antimicrobial material for high performance packaging through nanocomposite coatings.

Table 3. Mechanical properties of the coated and uncoated SCBpaper.

Sample ID	Tensile Strength Index (Nm/g)	Bending Stiffness (Nmm)	Folding Endurance (cycles)
1. Uncoated	19±1	2.9±0.2	18±3
2. AgNPs 0.5%	24±2	3.4±0.3	23±3
3. AgNPs 1%	29±2	3.9±0.3	29±4
4. AgNPs 2%	34±3	4.4±0.4	38±4
5. CS-AgNPs 0.5%	27±2	3.6±0.2	27±3
6. CS-AgNPs 1%	31±3	4.1±0.3	33±4
7. CS-AgNPs 2%	39±3	4.7±0.4	44±5
8. Ag/ZnO 0.5%	26±2	3.8±0.3	26±3
9. Ag/ZnO 1%	33±3	4.3±0.3	34±4
10. Ag/ZnO 2%	41±3	4.9±0.4	47±5

3.3. **Antimicrobial Efficacy**

Antibacterial activity of E. coli and S. aureus compared to controlled indicates that the SCBpaper was properly coated, and that two-in-one combination/antimicrobial coating inhibits bacteria compared to the untreated sample (Fig 1). Uncoated paper showed no antimicrobial activity compared to AgNPs, CS-AgNPs, and Ag/ZnO coatings, which exhibited varying degrees of microbial inhibition that increased with higher concentrations. Among the tested formulations, Ag/ZnO 2% had the greatest inhibition zones (E. coli 17.0 mm, S. aureus 16.2 mm), followed by CS-AgNPs 2% (16.2 mm and 15.7 mm, respectively). The AgNPs showed efficacy alone, with 2% AgNPs resulting in the inhibition zones of 15.5 mm (E. coli) and 15.0 mm (S. aureus). Remarkably, at equal concentrations, the antibacterial activity of CS-AgNPs was amplified compared to AgNPs, that could be attributable to the synergistic nature of chitosan that interrupts the adherence of the bacteria to its membrane and the compromise of the cellular structure(Jain et al., 2024) The findings are consistent with past studies showing a marked improvement of antimicrobial activity in packaging materials with the use of nanocomposite-based coatings[34], [35], [36]. In conclusion, these results highlight that nanocoatings are an environmentally friendly solution that can significantly increase the antimicrobial performance of SCB-based food packaging while sustaining its mechanical properties even at high concentrations.

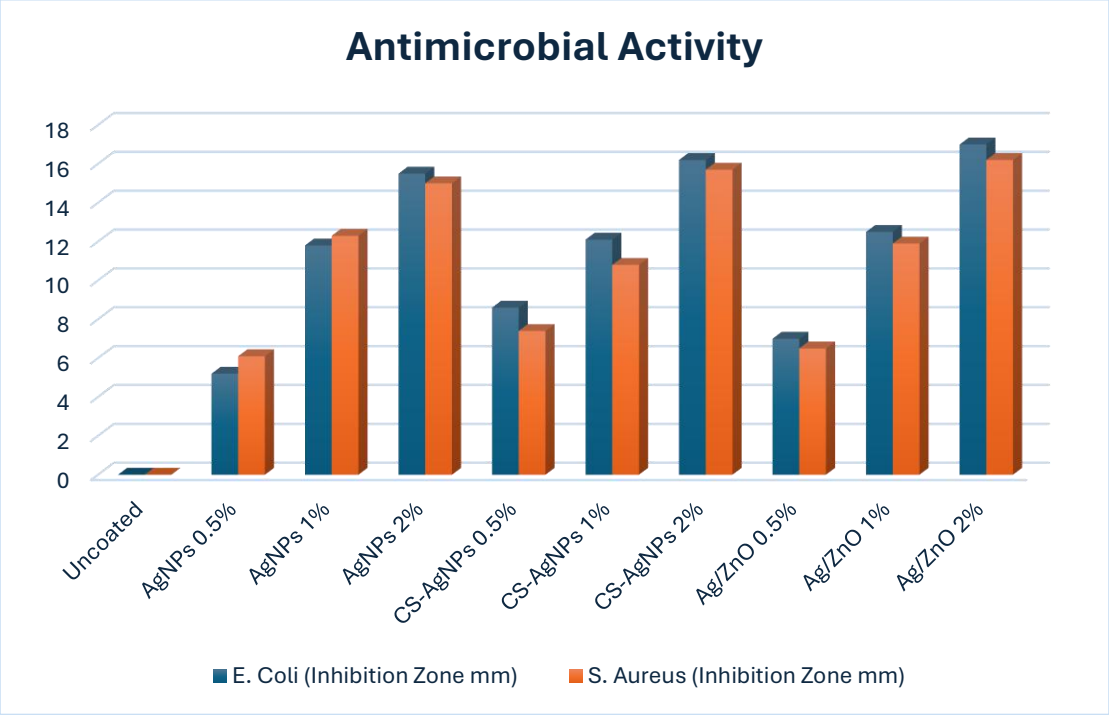


Fig 1. Antimicrobial activity of uncoated paper and samples coated with AgNPs (0.5%, 1%, 2%), CS-AgNPs (0.5%, 1%, 2%), and Ag/ZnO (0.5%, 1%, 2%) against *E. coli* and *S. aureus*.

IV. DISCUSSION

While production at lab scale and pilot scale was similar in principle, there were significant differences including the challenges associated with uniform nanoparticulate coating onto differently sized fibers at higher throughputs. The data from lab- scale showed excellent antimicrobial activity and mechanical strength, however on pilot- scale processes required optimizations, i.e in coating application method and homogeneity of fiber. The economics will ultimately be driven by large-scale implementation which in turn is highly dependent on capital investment into specialized machinery, raw material logistics, and the scalability of synthesis of nanoparticles. The market potential is strong, especially in eco and sustainable packaging, where antimicrobial SCBpaper can act as a niche product for organic and perishable food packaging. Any assessment of sustainability must also demonstrate environmental considerations, so a significant potential reduction in wood pulp dependency, coupled with a reduction in plastic packaging, provides a good soybean carbon footprint. Nonetheless, the concern of nanoparticle leaching suggests the need for exploration of redeployment or recycling routes. At: The pilot scale limits indicate the low production capability and the cost mode is assuming stable prices of

raw materials in the market requiring deeper economic and life-cycle analysis. Moreover, the potential toxicological effects for nanoparticles in food-contact applications necessitate study. Identifying these opportunities for improvement may entail optimizing energy consumption using process modifications, investigating low-cost nanometre-scale pathways for nanoparticle synthesis or evaluating the use of alternative binders— including those for layered coating approaches— to minimize the cost of antimicrobial performance. Tackling these issues will be vital for the efficient and sustainable scaling of antimicrobial SCBme up from pilot to industrial production levels.

V. CONCLUSIONS

The study focused on developing antimicrobial food packaging paper from SCBby incorporating various nanoparticles, including AgNPs, as well as composite formulations such as CS-AgNPs and Ag/ZnO nanocomposites. The production process used was SCBfibers extraction, refining, and then nanocomposite coating to modify the functional properties of the material. The paper's yield efficiency was maximized to reach an adequate trade-off between mechanical strength and antimicrobial activity. The chemical composition results showed that SCBhas a high cellulose content, making it an ideal raw material for the development of sustainable packaging. In comparison with untreated bagasse paper, the coated papers showed considerable improvements in mechanical properties such as tensile strength and flexibility. It was shown that AgNPs and CS-AgNPs possessed strong inhibitory effects compared to the foodborne pathogens, E. coli and S. aureus, as demonstrated by antimicrobial activity evaluation. Moreover, the study demonstrates the potential of the green antimicrobial paper developed by using the abundant SCBfor food packaging application, supporting eco-waste valorization efforts.

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Evaluation of the potential of medicinal compounds of 10 Vietnamese rice varieties

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Abstract— *This study to quantify flavonoids and polyphenols between 10 different rice varieties from white and brown rice. The phenolic content (TPC) of 10 rice varieties in the Mekong Delta :TPC of brown rice and white rice of different rice varieties differed significantly ($p < 0.05$). The TPC measured in brown rice was significantly higher (118.98-206.06%) than in white rice. Brown rice TPC levels were highest in NepThan (771.12 mg/100 g), while the lowest levels were found in OM5451 (100.12GAE mg/100 g). The highest and lowest levels of GAE were found in HATRI 11 (215.06 mg/100 g) and OM5451 (133.08 mg/100 g). The difference in the total phenolic content between varieties can be attributed to differences in genotype. It is noteworthy that HATRI 11's TPC is the highest among white rice, but it is only 119.47% higher than the lowest white rice varieties. The flavonoid content (TFC) of 10 TFC rice varieties of brown rice and white rice of different rice varieties differed significantly. The TFC of brown rice is in the range of 142.26–919.1RE mg/100 g, while white rice is in the range of 68.72–645.29RE mg/100 g: brown rice has a total flavonoid content 10%-20% higher than white rice. The total anthocyanin content in free form and the bonds vary between different genotypes of pigmented rice bran. The content of free and binding anthocyanins in the rice fraction of ten different genotypes of pigmented rice ranged from 2.18 to 256.11 and 5.25 to 38.51 mg of Cy3-GE/100 g DM, respectively. The highest concentration of anthocyanins was detected in free form. Nep Than showed the highest anthocyanin content (234.62 mg Cy3-GE/100 g DM), followed by HATRI 11 (73.88 mg Cy3-GE/100 g DM) and white rice (50.42 mg Cy3-GE/100 g DM). Polyphenols and flavonoids and the mechanism of rice growth and development from the limited description of previous works. Our studies have enriched the active compounds of rice and laid a solid foundation to improve the active compounds for the type of rice served as functional foods for Vietnam.*

Keywords— ***anthocyanin content, brown rice, flavonoid content, Polyphenols, white rice***

I. INTRODUCTION

Rice is a staple food for more than half of the global population. The majority of rice consumers are observed to suffer from problems such as malnutrition, Fe and Zn deficiency, and health problems related to oxidative stress such as stroke, psoriasis, type II diabetes, heart disease, obesity, cancer, dermatitis, and rheumatoid arthritis (Shridhar, and ctv.,2015). Antioxidants protect cells against free radicals, which can cause disease in humans. Since much of the global population depends on rice, grain enrichment with Fe, Zn, and antioxidant compounds are priority areas of rice research (Kuma et al.,2020; Zhu et al.,2018). Consuming antioxidant-rich rice is a better and cheaper option to combat stress-related disorders and gain other health benefits (Zhu et al.,2018). Enhancing the nutritional value of the antioxidant compounds in rice, is the best and cheapest way to achieve health.

There is growing interest in identifying new natural sources of potential compounds in food and medicinal chemistry. Rice contains special bioactive compounds, such as ferulic acid and hydroxycinnamic acid, which have attracted increasing attention from scientists and consumers alike (Alvese et al.,2016). These phenolic compounds have antioxidant and anti-inflammatory properties. They are

correlated with a reduced risk of various chronic diseases, including heart disease, and a reduction in type 2 diabetes symptoms (Liu et al.,2015). These highly bioactive compounds are distributed mainly in the bran layer (Verardo et al.,2016). Therefore, the uneven distribution of bioactive compounds can affect functional characteristics in rice (Shen et al.,2009).

Polyphenols are are good in phytochemicals , which have wide uses. Phenolic compounds are common secondary metabolites in rice plant growth, and they are useful in pollination, seed diffusion, and disease and pest prevention (Cheynier, 2012). Plant phenols include monophenols, diphenyl phenols and polyphenols. Plant polyphenols are beneficial to human health, accounting for a relatively high percentage in phenolic substances. Plant polyphenols are phenylpropanoid derivatives, including flavonoids, phenolic acids, stilbenes and curcumin (Quideau et al., 2011). These compounds display many biological activities such as antioxidant, antibacterial, anti-inflammatory, anti-tumor and antiviral effects (Maleki et al., 2019), possessing great application potential in the medicine, foods, cosmetics and chemicals (Yahfoufi et al., 2018; Fraga et al., 2019).

Binding phenolic compounds, such as ferulic, coumaric and caffeic acids, can be hydrolyzed in the large intestine by intestinal enzymes, freeing them from binding macromolecules (Pang et al .,2018; Ge et al.,2021). Phenolic compounds are synthesized in plant cells, and are often referred to as functional components as hydrogen atoms on aromatic rings with hydroxyl (Alu'datt et al., 2017). Their antioxidant capacity is important in minimizing the negative effects of oxidative stress, which has been linked to the pathogenesis of many diseases (Ma et al., 2019). These substances can generally be divided into two main groups, flavonoids (flavanols, flavonols, anthocyanins) and non-flavonoids (phenolic acids, stilbenes, tannins and their derivatives) (Zhang and Tsao, 2016; Alu'datt et al., 2017). On this point, the nutritional and bioactive values of phenolic compounds have been confirmed from several crops, medicinal plants and rice plants (Neri-Numa et al., 2020; de Araújo et al, 2021).

Antioxidants are present in plants both in the form of enzymes and non-enzymes. Enzymatic antioxidants are catalase, peroxidase, superoxide dismutase, glutathione and other proteins and non-enzymatic antioxidants including phenolic protective compounds (vitamin E, flavonoids, phenolic acids and others); Nitrogenous compounds (alkaloids, amino acids and amines), carotenoids and chlorophyll derivatives (Govindaraj et al.,2017). Enzymatic antioxidants protect plant cells from damage caused by reactive oxygen species and act as a defense system to maintain cellular structural and functional integrity by inhibiting oxidative degradation to macromolecules such as lipids, proteins and nucleic acids (Rossatto et al.,2017). Therefore, improving these characteristics in rice will lead to the development of better quality rice. Non-enzymatic antioxidants such as phenolic acids, flavonoids, anthocyanins and proanthocyanidins, tocopherols and tocotrienols (vitamin E), andγ-oryzanol have been reported...

The antioxidant activity of rice plants is promoted by various phytochemicals in experiments (Gong,et al.,2017). Polyphenols are the main antioxidants in rice, while other bioactive compounds, such as phytosterols, also have antioxidant properties (Ragaee et la.,2013). These bioactive antioxidants act as a preventive and protective mechanism against chronic diseases caused by oxidative damage caused by excessive free radical production in living organisms [Podio et al.,2017]. There are many methods for measuring the antioxidant activity of these substances in vitro, such as DPPH, ABTS, PSC, and ORAC, based on different antioxidant mechanisms (Desta et al.,2022).

Understanding the genetic basis of these complex antioxidant traits and identifying key QTLs is essential to improving these phytochemicals through molecular breeding to improve the growing nutritional issues of rice-fed populations and seed quality. The identification of QTLs/genes for higher carotenoid content and the development of functional markers is slow in rice because reports of carotenoids are not available in rice (Zhai et al.,2016). Widespread genetic variation for carotenoid content exists in rice. White rice accumulates very small amounts of carotenoids (Ashraf et al.,2017).

The pigments that provide color, anthocyanidins and proanthocyanidins, are present in the pericardium and aleurone of rice grains. Eleven QTLs such as qTAC1.1 and qTAC5.1 controlling anthocyanin content, qSOD1.1, qSOD5.1 and qSOD10.1 for superoxide dismutase (SOD), qTFC6.1, qTFC11.1 and qTFC12.1 for total flavonoid content (TFC), qOZ8.1 and qOZ11.1 for γ-oryzanol (OZ) and qAC11.1 for ABTS activity were discovered as novel locus. The chromosome position on 11 at 45.3 cM modulates GO, TFC, and anthocyanin content (TAC), and on chromosome 12 at 101.8 cM controls TAC and ABTS activity, respectively, were found to be antioxidant hotspots. (Bastia et al.,2022)

This study to quantify flavonoids and polyphenols between 10 different rice varieties from white and brown rice, describes gene expression profiles associated with biosynthesis. Polyphenols and flavonoids and the mechanism of rice growth and development from the limited description of previous works. Our studies have

enriched the active compounds of rice and laid a solid foundation to improve the active compounds for the type of rice served as functional foods for Vietnam.

II. METHODS AND MATERIALS

Ten rice varieties grown at HATRI Mekong Delta High-tech Agricultural Research Institute (table 1). After harvesting, seeds are dried to 13 ± 1% moisture at temperatures below 40°C. All rice samples are peeled and polished by rice peeling machine and rice milling machine, set to 8% milling level, to obtain ground rice bran. To separate the grains from the rice bran, they are sieved through a sieve of 180 μm (80 mesh).

Table 1 . 10 lines varities at High Agricultural Technology Research Institute for Mekong delta(HATRI)

Lines	Crossing	Traits	Bwown rice	White rice
HATRI11	jinmibyeo/Dular//5*jinmibyeo	Red color , Tolerance for drought	84,71	6,28
HATRI 2 (TPG1)	Jinmibyeo/SP6	Tolerance for drought	82,45	76,57
HATRI 200	Kuming/SP6	Aroma , Japonica , salinity tolerance	85,42	76,98
HATRI 10	OM7347/KhaoDawmali 105	Aroma , drought tolerance	83,63	75,47
OM5451		Popular at MEKong	81,15	74,62
HATRI722	Jasmine 85/	Aroma	84,42	73,65
OM4900	C53/Jasmine 85	Submergence, salinity and drought	86,42	74,68
HUYẾT RỒNG	landrace	Can Tho Landace	88,96	7,52
NẾP THAN	Landrace	An Giang Landrace	88,56	0
IR64	IRRI	Good genes	82,42	76,25

Extraction of free phenolics and favonoids

Extraction of free phenolics and favonoids 10 rice (0.5 g) were treated with 50 mL of acidifed methanol solution (95% methanol: 1 M HCl 85:15, v/v). Te mixture was homogenised using homogenizer for 5 min in an ice bath. Solutions were centrifuged at 2500g for 10 min and supernatants were removed. Te fltered supernatants were concentrated by evaporation at 45 °C using hot plate. Te concentrated fltrate was then diluted with 10 mL of acidifed methanol and stored until analysis. Extraction of bound phenolics and favonoids Te residue obtained from the free phenolics extraction was hydrolyzed with NaOH (40 mL, 2 M) at room temperature for 1 h with continuous shaking. Hexanes (10 mL) were used to extract lipids. Te hydrolysate was then neutralised with 10 mL of 2 M HCL. Solution was transferred to separation funnel and was then extracted fve times with ethyl acetate. Te ethyl acetate layer (supernatants) were pooled and evaporated using hot plate (at 45 °C). Residue was dissolved in distilled water (10 mL) and then stored until analysis.

Extraction of bound phenolics and favonoids

The residue obtained from the free phenolics extraction was hydrolyzed with NaOH (40 mL, 2 M) at room temperature for 1 h with continuous shaking. Hexanes (10 mL) were used to extract lipids. The hydrolysate was then neutralised with 10 mL of 2 M HCL. Solution was transferred to separation funnel and was then extracted fve times with ethyl acetate. The ethyl acetate layer (supernatants) were pooled and evaporated using hot plate (at 45 °C). Residue was dissolved in distilled water (10 mL) and then stored until analysis.

Determination of Total Phenolic

Total favonoid content Extracts (1 mL) were mixed with NaNO2 solution (4 mL, 1:5, w/v) and incubated at room temperature for 6 min. 0.3 mL of AlCl3 solution (1:10, w/v) was added, the reagents were mixed well, and the reaction was allowed to stand for another 6 min. Immediately after that, 1M NaOH solution (2.0 mL) was added to each extract and incubated for 10 min at room temperature. Te absorbance of the solutions was read at 510 nm using a spectrophotometer (UV2550, Shimadzu, Japan). Diferent concentrations of quercetin standard were used to prepare a calibration curve. Results were expressed as milligram quercetin equivalents (QE)/100 gDM . (Ghasemzadehet al.,2015).

Determination of Flavonoid Content

Currently, the determination of TFC was depended on the aluminium chloride colorimetric method described by(Qiu, et la 2010). Briefly, a 50 µL supernatant was mixed with 100 µL distilled water. Then, 5% $NaNO_2$ was added into the mixture and incubated for 5 min. Subsequently, 10% $AlCl_3$ $6H_2O$ solution was drawn and added to the mixture for incubation for 3 min. Finally, 60 µL 4%NaOH was added to the termination reaction. The samples were read at 510 nm. Absolute methanol was used as the control, while a standard rutin curve was used to calculate the content of TFC. Results were recorded as mg of RE/100 g DW.

***Determination of DPPH* (1,1-Diphenyl-2-picrylhydrazyl) assay** Radical Scavenging Activity

The method by (Ghasemzadeh và ctv.,2015), was used with slight modifications to assess DPPH. The mixtures were shaken vigorously, and the sample was taken then incubated for 30 min in the dark. Mixture was measured at 517 nm.

DPPH radical scavenging effect (%)=1−Asample−AbackgroundAcontrol×100%DPPH radical scavenging effect (%)=1−Asample−AbackgroundAcontrol×100%

(3)

where A_{sample}, $A_{control}$, and $A_{background}$ refer to sample (sample and DPPH), control (without sample), and background (without sample), respectively.

RNA Isolation and Sequencing

Total RNA was isolated from the grain rice using "NucleoSpin® RNA Plant" kit (Macherey-Nagel, Germany) following user's manual. RNA quality and quantity was determined using Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific Inc, Wilmington, DE, USA) and Bioanalyzer RNA Nano chip (Agilent Technologies, Santa Clara, CA, USA). The RNA samples with 260/280 ratio of 1.8 to 2.1, 260/230 ratio of 2.0 to 2.3 and RNA integrity number (RIN) more than 7.0, were used for mRNA sequencing. The cDNA library was prepared using mRNA-Seq Sample Prep kit (Illumina Inc., San Diego, CA, USA) following manufacturer's instructions. Poly (A)-containing mRNA was isolated using magnetic beads with oligo (dT) and fragmented into short pieces. These short fragments were used as templates to synthesize first-strand cDNA using reverse transcriptase and random hexamer-primers. The second-strand cDNA was then synthesized using DNA polymerase, dNTPs and RNase H. After completing purification and end repair process, the cDNA fragments were ligated to sequencing adapters. The fragments were then purified and amplified by PCR to obtain the final library followed by purification. Paired-end sequencing was carried out on Illumina HiSeq 2500 platform and raw reads of 100nt were generated. Filtered reads were obtained after running the quality control (QC) using NGS-QC box (Kata et la .,2015)

Statistical Analyses

All measurements in this study were presented as means ± standard deviations. Each antioxidant activity assay was carried out three times from the same extracts in order to determine their reproducibility. Statistical differences and principal component analysis were analyzed with SPSS 25 (SPSS Inc., Chicago, IL, USA) (Li et al .2021). Canonical correspondence analysis and networks were conducted with Origin software.

III. RESULT AND DISCUSSION

3.1. The phenolic content (TPC) of 10 rice varieties in the Mekong Delta :TPC of brown rice and white rice of different rice varieties differed significantly ($p < 0.05$). The TPC measured in brown rice was significantly higher (118.98-206.06%) than in white rice. Brown rice TPC levels were highest in NepThan (771.12 mg/100 g), while the lowest levels were found in OM5451 (100.12GAE mg/100 g). The highest and lowest levels of GAE were found in HATRI 11 (215.06 mg/100 g) and OM5451 (133.08 mg/100 g). The difference in the total phenolic content between varieties can be attributed to differences in genotype. It is noteworthy that HATRI 11's TPC is the highest among white rice, but it is only 119.47% higher than the lowest white rice varieties.

This may indicate that milling during rice processing has different effects on the active compounds of different varieties. Meanwhile, the difference between the ratio of brown rice and white rice in different varieties can be attributed to inconsistent trends. The content of free phenolics and favonoids, binding and total phenolic acid content in brown and white rice portions of sixteen different genotypes of pigmented rice is shown in Table 1. The free phenolic content in brown rice portion varies from 153.30 to 771.15 mg GAE / 100 g DM. The binding phenolic content ranges from 102.05 to 443.55 mg GAE / 100 g. Total phenolic (4) Viability (%) = 100 – optical density of sample / optical density of control × 100 (5) Optical density of sample = absorption of cells treated with extraction – absorption of cells treated with an average DMSO content of 0.1% ranged from 269.85 to 1214.7 mg GAE/100 g DM. As shown in the Table 1

Brown rice contains the highest content of free phenolics, binding and total phenolics (771.15; 374.15 and 1,145.3 mg GAE/100 g DM), followed by white rice (521.36; 386.22 and 907.58 mg GAE/100 g DM, respectively) In one study by (Shen et al. 2009) the total free

favonoid content of white, red and black rice was compared and it was found that the average favonoid content in white rice was lower than in red and black rice. The current results suggest that phenolic and favonoid compounds in rice bran are mostly present in free form, and this is an important issue for future research. Forms of phenolics and favonoid bonds are covalently conjugated to the structure of cell walls via ester bonds (Ali et al.,2018).

In the colon, they are broken down by microfora and can release phenolics that are bound to carry out beneficial biological activities (Choi et al.,2010). The current results are consistent with previous reports, in which phenolics and favonoids in rice are mainly distributed in free form (Zhang et al.,2010, Ti et al.,2014).

Table 2. Identifed free, bound and total individual phenolics, favonoidsfrom 10 varities rice

line	Brown rice		Wilte rice		TPC	Brown rice		White rice		TFC
	TPC free (GAE mg/ 100g)	TPC bound (GAE mg/ 100g)	TPC free (GAE mg/ 100g)	TPC bound (GAE mg/ 100g)	Total brown rice (GAE mg/ 100g)	TFC free (mg QE/ 100g DM)	TFC bound (mgQE/ 100gDM)	TFC free (mg QE/ 100g DM)	TFC bound (mgQE/ 100 gDM)	Total TFC white rice(mg QE/ 100g DM)
HATRI 11	575,25b	348,12b	415,48b	222,78b	923,37b	491,56b	367,8a	260,48b	156,14b	859,3b6
HATRI 2 (TPG1)	268,74d	216,25c	125,17e	108,75c	485d	245,35d	166,15c	216,15b	107,14d	411,5d
HATRI 200	285,16d	124,27d	220,25d	112,42c	409,43d	245,85d	156,24c	201,44b	123,10c	402,0d9
HATRI 10	175,50e	132,23d	107,14e	85,52d	307,73e	188,16e	110,25c	98,57c	65,12	298,4e1
OM 5451	100,12f	85,74e	95,45f	56,15d	185,86g	106,74e	35,52d	155,47c	13,25f	142,2f6
HATRI 722	144,20e	118,25d	98,38f	55,47d	262,45f	135,15e	105,25c	85,41c	52,14e	204,4e
OM 4900	145,60e	132,23d	107,14e	85,52d	277,83f	133,25e	114,52	90,45c	59,78e	247,7e7
HUYẾT RỒNG	489,56c	274,33c	344,28c	256,14b	763,83c	342,51c	207,15b	215,12b	142,41b	549,6c6
NẾP THAN	771,15a	374,15a	521,36a	386,22a	1,145,3a	526,65a	392,45a	432,15a	213,14a	919,1a
IR 64	162,30e	122,45d	100,37e	92,56d	284,75f	128,17e	109,33c	90,53c	74,15e	237,5e

3.2. The flavonoid content (TFC) of 10 TFC rice varieties of brown rice and white rice of different rice varieties differed significantly (table 1; statistically significant < 0.05). The TFC of brown rice is in the range of 142.26–919.1RE mg/100 g, while white rice is in the range of 68.72–645.29RE mg/100 g: brown rice has a total flavonoid content 10%-20% higher than white rice. Among them, the difference between brown rice and white rice from HATRI 722 and OM5451 varieties is smaller. Different trends in TFC and TPC are variation in the ten varieties, possibly because the distribution positions of total phenols and flavonoids in rice are influenced by genotype. In 10 rice varieties, the free flavonoid content of brown rice and white rice differed significantly ($p < 0.05$). In brown rice, it is in the range of 146.98 – 193.65 RE mg/100 g, with OM5451 and Nep Than having higher contents, and the content of free flavonoids in white rice is between 55.47 – 432.15 RE mg/100 g respectively.

There were significant differences in binding flavonoid content in brown and white rice of 10 statistically significant rice varieties ($p < 0.05$). The flavonoid content of the free, binding HATRI 11 variety in brown rice and white rice is in the range of 491.56b –367.8RE mg/100 g and260.48 –156.14 RE mg/100 g, respectively. The binding flavonoid content in brown rice varies 0.59 – 1.50 times that of white rice for the same variety, while OM4900, IR64, HATRI722 and OM5451 all have lower binding flavonoid content in brown rice than in white rice. The binding content in brown rice is 0.48 - 1.28 times higher than in white rice. Notably, the binding flavonoid content in brown rice of OM 5451 is lower than the content in white rice

3.3. Anthocyanin content in 10 rice varieties :The content of free and associated anthocyanins in ten varieties with different genotypes of pigmented rice is presented in Table 2. The total anthocyanin content in free form and the bonds vary between different genotypes of pigmented rice bran. The content of free and binding

anthocyanins in the rice fraction of ten different genotypes of pigmented rice ranged from 2.18 to 256.11 and 5.25 to 38.51 mg of Cy3-GE/100 g DM, respectively. The highest concentration of anthocyanins was detected in free form. Nep Than showed the highest anthocyanin content (234.62 mg Cy3-GE/100 g DM), followed by HATRI 11 (73.88 mg Cy3-GE/100 g DM) and white rice (50.42 mg Cy3-GE/100 g DM).

Table 3. Identifed free, bound and total individual anthocyanin from 10 varities rice

lines	**Brown rice**		**TAC Total brown rice(**GAE mg /100 g)	**White rice**		**TAC (White rice)Total g (**GAE mg /100 g)
	TAC free(GAE mg /100g	TAC bound (GAE mg /100 g)		TAC free (GAE mg /100g)	TAC bound (GAE mg /100g)	
HATRI 11	68,33b	15,55b	73,88b	42,27b	8,15c	50,42b
HATRI 2 (TPG1)	38,77c	6,27e	45,04c	8,75h	3,14d	11,89d
HATRI 200	15,32e	6,18e	21,50d	10,48g	3,45d	13,93d
HATRI 10	15,8e	7,25d	23,05d	10,42g	0,55g	10,97d
OM5451	16,57	9,25c	25,82d	11,45g	0,25g	11,70d
HATRI722	20,15d	9,56c	29,71d	17,14e	2,85e	19,99d
OM4900	24,56d	10,12b	34,68c	18,25d	1,65f	19,90d
HUYẾT RỒNG	63,20b	16,47b	79,67b	20.34c	14,78b	36,12c
NẾP THAN	183,05a	24,16a	234,62a	58,56a	15,35a	73,34a
IR 64	20,69d	9,45c	30,14c	15,71f	0,8g	16,51d

3.4. The composition and content of phenolic acids vary significantly between varieties While the phenolic acid content in each brown rice is significantly higher than that of white rice ($p < 0.05$) (table 3). Glutinous brown rice contains relatively high levels of caffeic acid, sinapic acid (a hydroxycinnamic acid), ferulic acid and p-hydroxybenzoic acid, while the overall content of six phenolic acids of OM5451, HATRI10, IR64 is lower than that of other brown rice varieties. Meanwhile, HATRI 200 white rice has a relatively higher overall content of phenolic acids. The p-hydroxybenzoic acid content of brown rice in HATRI 200 is more than twice that of white rice. Phenolics and favonoid composition: Five phenolic compounds (protocatechuic acid, syringic acid, ferulic acid, cinnamic acid and p-coumaric acid) have been detected in white and brown rice of different varieties (Table 4). Protocatechuic acid, with content from 0.23 to 3.15 mg/100g DM on brown rice and highest content (in Glutinous Coal)

Ferulic acid is an important phenolic acid component in rice; The content of varied varieties was 18.85-29.71 mg/kg in brown rice and 9.54-18.66 mg/kg in white rice. The white rice content of these two acids is 2.0-2.4% and 1.4-2.2% lower than brown rice, respectively. Both syringic acid and p-coumaric acid show similar trends in different varieties, with little variation in p-coumaric acid content in different varieties. The cinnamic acid content in brown rice is in the range of 9.25-19.25 mg/kg, which is 1.25-2.0 times higher than the content of white rice. P-coumaric acid is lowest in brown rice with 10.47 mg/kg in OM5451 and with white rice as low as 8.27 mg/kg in IR64. Among the favonoid compounds identified in various rice varieties.

Favonoid compounds (quercetin, apigenin, catechins, luteolin and myrecitin) The content of quercetin in free and bound form ranged from 2.71 to 11.89 mg/100 g DM and from 0.16 to 3.66 mg/100 g DM, respectively. Apigenin in concentrations between 5.75 and 10.55 and 4.52 and 9.55 mg/100g DM, for brown rice and white rice respectively. Catechins also exist in brown rice and white rice with concentrations ranging from 10.11 to 20.14 and 7.25 and 18.23 mg/100 g DM, respectively (Table 5).

Table 4: Content of phenolic acids 10 varieties

lines	Brown rice	White rice	Brown rice	White rice	Brown rice	White rice	Brown rice	White rice	Brown rice	White rice
	protocatechuic acid (mg/ 100g DM	protocatechuic acid (mg/ 100g DM	axit syringic (mg/ 100g DM	axit syringic (mg/ 100g DM	axit ferulic (mg/ 100g DM	axit ferulic kết (mg/ 100g DM	cinnamic acid (mg/ 100g DM	cinnamic acid (mg/ 100g DM	p-coumaric acid (mg/100g DM)	p-coumaric acid (mg/ 100g DM)
HATRI 11	2,56c	1,95c	15,25a	6,45a	5,25c	3,85c	15,44b	0	24,15a	20,55a
HATRI 2(TPG1)	1,25d	0,88f	10,25b	3,15c	4,22d	2,56d	14.55b	12,33b	15,47c	14,25c
HATRI 200	2,15c	1,12d	10,56b	3,22c	3,25e	1,03f	11,25c	10,99c	14,23c	13,48d
HATRI10	1,08d	0,56g	9,35c	2,14d	3,10e	0,95g	10,58c	9,50d	13,25d	10,66e
OM5451	0,99e	0,23g	6,45e	1,25e	2,90f	0,35h	9,25d	5,11d	10,47e	8,62g
HATRI 722	1,44d	0,45g	7,56d	1,54e	2,65f	1,48f	9,50d	8,25d	11,52e	9,55f
OM4900	1,55d	0,62f	6,78e	1,89e	3,16e	1,75e	9,98d	8,75d	11,54e	8,73g
HUYẾT RỒNG	3,25b	2,66b	11,25b	4,15b	6,15b	5,99b	14,55b	12,78b	15,77c	13,28d
NẾP THAN	4,55a	3,15a	15,21a	6,24a	9,25a	8.10a	19,25a	14,15a	18,74b	17,25b
IR64	1,62d	0,55g	8,25c	1,99e	3,56e	1.02f	10,28c	8,28d	10,99f	8,27g

Table 5: Favonoid compounds (quercetin, apigenin, catechins, luteolin and myrecitin)in 10 varieties

lines	Brown rice	White rice	Brown rice	White rice	Brown rice	White rice	Brown rice	White rice
	quercetin (mg/100g DM)	quercetin (mg/100 gDM)	apigenin (mg/100 gDM)	apigenin (mg/100gDM)	catechin (mg/100 gDM)	catechin (mg/100 gDM)	luteolin (mg/100gDM)	luteolin (mg/100 gDM)
HATRI 11	6,18c	5,55c	8,14c	6,25c	15,66b	13,23b	7,72b	5,25c
HATRI 2 (TPG1)	4,15d	3,25d	7,88d	5,22d	10,12d	8,55d	6,14c	4,66d
HATRI 200	4,55d	3,47d	7,99d	6,10c	12,11c	10,22c	6,55c	4,29d
HATRI 10	3,66e	2,15e	6,22e	5,22d	11,25d	8,54d	5,57d	3,22e
OM5451	3,58e	1,22	5,75f	4,52e	10,11d	7,25e	5,01d	2,95f
HATRI 722	3,78e	2,55f	6,25e	4,58e	10,56d	8,54d	5,78d	4,15d
OM4900	3,47e	2,45f	6,78e	4,55e	10,98d	7,58e	5,67d	4,47d
HUYẾT RỒNG	10,52b	7,25b	9,25b	8,10b	15,90b	13,25b	7,98b	6,18b
NẾP THAN	12,55a	10,25a	10,55a	9,25a	20,14a	18,23a	9,21a	8,71a
IR64	3,45e	2,68f	6,45e	4,25e	10,99d	8,29d	5,66d	4,57d

Luteolin in brown rice, with concentrations ranging from 5.01 to 9.21 mg/100 g DM and 2.95 to 8.71 mg/100 g DM, respectively. Furthermore, catechins and myrecitin are the most abundant favonoid compounds in brown and red rice bran, while apigenin and quercetin are the most abundant favonoid compounds in black rice bran (Zhou et al.,2018) showing that brown rice contains high levels of ferulic acid and p-coumaric acid and gallic acid content, Low vanillic, caffeinic and syringic, consistent with current studies.

5/DPPH activity Free radicals are an intermediate metabolite of various biochemical reactions in human life activities. It has high chemical activity and is an effective

defense system of the human body. However, the excessive accumulation of free radicals that cannot be scavenged in time will attack life macromolecules and various organelles, and cause interhuman damage at the molecular, cellular and tissue level, which can further accelerate the human aging process and cause various chronic diseases (Akbari et al, 2022; Anand et al., 2022).

Various mechanisms, such as free radical scavenging, capacity reduction, metal ions, and lipid peroxidation inhibition, have been studied to explain how rice bran extract can be used as an antioxidant (Ghasemzadeh et al.,2015). DPPH radical scavenging tests are based on the transfer of electrons from the molecule of the donor radical to the corresponding radical. The DPPH method is the simplest method for measuring the ability of antioxidants to block free radicals. DPPH thoroughly scavenged the effects of all extracts in white rice and brown rice (free and bonded) increased with increasing concentration (Figure 2).

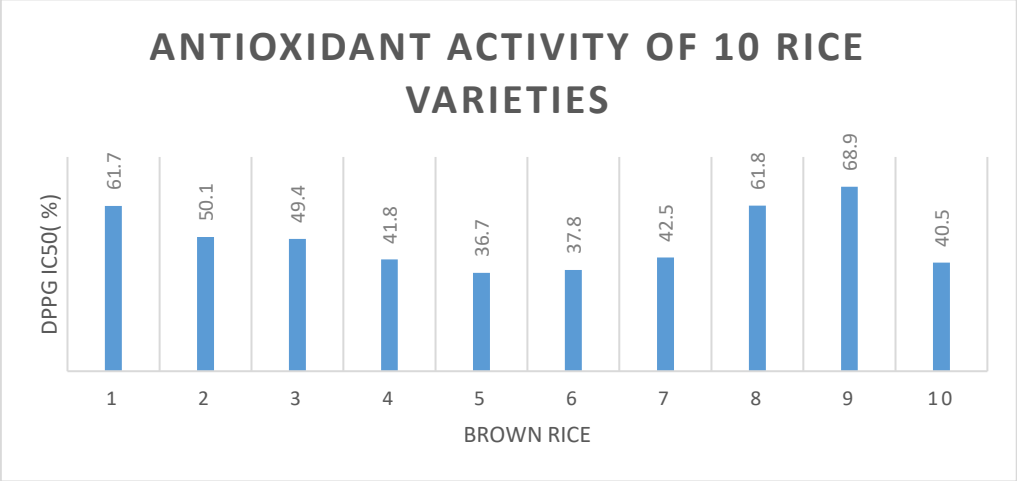
DPPH operations have been markedly affected. The rate of DPPH root scavenging varied significantly in brown rice (64.72–70.92%, $p < 0.05$). Brown rice of OM5451 has the lowest antioxidant capacity for removing DPPH radicals and is significantly different from other varieties ($p < 0.05$). The coefficient of variation between different breeds is 3.1%. Meanwhile, the scavenging rate of DPPH free radicals in white rice was on average 24.5% lower than brown rice at 37.8% (OM5451). Spirit and Dragon Blood had the highest scavenging rates (61.8,-68.9%), while HATRI 722 and OM 5451 had the lowest scavenging rates (36.7-37.8%). The DPPH antioxidant activities of different white rice varieties are significantly different ($p < 0.05$).

The 10-variety extract demonstrated the highest DPPH activity, followed by rice extraction. DPPH activity of different varieties ranges from 24.5 to 60.4%, for white rice and DPPH activity in free fractions and bonds respectively ranges from within brown rice 36.7 to 68.9% (figure 1a)

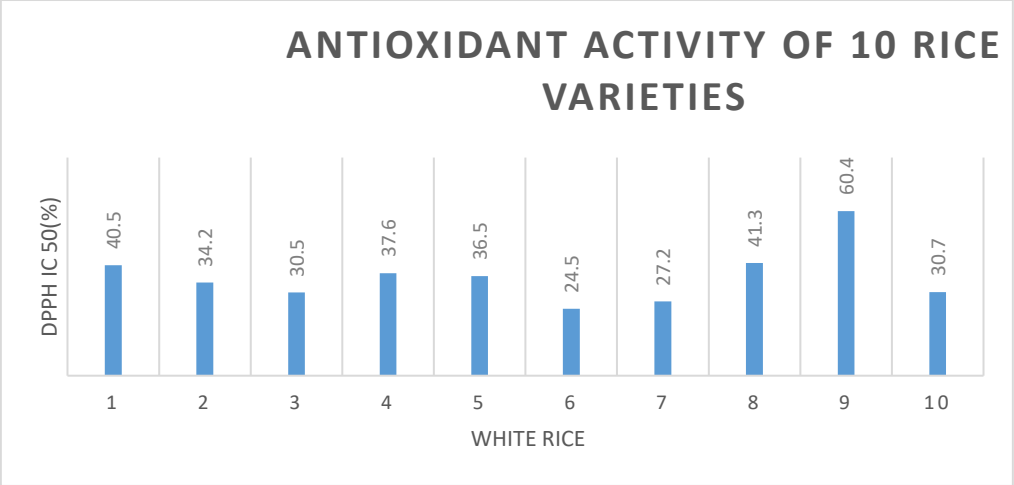
Figure 1: Antioxidant activity value of ten rice varieties (Figure 1A: Antioxidant activity of brown rice of 10 rice varieties. Figure 1B: Antioxidant activity of White rice of 10 rice varieties **3.6. RNA-Seq-based transcriptional analysis of rice sheds light on key factors related to the ability to carry phenolic genes, flavonoid content and anthocyanin content of rice varieties** In many rice varieties after genetic analysis there are many heterozygous factors and many hypotheses. Among them, the dominance hypothesis, the dominant hypothesis, and the epistasis hypothesis have been widely accepted and underlie heterozygous research (Shasidhar et al.,2020). The heterogeneity of plants is not fully and logically explained by any of these hypotheses or views, no matter how different they are. Thus, SSR molecular markers and quantitative trait locus mapping (QTL) are increasingly becoming a standard tool for testing the genetic basis in breeds due to Yu et la 2017

Gene total phenolics content(TPC) liên kết với RM24616 trên nhiễm sắc thể số 9 summarized QTL's effect on heterozygosity based on 35 studies and found that dominance and epistasis were equally proportional in these studies, suggesting that QTL mapping results varied between species and even within different groups of the same specimen. Therefore, SSR and QTL markings are not sufficient to comprehensively explain the heteromorphism. Genes associated with traits established with TPC on chromosome 9 are recorded on rice varieties Genes associated with traits established with TPC on chromosome 9 are recorded on rice varieties (Krishnendu et al.,2022) Total phenolics content (TPC) gene binds RM24616 on chromosome 9

A



B



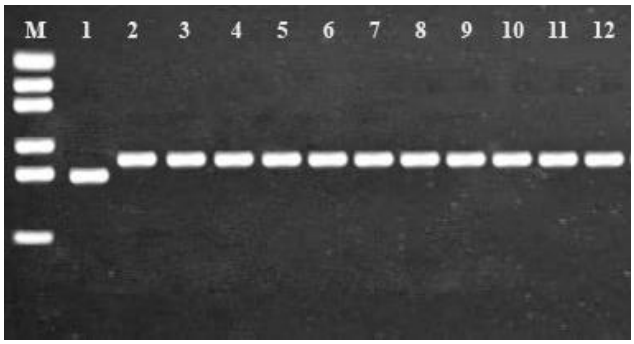


Fig.2: The PCR producted of RM24616 (chromsome 9)

Total flavonoid content (**TFC) with RM 17115 on chromosome 4**

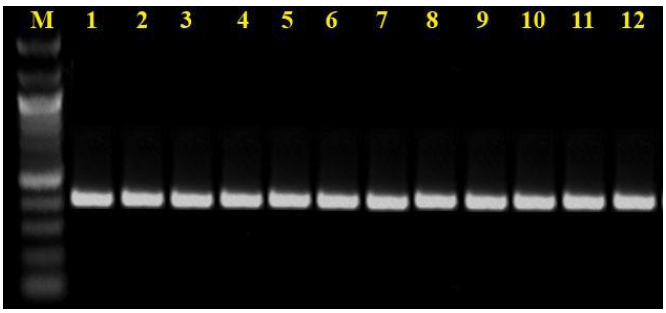


Fig.3: The PCR producted of RM 17115 on chromosome 4

Total anthocyanin conten(TAC) linked with RM285 omn chromosome 9 and RM28828 on chromosome 12 (figure 4)

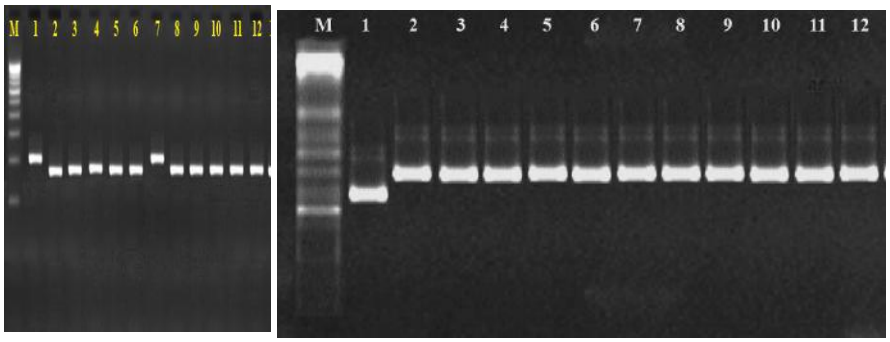


Fig.4 The PCR producted of RM 285 (chromsome 9) and RM28828 on Chromosome 12 on agarose gel.

Comprehensive RNA-Seq analysis of rice varieties and can identify many genes involved in biosynthesis The results of RNA-Seq analysis have been confirmed using qRT-PCR for several genes. Our results help explain the accumulation of secondary metabolites. The transcription data reported here will facilitate future studies of the molecular mechanisms of polysaccharide biosynthesis and provide new insights into rice plants Comparison of RNA-sequencing code sequences of 10 rice varieties: DNA sequences of 10 cladogram rice varieties (Figure 5), And they will be described the similarity between joinings, to show a large number of different nucleotides. The difference in the number of nucleotide sequences between varieties is shown in Figure 5

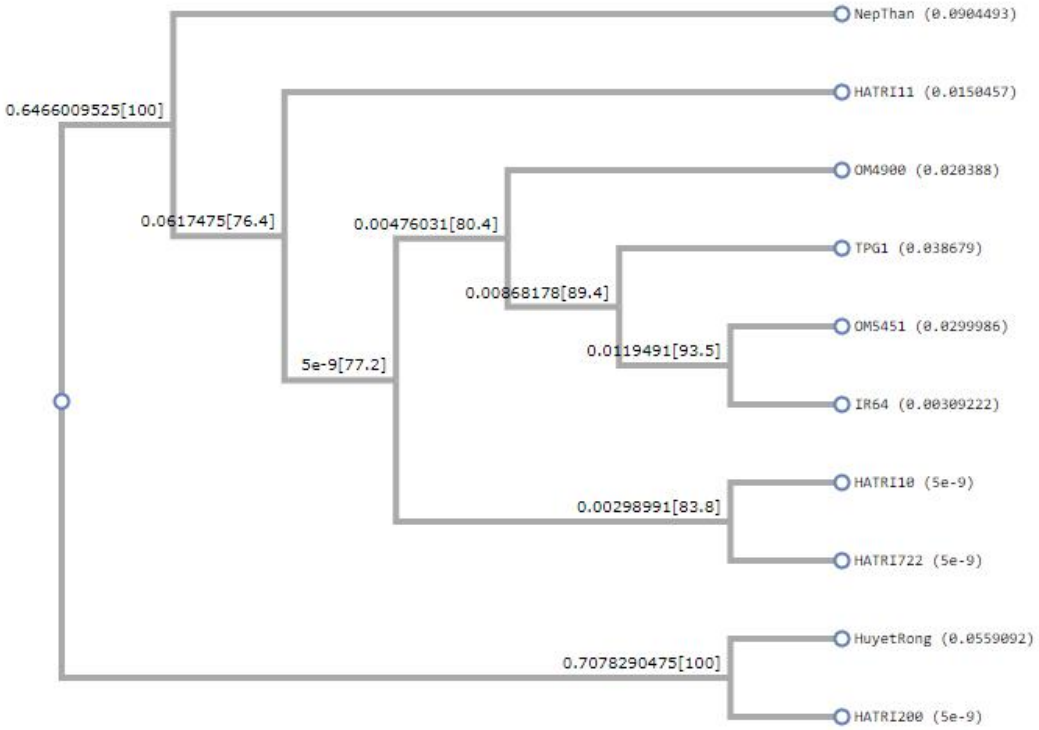


Fig.5: Phylogenetic plants based on TPC sequence using analysis of 10 different rice varieties

IV. CONCLUSIONS

Looking at a series of analytical results, the determination of polyphenols in rice extract aims to determine the quantitative profile of the quality of rice grains. Significant strides have been made in elucidating the chemical structure of these bioactive compounds but while mass spectrometry based techniques certainly represent a powerful tool for defining brown rice phenolic profiles, white rice. We strongly believe that the research efforts undertaken to date constitute an excellent starting point towards the development of analytical tools aimed at investigating the phenolic fraction of rice for demand in medicinal chemistry.

V. FUTURE PROSPECTS

The use of phenolic compounds and flavonoids is a potential candidate of bioactive agents in the field of pharmaceuticals and pharmaceuticals to enhance human health, prevent and cure various diseases. In order to explore and conduct alternatives using plant chemical compounds, medicinal plant surveys along with robust profile research need to be undertaken. The targeted compounds should be used in biomedical and pharmaceutical research ranging from in vitro, in vivo and step clinical trials to evaluating the safety, efficacy and also side effects of the candidate compounds tested.

1. Consequently, the flavonoid and phenolic compounds which are abundant found in a large number of rice and other plants may possible be an interesting choice of molecules for drug and medical product development.
2. In the same species of medicinal rice , the different cultivars may provide different amount of flavonoid and phenolic compounds as well as the biological activities. Thus, the cultivars of medicinal plant should be taken into account for

the future medical and pharmaceutical research studies.

3. The geographic areas of raw plant material should also be analyzed and compared in the future research. Since the environmental factors e.g., nutrients and mineral in soil are also effect on the quality and quantity of phytochemical compounds in some species of medicinal plant and rice.
4. The molecular mechanism and signaling pathway of many known flavonoid and phenolic compounds are need to be done in the future, so as to apply this knowledge to the drug development processes.
5. The need of purified compounds to confirm data obtained with the plant extracts.

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Effects of Leaf Reserved and clipped on Axillary Bud Quality in Umbrella-Shaped *Hevea brasiliensis* 'Reken 628' Bud-sticks

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Abstract—*The insufficient supply of bud-sticks from traditional single-stem rubber trees (Hevea brasiliensis) remains a bottleneck for mini-seedling budding in commercial rubber plantations. To address this issue, we investigated the impact of leaf reserved versus leaf clipped on axillary bud development in umbrella-shaped 'Reken 628' rubber trees. After removing apical dominance by topping, the plants developed multiple branches, yielding 4–6 times more bud-sticks than conventional methods. Bud-sticks were harvested when petioles detached naturally and leaf scars turned brown. Axillary buds (scale buds and petiole buds) from the second (2nd) and third (3rd) leaf whorls were analyzed for quantity, moisture content, and morphological traits (bud scar dimensions, bud eye size). Leaf reserved 2nd buds exhibited superior quality, with significantly higher moisture content (9.40–10.69%), larger bud scar width (43.21%), and thicker bud scars (19.69%) compared to their clipped counterparts ($P < 0.05$). Leaf-clipped reduced physiological consistency, increasing variability in leaf length (CV: 21.40–25.86%), leaf width (20.79–23.36%), and stem moisture (5.89%). Correlation analysis revealed strong synergies between leaves reserved, stem thickness, and bud moisture, critical for grafting success. We conclude that reserved leaves on 2nd whorls of umbrella-shaped trees optimizes bud-sticks quality for mini-seedling budding. Post-topping management should prioritize frequent irrigation and balanced fertilization to sustain nutrient supply. This strategy enhances bud-sticks yield, grafting efficiency, and survival rates, offering a scalable solution for high-demand rubber nurseries.*

Keywords—***Hevea brasiliensis, umbrella – shaped bud sticks, leaf reserved, axillary bud, quality.***

I. INTRODUCTION

In recent years, the persistently low price of natural rubber and its suboptimal economic returns have significantly diminished the willingness of rubber farmers and cultivation entities to invest in new rubber saplings. This has precipitated a sharp decline in demand for rubber seedlings, thereby disrupting the sales dynamics of rubber nursery stocks. Concurrently, reduced market demand has further eroded the enthusiasm of nursery production units to cultivate rubber seedlings. As a critical agricultural commodity and strategic resource, natural rubber production in China faces constraints from resource limitations and socioeconomic development. Notably, the national self-sufficiency rate of natural rubber has steadily declined from approximately 50% in the 1990s to 13.7% by 2020, with a sustained deficit below 20% over seven consecutive years, positioning China as a major importer of natural rubber [1]. This insufficient self-sufficiency poses strategic security risks, prompting governmental

interventions. The 2018 No.1 Central Document mandated the establishment of a 12-million-acre natural rubber production protection zone across Hainan, Yunnan, and Guangdong provinces to safeguard domestic supply. Subsequent policies, including the 14th Five-Year Plan for Natural Rubber Production Capacity Development (2021) and the 2023 No.1 Central Document, reinforced support mechanisms. In December 2023, the Comprehensive Insurance Policy for Natural Rubber was jointly issued to stimulate production incentives. The 2024 agricultural policy further emphasized industrial consolidation through intelligent harvesting technologies, aging plantation renewal, and specialized rubber garden development, driving nationwide standardization of 58,000 acres of specialized plantations to enhance production efficiency.

The surging demand for rubber seedlings has exposed critical bottlenecks in conventional propagation methods. Natural rubber cultivation remains a vital economic pillar for tropical regions like Hainan, Yunnan, and Guangdong, sustaining millions of livelihoods [4]. However, traditional nursery practices—characterized by extended production cycles (≥18 months for rootstock-to-grafted seedling development), escalating land use costs, and labor-intensive operations—urgently require modernization to improve efficiency and reduce costs.

Mini-seedling budding technology has emerged as a pivotal industrial propagation method [5–6]. This technique employs nutrient-rich rubber seeds for early-stage indoor grafting, enabling bud union on juvenile rootstocks prior to full leaf expansion. Compared to conventional approaches, it offers multiple advantages: shortened cultivation cycles (miniaturized seedlings), reduced labor inputs, higher spatial efficiency, enhanced root system development, and improved post-transplantation survival rates with earlier tapping maturity [7–9]. Consequently, it has gained widespread adoption in Yunnan and Guangdong [10–12]. Nevertheless, Hainan's rubber industry faces mounting pressures from stagnant rubber prices, rising labor costs, and infrastructure demands from the Hainan Free Trade Port initiative. Technical challenges persist, including inconsistent survival rates due to variable rootstock/scion quality and environmental factors, which escalate production costs through wasted materials and additional management labor. Furthermore, suboptimal post-grafting care often yields inferior seedlings, diminishing market acceptance.

Current bud-sticks production methods exhibit limitations: (1) Repeated pruning of field-grown plants yields green bud-sticks with dimensional incompatibility to rootstocks [13]; (2) Micro-propagation via anther culture incurs high technical and capital costs [14]; (3) Shoot tipping of field plants produces limited quantities of juvenile bud-sticks [15]; (4) Root-restricted greenhouse cultivation achieves small-stem bud-sticks at elevated costs [16]. Empirical evidence suggests defoliation enhances budding success in rubber trees [7–8], though analogous practices in other tree species may induce stress responses [17–18]. Existing studies have investigated external factors influencing grafted seedling growth [19–21], bud-stick selection criteria [22–23], and apical dominance disruption for multi-branch bud-sticks production [24-25]. However, systematic analyses of pre-grafting axillary bud quality—a critical determinant of success—remain lacking.

To address these gaps, this study innovates a protocol for efficiently producing small-stem defoliated bud-sticks compatible with mini-seedling grafting. Using *Hevea brasiliensis* clone 'Reken 628' as propagation material, we developed umbrella-shaped bud-sticks by tipping single-stem plants at four-whorl maturity to induce stable three-whorl lateral branches. Defoliation was performed on secondary and third whorl petiole buds, followed by bud-stick collection after petiole abscission and scar lignification. Comprehensive metrics—including bud counts, moisture content, scar dimensions (length, width, thickness), and bud eye morphology were quantified and compared to non-defoliated controls. This investigation elucidates the relationship between defoliation practices and axillary bud quality, aiming to optimize mini-seedling budding productivity, survival rates, and economic viability while providing theoretical and practical guidance for industrial propagation.

II. MATERIAL AND METHODS

2.1 Plant materials and experimental site

The experiment utilized *Hevea brasiliensis* clone 'Reken 628', planted in December 2023 at the Rubber Research Institute Nursery Base (109°29′62″E, 19°30′12″N; elevation: 116.9 m) of the Chinese Academy of Tropical Agricultural Sciences in Danzhou, Hainan Province, China.

2.2 Treatments and growth management

In June 2024, single-stem shoots with four stabilized leaf whorls and actively elongating apical buds underwent manual apical dominance removal (de-topping). By September 2024, umbrella-shaped shoots with three stabilized leaf whorls had developed from the de-topped positions.

2.3 Physiological measurements

In August 2024, phenological stages of the top whorl leaves on umbrella-shaped 'Reken 628' shoots were monitored. Leaf length and width were measured using a transparent ruler. Chlorophyll content, nitrogen

concentration, leaf surface humidity, and temperature were quantified with a SPAD meter (Jinkelida TYS-4N). Plant height and stem diameter were recorded using a measuring tape (1 mm precision) and digital vernier caliper (0.01 mm precision), respectively.

2.4 Experimental design and sampling

Six treatments were established in a randomized complete block design with three replicates per treatment. Upon stabilization of the top whorl leaves, defoliation treatments were applied: leaves on petiole buds of the second and third whorls—excluding densely noded buds—were excised. After petiole scars transitioned from green to brown, stems from 2nd and 3rd positions were harvested. Leaf blades, petiole buds, and scale buds from these positions were collected separately.

2.5 Biometric and hydration analyses

Fresh weights of leaves, buds, and stems were recorded using an analytical balance (0.01 g). Leaf dimensions were measured with a ruler (1 mm), while bud scar dimensions (length, width, thickness) and bud eye morphology (length, width) were quantified via digital vernier caliper (0.01 mm). Stem diameter and length were assessed using calipers and a tape measure, respectively. Following fresh weight measurements, samples were oven-dried to constant weight at 65°C for dry mass determination and water content calculation.

2.6 Statistical analysis

Data were processed using DPS software (v20.05 Advanced Edition) for Student's t-tests (two-sample comparisons) and one-way ANOVA with Duncan's multiple range test ($\alpha = 0.05$). Graphical representations were generated using GraphPad Prism (v8.3.0), and correlation analyses were performed via the Tutools Platform (http://www.cloudtutu.com).

III. RESULT AND DISCUSSION

3.1 Plant growth performance (leaf length, leaf width, leaf number, leaf water content, plant height, stem diameter, stem water content)

3.1.1 umbrella-shaped leaf- reserved 2nd and 3rd leaf whorls

In the leaf- reserved umbrella-shaped treatment, the 2nd leaf whorl exhibited significantly greater leaf width (Fig. 1B, +9.32%, $P < 0.05$), leaves (Fig. 1C, +18.92%, $P < 0.05$), leaf moisture (Fig. 1D, +6.30%, $P < 0.05$), and stem moisture (Fig. 1E, +8.33%, $P < 0.05$) compared to the 3rd leaf whorl. Conversely, the 2nd whorl showed significantly reduced plant height (Fig. 1F, −34.83%, $P < 0.05$) and stem diameter (Fig. 1G, −2.36%, $P < 0.05$). No significant differences were observed in other parameters. These results indicate that the 2nd leaf whorl outperformed the 3rd in leaf width, leaf number, and water retention, while the 3rd whorl exhibited superior vertical growth (plant height) and stem thickening.

3.1.2 Umbrella-shaped leaf clipped 2nd and 3rd leaf whorls

Under clipped treatment, the 2nd leaf whorl displayed significantly greater leaf length (Fig. 1A, +6.56%, $P < 0.05$), leaf width (Fig. 1B, +9.60%, $P < 0.05$), and stem moisture (Fig. 1E, +3.78%, $P < 0.05$) compared to the 3rd whorl. However, the 2nd whorl exhibited a significantly smaller stem diameter (Fig. 1G, −19.75%, $P < 0.05$). Other parameters showed no significant differences. This suggests that leaf-clipped treatments amplified physiological advantages in the 2nd whorl for leaf expansion and water retention but compromised stem thickening.

3.1.3 Comparative Analysis: leaf- reserved vs. clipped 2nd and 3rd leaf whorls

Leaf-reserved 2nd whorls exhibited significantly higher leaf length (Fig. 2A, +11.29%, $P < 0.05$), leaf width (Fig. 2B, +12.25%, $P < 0.05$), leaves (Fig. 2C, +36.22%, $P < 0.05$), leaf moisture (Fig. 2D, +15.54%, $P < 0.05$), and stem moisture (Fig. 2E, +13.12%, $P < 0.05$) compared to clipped counterparts. Similarly, leaf-reserved 3rd whorls showed superior performance in leaf length (+15.64%, $P < 0.05$), leaf width (+12.52%, $P < 0.05$), leaves (+39.34%, $P < 0.05$), leaf moisture (+10.96%, $P < 0.05$), plant height (Fig. 2F, +28.26%, $P < 0.05$), and stem moisture (+8.81%, $P < 0.05$). These findings collectively demonstrate that leaf-reserved treatments consistently outperformed clipped treatments across key growth metrics, highlighting the critical role of intact foliage in maintaining physiological stability and growth vigor.

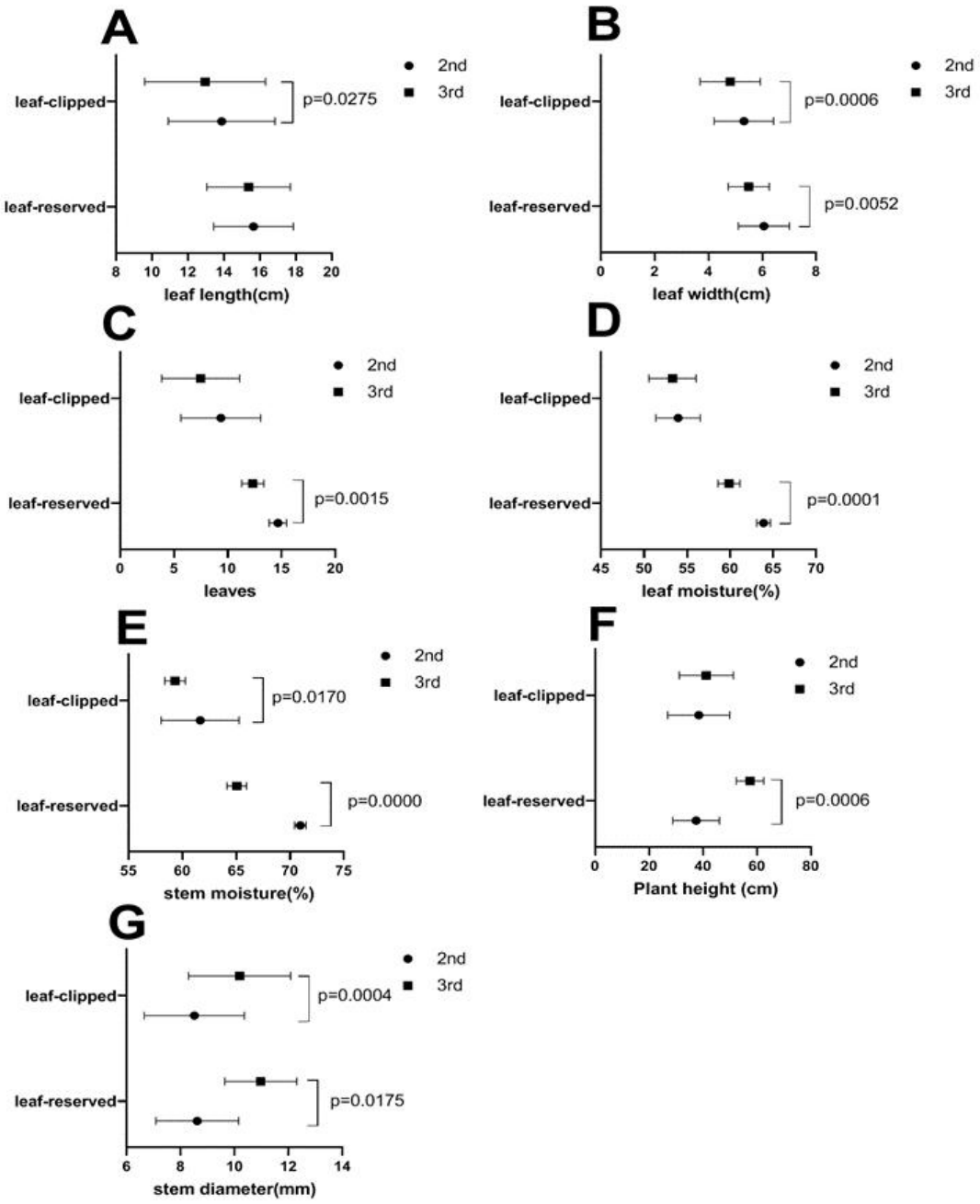

Fig. 1. Comparison of Leaf length, leaf width, leaves, leaf moisture, stem moisture, plant height, and stem diameter between 2nd and 3rd leaf whorl

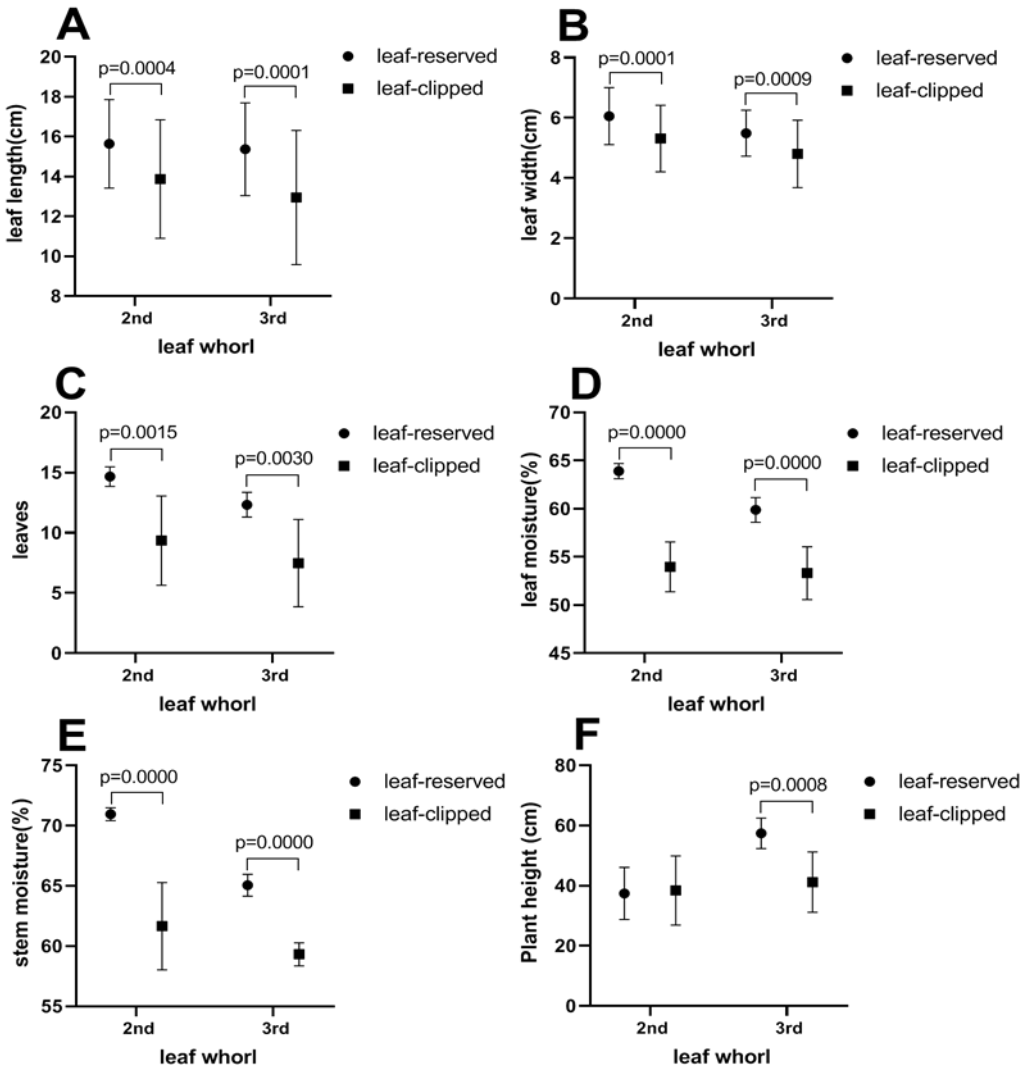

Fig. 2. Comparison of leaf length, leaf width, leaves, leaf moisture, stem moisture, plant height between leaf reserved and clipped treatment in the same leaf whorl

3.2 Axillary bud quality

3.2.1 Comparison of petiole and scale bud numbers between leaf -reserved and clipped treatments

In leaf- reserved plants, the 2nd leaf whorl produced significantly fewer petiole buds (Fig. 3A, −31.11%, $P < 0.05$) but more scale buds (Fig. 3B, +27.59%, $P < 0.05$) compared to the 3rd whorl. Notably, non-defoliated 2nd whorls exhibited 15.17% more scale buds than clipped 2nd whorls (Fig. 3C, $P < 0.05$). These trends suggest a whorl-specific trade-off between petiole and scale bud development, with defoliation preferentially suppressing scale bud proliferation.

3.2.2 Axillary bud quality (scar dimensions, bud eye morphology, moisture)

3.2.2.1 Leaf reserved treatment: intra-plant variation between 2nd and 3rd leaf whorls

For scale buds (Fig. 4), the 2nd whorl exhibited significantly larger scar width (Fig. 5A, +43.21%, $P < 0.05$), scar thickness (Fig. 5B, +19.69%, $P < 0.05$), and bud eye width (Fig. 5C, +20.19%, $P < 0.05$) but shorter scar length (Fig. 5D, −24.99%, $P < 0.05$) compared to the 3rd whorl. For petiole buds, the 2nd whorl demonstrated reduced scar length (Fig. 5a, −23.61%, $P < 0.05$) and width (Fig. 5b, −31.85%, $P < 0.05$) but greater scar thickness (Fig. 5c, +11.89%, $P < 0.05$) and bud eye length (Fig. 5d, +24.47%, $P < 0.05$). These contrasting patterns underscore the complexity of bud quality assessment, as no single morphological parameter reliably predicts overall bud viability.

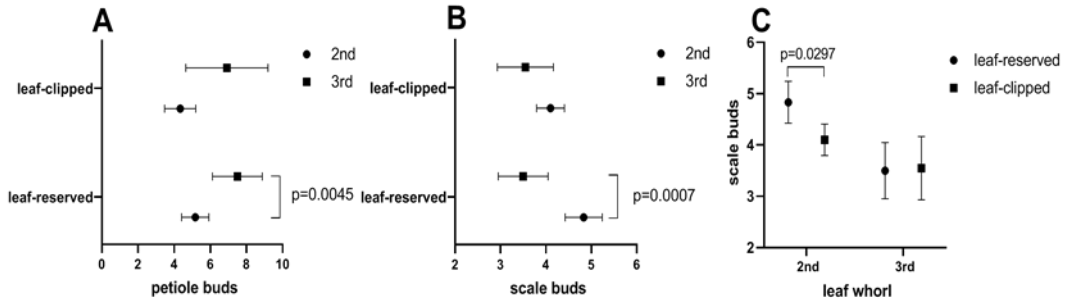

Fig. 3. Comparison of the number in petiole buds and scale buds between the 2nd and 3rd leaf whorl, and scale buds between leaf reserved and clipped treatment in the same leaf whorl

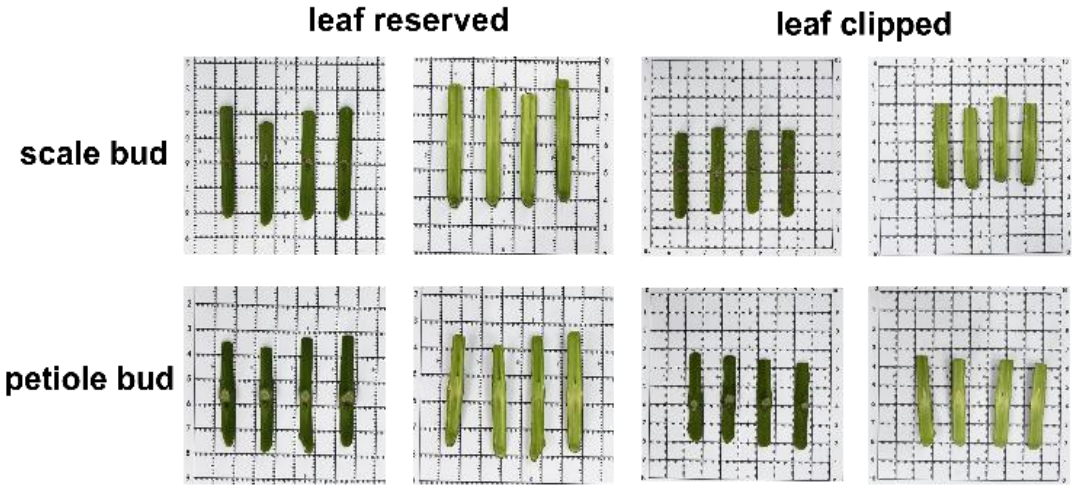

Fig. 4. Positive and negative side view of petiole bud and scale bud on 2nd leaf whorl

3.2.2.2 Comparison of umbrella-shaped leaf- clipped among different leaf whorls of the same plant

In the umbrella-shaped leaf clipping treatment, the bud scale scar width (Fig. 5A) and thickness (Fig. 5B) of scale buds in the 2nd leaf whorl (2nd) were significantly larger than those in the 3rd leaf whorl (3rd) by 24.14% ($P < 0.05$) and 14.27% ($P < 0.05$), respectively. Conversely, the bud eye width (Fig. 5C), scar length (Fig. 5D), and eye length (Fig. 5E) of scale buds in 2nd were significantly smaller than those in 3rd by 6.40% ($P < 0.05$), 32.21% ($P < 0.05$), and 12.06% ($P < 0.05$), respectively. For petiole buds, 2nd exhibited significantly greater bud scar width (Fig. 5b), scar thickness (Fig. 5c), eye length (Fig. 5d), and eye width (Fig. 5e) compared to 3rd, with increases of 15.94% ($P < 0.05$), 25.98% ($P < 0.05$), 8.12% ($P < 0.05$), and 14.21% ($P < 0.05$), respectively, while other parameters showed no significant differences.

These results indicate that scale buds in 2nd displayed larger scar dimensions (width and thickness) but smaller scar length and eye dimensions compared to 3rd. In contrast, petiole buds in 2nd consistently outperformed 3rd in scar width, thickness, and eye dimensions, suggesting superior quality of petiole buds in 2nd after leaf clipping.

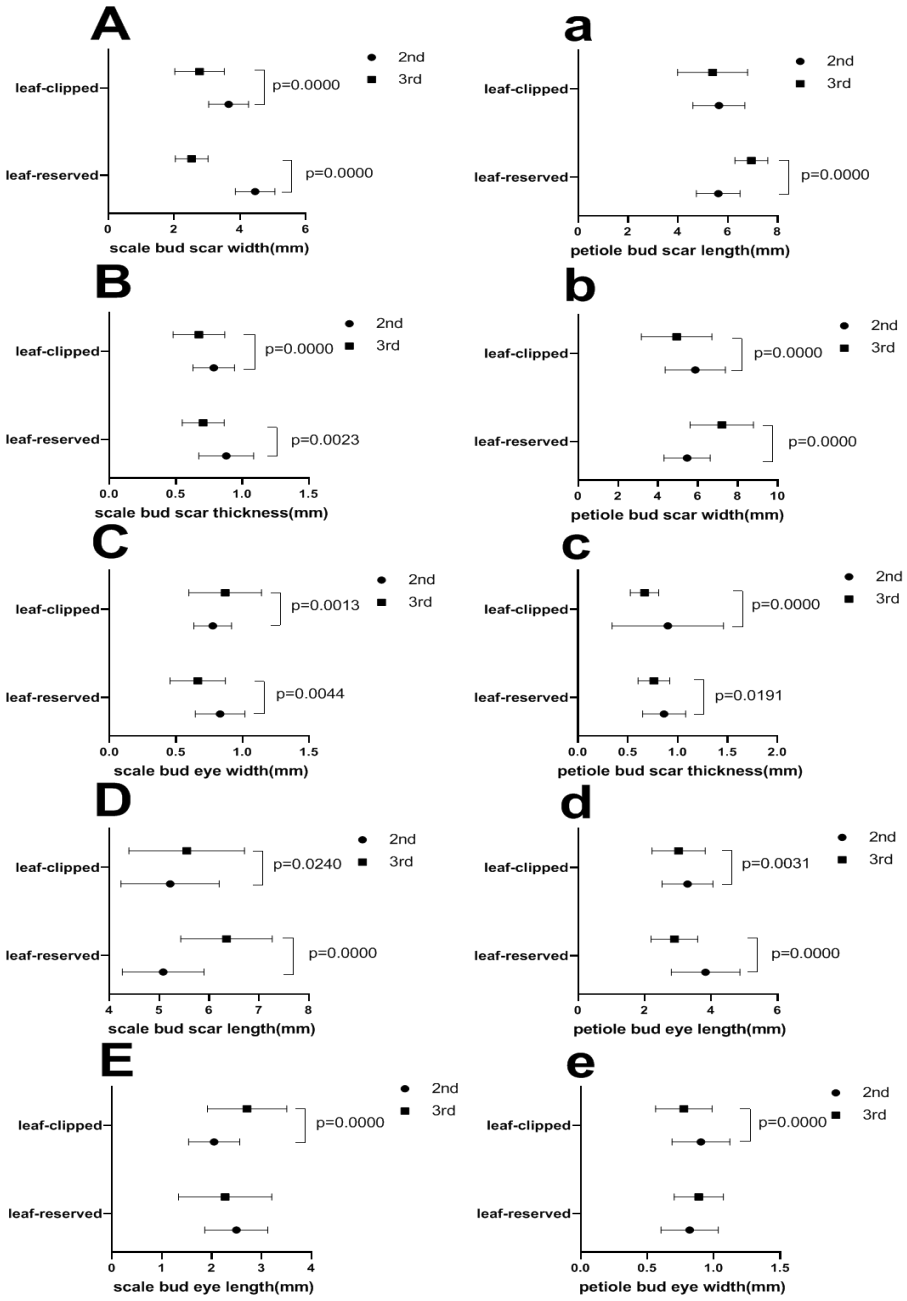

Fig. 5. Comparison of scar dimensions, bud eye morphology between the 2nd and 3rd leaf whorl

3.2.2.3 Comparison of umbrella-shaped leaf-reserved vs. leaf-clipped within the same leaf whorl

For scale buds in 3rd, the scar length of reserved leaves (Fig. 6A) was 12.55% larger than that of clipped leaves ($P < 0.05$). In 2nd, reserved leaves exhibited 18.03%, 10.63%, and 17.80% greater scar width (Fig. 6B), thickness (Fig. 6C), and eye length (Fig. 6D), respectively, compared to clipped leaves ($P < 0.05$). Conversely, the eye length and width of reserved 3rd scale buds (Fig. 6E) were 19.41% and 31.06% smaller than those of clipped 3rd ($P < 0.05$). For petiole buds, reserved 3rd showed 22.38%, 31.47%, and 12.34% increases in scar length (Fig. 6a), width (Fig. 6b), and thickness (Fig. 6c) compared to clipped 3rd ($P < 0.05$), while reserved 2nd exhibited a 14.16% increase in eye length (Fig. 6d) relative to clipped 2nd ($P < 0.05$).

Leaf- reserved generally enhanced morphological development in petiole buds. Reserved 2nd demonstrated larger scar width, thickness, and eye length in scale buds, while reserved 3rd showed longer scars but smaller eyes compared to clipped counterparts.

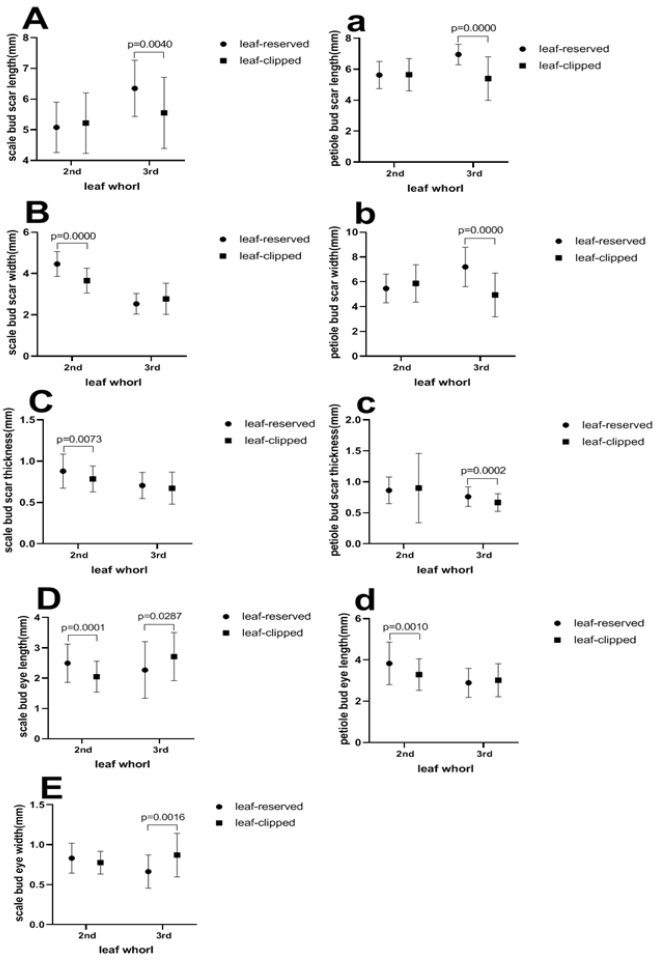

Fig. 6. Comparison of scar dimensions, bud eye morphology within the same leaf whorl

3.2.2.4 Moisture content comparison between leaf-reserved and leaf-clipped treatments

The moisture content of scale buds in reserved 2nd (Fig. 7A) and 3rd was 9.40% and 8.28% higher, respectively, than in clipped treatments ($P < 0.05$). Similarly, petiole bud moisture content in reserved 2nd (Fig. 7B) and 3rd exceeded clipped treatments by 10.69% and 8.55% ($P < 0.05$). These findings confirm that leaf-reserved positively maintains moisture levels in both bud types.

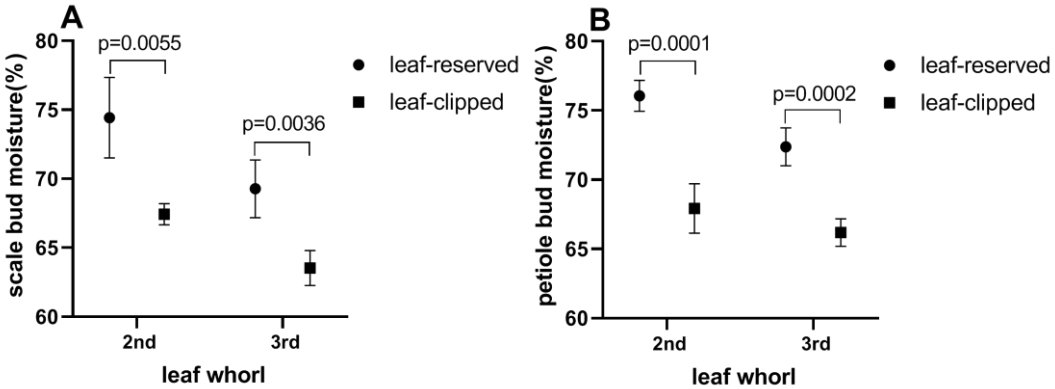

Fig. 7. Moisture content comparison between leaf-reserved and leaf-clipped treatments

3.3 Coefficient of variation analysis

Leaf clipping increased CV values for leaf length, width, number, moisture content, and stem moisture in both whorls (2nd: 21.40%, 20.79%, 39.67%, 4.80%, 5.89%; 3rd: 25.86%, 20.79%, 48.39%, 5.14%, 24.41%), indicating reduced uniformity. Reserved 2nd exhibited lower variability in petiole bud number (CV = 14.57%) and scale bud number (CV = 8.45%) compared to 3rd (18.38% and 15.65%, respectively). Moisture-related CVs for reserved treatments (scale buds: 1.13–3.92%; petiole buds: 1.46–2.62%) were consistently lower than clipped treatments. Morphological CVs for buds (scar/eye dimensions) ranged from 9.47% to 62.08%, with clipped treatments generally showing higher variability.

Table 1. Coefficient of variation (%) between all parameters of umbrella shaped under leaf reserved and clipped treatment

parameter		**reserved leaves**		parameter		**clipped leaves**	
		2nd	3rd			2nd	3rd
growth index	leaf length	14.21	15.12	growth index	leaf length	21.40	26.00
	leaf width	15.62	13.92		leaf width	20.79	23.36
	plant height	23.18	8.84		plant height	29.92	24.41
	stem diameter	17.79	12.17		stem diameter	21.82	18.58
number	leaves	5.57	8.37	number	leaves	39.67	48.39
	scale bud	8.45	15.65		scale bud	7.47	17.38
	petiole bud	14.57	18.38		petiole bud	19.88	32.88
moisture	leaf	1.24	2.13	moisture	leaf	4.80	5.14
	stem	0.75	1.39		stem	5.89	1.62
	scale bud	3.92	3.01		scale bud	1.13	2.00
	petiole bud	1.46	1.89		petiole bud	2.62	1.50
scale bud	bud scar length	16.16	14.45	scale bud	bud scar length	18.90	20.87
	bud scar width	13.46	19.67		bud scar width	16.58	27.20
	bud scar thickness	23.44	22.39		bud scar thickness	19.89	28.83
	bud eye length	25.31	41.25		bud eye length	24.92	29.31
	bud eye width	22.45	31.39		bud eye width	18.24	31.41
petiole bud	bud scar length	15.61	9.47	petiole bud	bud scar length	18.45	25.99
	bud scar width	21.20	22.03		bud scar width	25.68	35.80
	bud scar thickness	24.94	20.76		bud scar thickness	62.08	21.33
	bud eye length	26.91	24.23		bud eye length	23.27	26.44
	bud eye width	26.21	20.82		bud eye width	24.09	27.43

3.4 Correlation analysis

As shown in Figure 8, strong positive correlations ($p < 0.01$) were observed between leaf number and petiole bud moisture, stem diameter and scale bud moisture, and scale bud number with petiole eye length. Significant positive correlations ($p < 0.05$) included leaf width with scale bud moisture and multiple morphological parameters between bud types. Negative correlations emerged between scale eye width and petiole scar width ($p < 0.01$), as well as scale eye length and petiole eye width ($p < 0.05$), suggesting coordinated growth regulation.

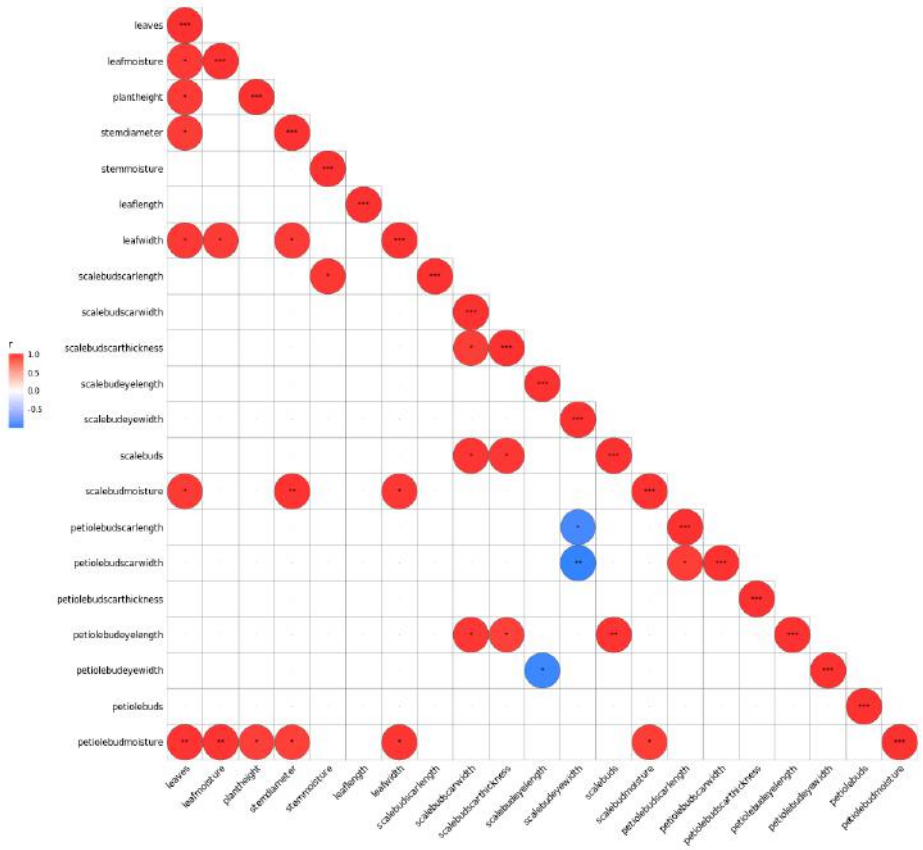

Fig. 8. Correlation analysis of all growth indexes observed

3.5 Comprehensive analysis

Using Technique for Order Preference by Similarity to Ideal Solution (TOPSIS) with stem diameter and plant height as low-priority indicators, the ranking of treatments was: reserved 2nd > reserved 3rd > clipped 2nd > clipped 3rd. Reserved 2nd demonstrated optimal bud quality, supported by superior physiological metrics (leaf number, stem thickness, moisture content).

Table 2. Comprehensive analysis based on growth index

Leaf whorl - leaf status	Statistic CI	Rank
2nd-leaf reserved	0.6773	1
3rd -leaf reserved	0.4963	2
2nd- leaf clipped	0.4197	3
3rd-leaf clipped	0.3711	4

CI, approximation to the Optimal Vectors.

IV. CONCLUSION

In *Hevea brasiliensis* clone Reken 628, leaf-reserved buds from the 2nd leaf whorl (2nd) exhibited the highest quality for seedling grafting. Leaf-reserved promotes moisture retention and morphological development in both scale and petiole buds, while the clipped increases phenotypic variability. For practical propagation, apical pruning without leaf removal is recommended to enhance branching. Priority should be given to 2nd-derived buds due to their stability and positive correlations with key growth parameters (leaf number, stem thickness, plant height), which collectively improve grafting success rates.

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Genome-Wide Identification and Characterization of Superoxide Dismutase (*SOD*) Gene family in Finger Millet (*Eleusine coracana*)

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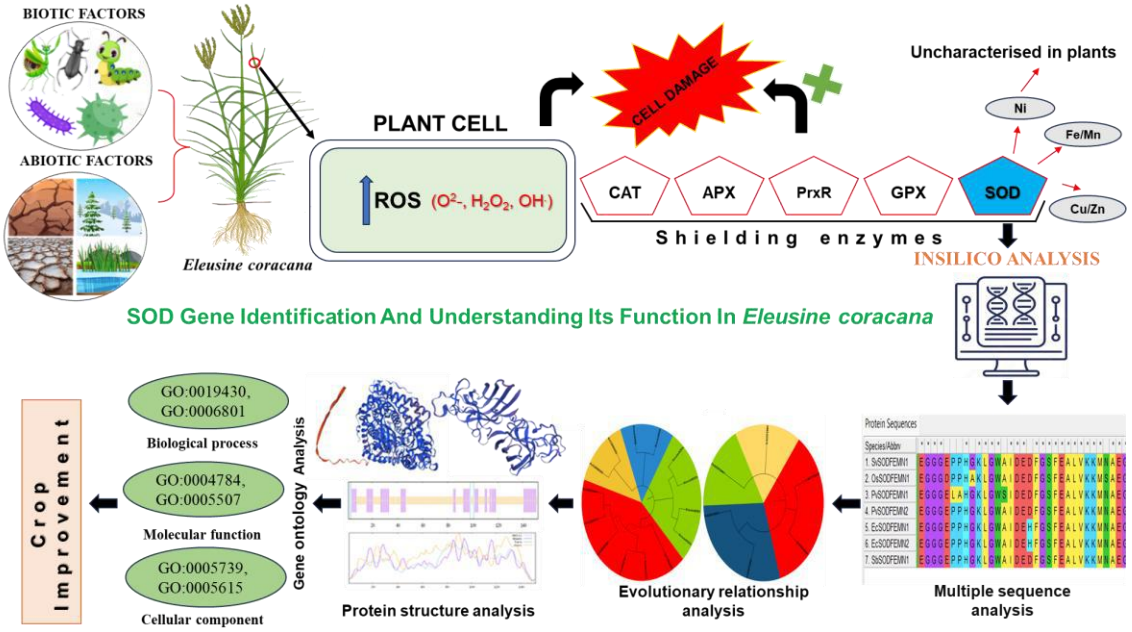
Abstract— *The metallo-enzyme superoxide dismutases (SODs) are significant in protecting plants from environmental challenges as well as regulating their growth and development. Although many plants have been determined to possess SOD gene families in their genomes, it is known, very scarcely, about such gene families in finger millet (Eleusine coracana). This study explored the SOD gene family across the entire genome of finger millet. A total of 10 SOD genes were discovered, comprising eight Cu/ZnSODs and two Fe/MnSOD. These EcSODs are spread irregularly throughout 3A and 8B chromosomes. Phylogenetic analysis revealed that SOD proteins in plants possibly classified into three primary groups for both EcCu/ZnSODs and EcFe-MnSODs. The motif and exon/intron makeup of SOD genes are conserved within the same subgroup. Protein structure prediction showed all homologs contains highest similarity with SOD peptide structure. Furthermore, numerous cis-elements that react to distinct stresses were distributed differently. The various biological processes associated with background molecular roles of SODs are further demonstrated by gene ontology analysis. The transcriptional factors discovered indicate that SODs are mostly connected to external environmental and biotic stress. This study lays the groundwork for future cloning, Genetic manipulation of SOD gene in finger millet which contributes towards finger millet breeding programs.*

Keywords— ***Finger millet (Eleusine coracana), Superoxide dismutase (SOD), ROS, Abiotic and Biotic stress.***

GRAPHICAL ABSTRACT



I. INTRODUCTION

Millets were among the initial small-seeded nutri-cereals annual grass species to undergo domestication. Their exceptional nutritional value with climate-resilience properties enables them to serve as a traditional food source in many countries like Africa and Asia. These staple grains are a part of daily routine in most of the developed and under developed countries and consumed by more than 590 million people. As more people seeking healthy diet options, the global market of millet is projected to grow, potentially reaching $12 billion by 2025 (1). India contributes 11.42 (37%) million tons of the 30.73 million tons of millets produced worldwide currently and sustaining its place as the top producer (2). Originating in the highlands of Ethiopia, finger millet is now extensively cultivated in over 25 nations (3). *Eleusine coracana L. Gaertn.* is regarded as an orphan cereal (4). which is used for food as well as fodder. It is also known as hardy crop due to its widespread farming in barren and harsh environments as well as in poor, dry, and less fertile soils. Thus, finger millet serves as a boon in areas of extreme poverty (5,6,7). Finger millet is said to be more nutritious than wheat, rice, and maize because its grains are high in dietary fiber, proteins, minerals, vital amino acids, vitamin B complex, iron, and calcium and many more, (5). It is free from gluten and provides an added benefit for those with digestive problems. It also offers a wide range of health benefits, which includes anti-diabetic (type 2 diabetes mellitus), anti-diarrheal, antibiotics, anti-allergic, atherosclerogenic, anti-ulcer, antitumorigenic (for K562 chronic myeloid leukemia), and antioxidant qualities (8).

Finger millet is constantly subjected to environmental stresses which hampers its yield. Abiotic stress includes heat, cold, drought, salt, metal stress, ozone, UV radiation, and nutrient deficiencies (9,10). Biotic stress includes following diseases- blast, foot rot, and pests like pink stem borer and root aphid (11). An especially destructive disease that significantly reduces finger millet output is *Magnaporthe grisea* (anamorphic stage: *Pyricularia grisea*), an ascomycete filamentous fungus that causes finger millet blasts (12). Blast causes yield losses ranging from 7.32% to 90% in finger millet depends on which tissue it occurs (13). A yield loss of 28% to 36% has been observed in India (14), and up to 80% elsewhere (15). To ensure global food security, there is high need to improve the genetic resources of finger millet. The most efficient, environmentally friendly, sustainable, and farmer-beneficial approaches to handle this finger millet yield loss is by using genetic manipulation and genomic improvement techniques based on deep study on interactions between plant and stress.

Whenever a plant encounters a stress, excessive accumulation of Reactive oxygen species (ROS), byproducts of cell metabolism and specialized with oxidizing properties which results in release of hydroxyl radical (OH), and superoxide (O^{2-}), along with hydrogen peroxide (H_2O_2). When ROS levels are appropriate, organisms require it. It can influence several plant physiological processes in various species by acting as a signalling molecule (16). Overproduction of ROS can cause membrane lesions, metabolic disruption, and even cell death. It can also harm biological macromolecules lipids,

proteins, and nucleic acids (17). Plants have evolved effective defense mechanisms against ROS induced damage in order to combat its toxicity by means of elimination of surplus of ROS. Some shielding enzymes, including superoxide dismutase (SOD), peroxiredoxin (PrxR), catalase (CAT), glutathione peroxidase (GPX), and ascorbate peroxidase (APX), can keep the dynamic equilibrium of ROS levels in plants (18). Among the mentioned enzymes, *SODs* are mentioned as the primary enzyme involved in plant defense system and have the potential to mitigate ROS-induced damage by catalysing the breakdown of superoxide radicals into H_2O_2 and O_2 (19). They mitigate the risks associated with ROS by forming molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) from superoxide (O_2^-) in a oxidative stress (20). As metalloenzymes, *SODs'* peptides need metal cofactors in order to function as catalysts. Plant *SODs* are divided into three classes based on their metal cofactor which are iron *SODs (FeSODs)*, manganese *SODs (MnSODs)*, and copper/zinc *SODs (Cu/ZnSODs)* (21). Nickel superoxide dismutase (*NiSOD*), another class of *SOD*, has not been found in any plant (22).

Verma D et al, 2019 stated that *SOD* gene serves its critical functions in plants by responding to biotic and abiotic stress. Certain research has looked at sea-grasses unique reaction to oxidative stress and the responses of *SOD* in hot conditions (24). It has been demonstrated that the *ZmMnSOD* enzyme is crucial in reducing oxidative damage response during temperature stress (25). In recent study by Madanala et al. (2011) (26) it was found that *Withania somnifera* plant species contains a highly stable *Cu/Zn-SOD* gene in their chloroplast and this gene shows a greater resistance to ethanol and detergent (27) like external substances. *MnSOD's* higher expression in tomatoes confers resilience against salt and oxidative damage (28). Advances in sequencing technologies uncovered multiple activities of *SOD* genes and led to the identification of *SOD* gene families across the complete genomes of different plant species (29).

Finger millet has a high degree of genetic diversity in terms of agronomic features, dietary value, and root properties (30). Hence, genomic techniques and databases are critical for phenotypic exploration, gene mining, and marker-assisted breeding (31). Transcriptomics has been utilized in finger millet to annotate the genome, identify markers, and locate candidate genes (32). Genome assets are most important tools for designing high yielding stable finger millet cultivars and increasing genetic diversity in response to changing environmental circumstances.

The present analysis is a comprehensive genome-wide analysis of the *SOD* gene family in *Eleusine coracana* and compared the findings with previous findings of *Arabidopsis thaliana* in order to explore the significance, functionality, and evolutionary relationships of the *SOD* gene family.

II. MATERIALS AND METHODS

IN-SILICO EcSOD GENE IDENTIFICATION, AND CHARACTERIZATION

EcSOD genes were retrieved from finger millet using protein sequences obtained from phytozome database (http://www.phytozome.net/) (33). *Arabidopsis's AtSOD* gene was utilized to BLAST P against the *Eleusine coracana* protein database. The hits were selected based on a threshold E value of <1E-5. The selected genes were provided to the motif finder tool, which revealed the domain in protein sequences. The genes containing the SOD Domain were selected from the findings, whereas others were declared redundant.

GENE STRUCTURE PREDICTION, MOTIF STUDY and SUB CELLULAR LOCALIZATION

Eleusine coracana GTF data and Gene Structure Display Server 2.0 were employed to determine structural patterns of exons and introns. (https://gsds.gao-lab.org/index.php) (34). To predict conserved motifs, a set of parameters were applied: a maximum of 10 motifs, 2–20 motif positions, and 6–20 widths using MEME (http://tools.meme-suite.org/) (35). The conserved domain analysis was done using NCBI-CDD search. WOLFPSORT is used for protein Subcellular Localization Prediction. (https://www.genscript.com/wolf-psort.html) (36).

PHYLOGENETIC, SYNTENY AND PROMOTER/ CIS-ELEMENTS ANALYSIS

The amino acid sequences of *SOD* genes from Rice and Sorghum were aligned using Mega version 11.0. (megasoftware.net). Mega-program was used to create the phylogenetic genetic tree using a neighbour joining technique and a thousand bootstrap replications (5). Paralog genes synonymous and non-synonymous ratios were computed using the Ka/Ks calculator. (KaKs_Calculator 3.0: calculating selective pressure on coding and non-coding sequences. Genomics Proteomics Bioinformatics 2021). The candidate *EcSOD* gene homologs were mapped to *S. bicolor* and *O. sativa* genomes, and synteny maps were developed using TB tools. The multiple circos similarity analysis was performed using circoletto online server. (http://bat.infspire.org/tools/circoletto) through bit score and % identity methods.

The phytozome database included the promoter sequences for every gene that was chosen for further analysis. These sequences are 1500 bases upstream from the matching genomic sequence of each gene. With the use of these downloaded promoter sequences, the PLANT CARE tool anticipates CIS regulatory components. (http://webtools/plantcare/html/bioinformatics.psb.ugent.be).

PHYSICAL MAPPING OF SOD GENES ON CHROMOSOMES

The physical mapping of genes on finger millet chromosomes was done through Phenogram tool. (https://visualization.ritchielab.org/phenograms/plot) The results were downloaded with exception of empty chromosomes.

GENE ONTOLOGY, TRANSCRIPTION FACTOR ANALYSIS and CpG Island PREDICTION -

Since PTFDB plant transcription factor database(http://gao-lab.org/planttfdb/) does not have data on finger millet, it was necessary to pick closely similar species, such as *Seteria viridis*, in order to estimate the connection sites of all *EcSOD* protein transcription factors. With the aid of these data, network was created with the Cytoscape application (https://cytoscape.org). Using Cytoscape Tool, the data are captured and shown as a network of *EcSOD* genes. The homologous gene ontology has been analyzed using PANNZER2.0. The upstream 200-2000bps promoter sequence was taken with default parameters. CpG islands for EcSOD homologs were identified using methprimer tool (MethPrimer-Design MSP/BSP primers and predict CpG islands - Li Lab, PUMCH (54). The promoter regions 200-2000 bp upstream sequences as well as gene body sequence was taken for analysis.

PROTEIN STRUCTURE PREDICTION, PROTEIN PARAMETERS and PPI INTERACTIONS

Using the Swiss model server a fully automated protein structure homology modelling server, the 3D protein structures of the *EcSOD* transcripts were estimated. (https://expasy.org/swissmodel/) Using Ramachandran plots, the PSVS protein structure verification site (https://saves.mbi.ucla.edu/) is used to verify and analyze the stability of predicted protein structures. The 2D structure of *EcSOD* homologs was identified using SOPMA server (37).

(https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html).

The STRING database was used to predict and observe the PPI protein-protein interactions of these homologs. The URL is https://string-db.org. The protParam program predicts properties of proteins, including the isoelectric point, gravy (grand average of hydropathy), and aliphatic indices.

INSILICO EXPRESSION ANALYSIS-

The expression data of *EcSOD* genes under drought stress were retrieved from Milletdb (http://milletdb.novogene.com/home/) database and TKM values of control and treated plants were mapped by TB tools.

III. RESULTS

GENE - IDENTIFICATION AND CHARACTERIZATION.

The sequence from *Arabidopsis thaliana* is taken and used as query for BLAST P search against *Eleusine coracana* genome to identify of *EcSOD* genes. From all the hits obtained, we carefully chosen 10 genes out of which 8 genes contain CU-ZN domain and 2 genes contains FE-MN domains. These genes were subjected to motif Finder tool for identification of SOD domain. We got 10 sequences containing SOD domain which are renamed as *EcSODCUZN1, EcSODCUZN2, EcSODCUZN3, ……. EcSODCUZN8,* and *EcSODFEMN1, EcSODFEMN2* with regard to their ligand and location on pseudo chromosomes. (Table1). Gene characterization was done and proteins with longest peptide sequence were *EcSODFEMN1* and *EcSODFEMN2* and protein with shortest were *EcSODCUZN1, EcSODCUZN5* and *EcSODCUZN6.* Analysis was done to obtain molecular weight of proteins and the outcome was all 10 *EcSOD* proteins are in between 15kDa to 21KDa. We analysed protein parameters which show that Pi ranged from 5.34 to 7.92, GRAVY ranged from -0.075 to -0.272. (Table2). Gene structure predictions revealed that among 8 *EcSODCUZN* genes, *EcSODCUZN4* and *EcSODCUZN5* has 7/8 intron to exon ratio; *EcSODCUZN1, EcSODCUZN2, EcSODCUZN3, EcSODCUZN6, EcSODCUZN7, EcSODCUZN8* has 6/7 intron to exon ratio; All the genes have no UTR regions. *EcSODFEMN1* and *EcSODFEMN2* has 5/6 intron to exon ratio, was nearly similar among same group members. (Figure 1b). The subcellular localization of *EcSODCUZN* homologs were found to be in cytoplasm for *EcSODCUZN1, EcSODCUZN2, EcSODCUZN5, EcSODCUZN6, EcSODCUZN7, EcSODCUZN8;* whereas *EcSODCUZN3, EcSODCUZN4* were in chloroplast. The subcellular localisation of *EcSODFEMN1* and *EcSODFEMN2* were in Mitochondria.

PREDICTION OF MOTIFS AND CONSERVED DOMAIN.

MEME tool was used to spot different types of motifs. Out of 10 motifs found, most of them are present among genes

and in same patterns within the same group. (Figure 8a). Results from conserved domain and motif patterns of candidate gene revealed that Super oxide dismutase domain is conserved among all genes.

The three domains were observed but domain with ID-PLN02386 also belongs to superoxide dismutase -Cu/Zn superfamily (cl00891), the protein homologs with *EcSODFEMN* showed domain specific for metal Mn/Fe superoxide dismutase. (Figure1c). The pattern of copper or zinc SOD domain was similar in all proteins. The *EcSODCUZN* genes were having extra motif of *Cu/Zn SOD*. The motif sequence pattern of these is shown in figure 8b and 8c. This identical patterns in motifs of genes among other groups relates with evolutionary relationships

MULTIPLE SEQUENCE ALIGNMENT, PHYLOGENY and SYNTENY and SIMILARITY AMONG SPECIES AND Ka/Ks ANNOTATION.

Multiple sequence alignment of *EcSODFEMN* gene homologs (Fig 2a) with protein sequences of *OsSODFEMN1, SvSODFEMN1, PvSODFEMN1, PvSODFEMN2, SbSODFEMN1 and EcSODCUZN* homologs (Fig 2b) with peptide sequences of *OsSODCUZN1, SvSODCUZN1, SvSODCUZN2, PvSODCUZN1, PvSODCUZN2, SbSODCUZN1* were analysed using Mega 11.0 by ClustalW and Visualised through Snapgene viewer, the alignment showed that there are identical frequencies, which means they are conserved among the species.

The evolutionary relationship analysis among *EcSOD* homologs exposed 5 paralog pairs with 3 groups. *EcSODCUZN1, EcSODCUZN3; EcSODCUZN5, EcSODCUZN8; EcSODCUZN3, EcSODCUZN4; EcSODCUZN2, EcSODCUZN7;* and *EcSODFEMN1, EcSODFEMN2*; are the paralog pairs formed (Fig 1a).

The phylogenetic analysis of candidate genes with closely related species like *Seteria viridis, Panicum virgatum, Sorghum bicolor, Oryza sativa* gave 4 groups with both *CUZN* and *FEMN* homologs respectively. *SvSODCUZN1, PvSODCUZN1, SbSODCUZN1, OsSODCUZN1, EcSODCUZN1, EcSODCUZN6* formed orthologous pairs with in one group. *EcSODCUZN2, EcSODCUZN3, EcSODCUZN4,* and *EcSODCUZN7* shown no orthologous pairs with other species. *EcSODCUZN5 and EcSODCUZN8; SvSODCUZN2, PvSODCUZN2*; formed into two other groups. The SODs with metal factor Fe/Mn from finger millet goes into one group forming no paralog pairs with other species (Figure 3a and 3b).

The similarity analysis by multiple circos among species was performed and the results were downloaded. Red colour (Fig 9a) indicating higher bit score value (>0.75) between the genes compared to others and orange colour (Fig 9b) indicated higher %identity (99.99%) among protein sequences. The *EcSOD* genes shown similarity with *Sorghum bicolor* and *Seteria viridis* species. Three paralog pairs are identified from Ka/Ks annotated evolutionary tree analysis results. All paralogs of *EcSOD* showed Ka/Ks c ratio not more than 0.21 (<1) which signifies specific purifying selection. The tabulated Ka/KS values and paralog pair given in Table3. Ka/Ks ratios of >1, =1, and <1 indicate positive, purifying, and neutral evolution, respectively.

Synteny analysis with *S. bicolor* shown candidate genes were distributed across the genome (Fig 2c). The genes on chromosome 8A and 8B of *E. corcana* shows homology with chromosome 07 of *S. bicolor.* chromosome 5A, 5B of *E. corcana* shows homology with chromosome 09 of *S. bicolor.* the genes distributed on *E. corcana* chromosome 3A, 3B collinear with chromosome 01 in *S. bicolor*. Genes on chromosome 7A and chromosome 7B of *E. corcana* shows collinearity with S. bicolor chromosome 01. Results of synteny between *E. corcana and O. sativa* revealed chromosome 8A, 8B similarity with chromosome 08; chromosome 5A, 5B with chromosome 05; chromosome 3A, 3B with chromosome 03; chromosome 3A, 3B, 7A, 7B with chromosome 07 respectively (Fig 2d). Collinearity was observed in between *M. sinesis* and *E. corcana.* Chromosome 8A, 8B homologs with chromosome 03, 07 on *M. sinesis*. Chromosome 5A, 5B homologs with chromosome 16, 17 on *M. sinesis.* Chromosome 3A, 3B homologs with chromosome 01 on *M. sinesis.* Chromosome 02 of *M. sinesis* has similarity for genes on chromosomes 3A, 3B, 7A, 7B of *E. corcana* (Fig 2e). There is no collinearity among *E. corcana* and *S. viridis*.

Physical Mapping, Cis-Elements Prediction, and their Distribution -

Transcription is a crucial step to start the production of genes and it is also a point where RNA polymerase binds with promoter like regulatory regions. The structure of the promoter is essential for RNA polymerase binding affinity, which in turn affects the degree of gene expression (38). Promoter analysis of *EcSOD* homologs revealed the occurrence of Abiotic stress, light responsive and Phyto-hormone responsive putative cis-regulatory elements (Fig 7b). The frequency of occurrence of cis elements were tabulated and presented as a heatmap (Fig 7a). Light responsive elements and MEJA were highly distributed among the genes. MYB- Related, ABA responsive elements are moderately present in most of them. The regulatory regions of *EcSOD* gene homologs contain a variety of cis-regulatory elements, including those associated to MYB-flavonoid genes, MYB-drought responsive, MYB-light responsive, and defense responsive. MEJA, GA, SA, and

AR are phytohormone-responsive elements found in the majority of *SOD* homologs. Across the Finger millet genome, the locations of *EcSOD* homologs were mapped (Fig 10a).

PROTEIN MODELING- 2D,3D AND PROTEIN-PROTEIN INTERACTIONS (PPIs)

The Swiss model is used here which predict 3D structure of various proteins based on PDB structures. High similarity models were selected from standard structures available in database. The appropriate template ID, protein ID, percentage identity were noted and tabulated (Table 2). 3D structures are given in (Fig 4).

The results of secondary structure were tabulated in Table 4. Beta turn percentage was zero in all the *SOD* proteins. In case of *EcSODFEMN* homologs the % of alpha helices was higher approx. 50% compared to *EcSODCUZN* homologs. The structure of *EcSODCUZN*1 and *EcSODFEMN1* are saved (Fig 5a, 5b).

The structure ID B4F925.1.A with superoxide dismutase from mitochondria, showed structure similarity with *EcSODFEMN* homologous. Structure ID 3Km2.3. A Superoxide dismutase with copper/zinc from chloroplast region, Shown similarity with *EcSODCUZN1, EcSODCUZN5, EcSODCUZN6, EcSODCUZN8*. Structure P93407.1.A – SOD (Cu-Zn), Showed similarity with *EcSODCUZN3, EcSODCUZN4. EcSODCUZN2* shown highest similarity with K4AFE1.1. A – SOD (Cu/Zn). The structure similarity ranged between 64 to 94% among homologs.

A network displaying both direct and indirect links to the candidate proteins was built using an online database-String database. The interpretation of the results reveals interactions with A0A368QPZ2, A0A368RQ49, A0A368SFS1, A0A368STV7, K3YIU7_SETIT, K3Z9A6_SETIT, K3ZXQ6_SETIT, and K4AFE1_SETIT, which are primarily involved in the metabolism of ROS, carbohydrates, the glyoxal metabolic pathway, and superoxide dismutase activity within cells in response to oxidative stress (Figure 5c).

TRANCRIPTIONAL FACTORS PREDICTION-

The results of transcription factor analysis showed that 35 different kinds of transcriptional factors are associated with candidate genes. Abiotic stress and biotic-related transcriptional factors, such as MYB, WOX, EIL, TALE, Trihelix, CAMTA, C2H2, LBD, ERF, MYB-related, HD-zip, BZIP, GATA, SBP, MIKC-MADS, AP2, NAC, WRKY, TCP, bHLH, and G2-like, are primarily engaged in plant growth and development. (Figure 6). (39), (40). Certain transcriptional factors, such as Dof and GATA, are associated with growth and development of plants, while some are hormone-responsive Tfs.

INSILICO EXPRESSION ANALYSIS –

Expression data reveals that *SOD* gene levels increased under drought stress compared to untreated ones in both the cultivars (IE7079, IE6537). Among the homologs *EcCUZNSOD1, EcCUZNSOD3, EcFEMNSOD1* were highly expressed under drought treatments. Results show that *SOD* is upregulated under stress and a stress responsive gene (Fig 10b).

OUTCOME OF CPG ISLANDS PREDICTION AND GO ANALYSIS -

Methylated regions in promoter and gene body were identified and results were tabulated (Table 6). *EcCUZNSOD2, EcCUZNSOD5, EcCUZNSOD8, EcFEMNSOD1* showed three CpG Islands in their promoter regions. Maximum number of CpG rich sites were found in *EcCUZNSOD7* whereas *EcCUZNSOD1, EcCUZNSOD4, EcCUZNSOD6* shown no CpG rich regions in the promoter regions. *EcCUZNSOD3, EcFEMNSOD2* got two CpG islands. *EcSODCUZN* homologs are mainly involved in superoxide metabolic activity, response to ozone, oxidant detoxification and major responses in according to abiotic, salt, high intensity light, metal responses whereas *EcSODFEMN* homologs are involved in removal of superoxide radicals, oxidative stress, response towards xenobiotics and herbicides. GO in molecular function shows copper ion binding for *EcSODCUZN* homologs but for *EcSODFEMN* its metal ion binding. The cellular components for *EcSODCUZN* homologs were cytoplasm, chloroplast, and peroxisomes whereas *EcSODFEMN* located in mitochondria. The results of GO analysis come in accordance with results of prediction of subcellular localisation for the *EcSOD* protein homologs.

IV. DISCUSSION

Environmental stress causes a significant challenge to plants, leading to troubles in both morphological and physiological growth processes, toxic ROS are frequently produced in plants upon interaction with these stresses. Overexposure to ROS can cause membrane lipids, DNA strand breaks, and enzyme inactivation (41). Members of the SOD family play a preliminary plus vital role in protecting against ROS. All creatures that exist in the presence of O2 are assumed to include the enzyme antioxidant *SODs*, which disproportionately convert reactive O2•− in to H2O2 and O2 upon interactions with co-factors like Cu, Fe, or Mn. (42). Because the active core of the enzyme reaction cycle involves the interaction of two O2•− free radicals and the short-term retention of them, two

redox-active metal ions are required as cofactors (43). Most of the previous studies of plant species follow a traditional methodology such as Blast search and pfam search of known proteins of related families to identify SOD gene family target species (23). It is crucial to characterize the SOD gene family in finger millet because to its notable resistance to Environmental stresses.

Ten *SOD* genes total—eight *Cu/Zn-SODs* and two *Fe/Mn-SODs*—covering the two main categories of *SOD* genes were found in finger millet in the current study (Table 1*).SOD* gene number varies from plant to plant and various previous studies mentioned that Arabidopsis have 8 *SODS* (3 *Cu/Zn-SODs*, 2 *Mn-SODs*, and 3 *Fe-SODs)* whereas sorghum contains 8 (5 Cu/Zn-SODs, 1Mn-SOD, and 2 Fe-SODs) and tomato having 9 *SODS (4 Cu/Zn-SODs, 1 Mn-SOD, and 4 Fe-SODs*).This difference in number of SOD genes in each plant may be due to the differences in their genome size, evolutionary divergence, and Environmental Adaptation. However, not only limited to these. Gene duplication, which includes tandem and sectoral duplications are crucial for the growth of *SOD* gene diversity, might be the root cause of these variations (44), (45).

Phylogenetic investigation showed a close association among *Cu/Zn-SODs* and *Fe-SODs/Mn-SODs* members. Based on the bootstrap values, relative phylogenetic analysis of *SOD* proteins of finger millet and other crops or plants (Sorghum, Panicum, Seteria, and rice) mutually formed four distinct groups in both cases; these findings are compatible with earlier research; Regarding the subcellular location of *SODs*, most of the data supported the evolutionary conclusions. It was anticipated that the cytoplasm, mitochondria, and chloroplast would contain *SODs.* According to prior research (Fink and Scandalios 2002), the majority of cytosolic and chloroplast genes include seven introns, and intron–exon arrangements of plant *SO*D genes were shown to be well conserved (21). Furthermore, similar sequences for the majority of *EcSODs* were discovered through phylogenetic analysis of other *SOD* species, indicating that *EcSODs* most likely serve the same purposes as *SODs* in other plant species. According to earlier research, *SOD* protein clusters may be related to the subcellular placements of *SODs;* in our investigation, individuals that grouped within the same subgroup likely to have similar subcellular localizations (46, 47).

Seven intron regions have been found among the ten *EcSOD* genes through gene structure analysis. In a previous study it was revealed that *SOD* genes in plants had highly conserved introns while chloroplast *SOD* as well as most of the cytosolic *SODs* retained seven introns (21).

The three main mechanisms that may be causing this discrepancy are gain/loss of exon/intron, insertion/deletion, and exonization/pseudo-exonization according to recent research. Their enzymatic activity and expression pattern that adapts to different stress circumstances may be impacted by structural divergences (48). The examination of conserved motifs in *SOD* proteins corroborates the evolutionary information (Fig. 2). Similar concepts were shared by the same grouping. Interestingly, *SOD* proteins that were concentrated in each subgroup found to have similar motif distributions, dimensions, and locations (48).

The protein structure predicted aligns with phylogenetical relationship of candidate proteins, paralog proteins are having similar 3D structure with identical percentages of alpha helices, beta turns and extended strands of them (Fig 4 and Fig5a,5b).

A complex regulatory mechanism is required to control gene expression in response to different abiotic and biotic stress. Understanding the transcription factors and cis-elements present in the promoter sequences provides information on how *SiSODs* are regulated upstream. Our findings demonstrate that a large number of cis-elements linked to stress-responsive events were found in the promoters of *SiSODs*. MYB is a class of transcription elements that has been identified to be involved in control of physiological metabolism, organogenesis, cell morphogenesis, and growth and development in plants (49). Furthermore, biotic, and abiotic stress responses in plants were linked to several *MYB* genes (50). A large portion of *MYB TFs* helps in the establishment of host resistance against various pathogenic fungi (51). In order to protect themselves from biotic and abiotic stress, plants develop a variety of secondary metabolites. The combined activity of bHLH and MYB TFs controls the synthesis of tissue-specific flavonoids, such as phenylpropanoid (52). Plants which grow in stress free environment showed very little induction of *CAMTA* genes. This may be because the TF genes in this family have redundant functions or that the genes in this class are expressed in particular environments (53).

The cis-regulatory elements existing in the promoter regions were the binding sites of *SOD*s gene with other proteins to play an essential role in regulating gene transcription. There were a huge number of light responses associated regulatory elements, Phyto-hormone responsive elements which involves in plant defense mechanism, growth, and drought stress reactive elements. Among all the *EcSOD* homologs, MYB had the greatest number of elements, suggesting that it is associated with the production of lignin and plays a role in stress tolerance. Defense-responsive elements found in *EcSOD* homologs

suggest that these proteins are involved in defense related to biotic stress. MeJA, GA, SA, and AR are phytohormone-responsive elements found in the majority of *SOD* homologs. The defense response elements MeJA and SA have been discovered to be the most abundant among phytohormone responsive elements. They have also been confirmed to be present in all *EcSOD* homologs, suggesting that they are involved in the defense mechanism against biotic stress. The promoter regions of *EcSOD* genes also contain light-responsive elements. This discovery suggests that light might affect the Finger millet's *SOD* genes. Studies involving cis-elements are crucial as they have the potential to reveal the functional control of members of the *EcSOD g*ene family. Gene expression patterns and gene functions that are comparable between homolog sequences may be greatly influenced by similar Cis-regulatory regions. The abscisic acid-related motif ABA and the methyl jasmonate-related MEJA motif were present in a significant proportion of *EcSODs*. Different subgroups' unique regulatory elements may cause the genes in those subgroups to act differently. Expression analysis shows *SOD* gene as a stress responsive and upregulated under stress conditions in finger millet cultivars. The gene ontology studies shows that *EcSODs* are mainly related in responses to oxidative radicals, oxidative stress management and other abiotic stresses which makes *EcSOD* gene as a potential target for producing crop varieties resilient to environmental conditions and biotic infections.

Table 1: Gene characterisation of EcSOD homologs.

TRANSCRIPT ID	GENE	CHR	LOCATION START END	STRAND	Localization
ELECO.r07.5BG0424640.1	>EcFEMNSOD1	5B	11423066..11427964	Reverse	Mitochondria
ELECO.r07.5AG0377540.1	>EcFEMNSOD2	5A	13454562..13459494	Reverse	Mitochondria
ELECO.r07.3BG0279950.1	>EcCUZNSOD1	3B	51383645..51385320	Reverse	Cytoplasm
ELECO.r07.3BG0288910.1	>EcCUZNSOD2	3B	58305000..58307819	Forward	Cytoplasm
ELECO.r07.8BG0669600.1	>EcCUZNSOD3	8B	62759715..62761705	Forward	Chloroplast
ELECO.r07.8AG0641100.1	>EcCUZNSOD4	8A	47311060..47313528	Forward	Chloroplast
ELECO.r07.7AG0580610.1	>EcCUZNSOD5	7A	46693478..46694625	Reverse	Cytoplasm
ELECO.r07.3AG0248280.1	>EcCUZNSOD6	3A	48098898..48101342	Forward	Cytoplasm
ELECO.r07.3AG0239070.1	>EcCUZNSOD7	3A	40631296..40634115	Reverse	Cytoplasm
ELECO.r07.7BG0611780.1	>EcCUZNSOD8	7B	57514079..57515197	Reverse	Cytoplasm

Table 2: Protein parameters of SOD genes.

TRANSCRIPT ID	**GENE**	**A.AS**	**WEIGHT**	**PI**	**Gravy**
ELECO.r07.5BG0424640.1	>EcFEMNSOD1	236	25.51 kb	7.9	0.153
ELECO.r07.5AG0377540.1	>EcFEMNSOD2	236	25.49 kb	7.92	0.179
ELECO.r07.3BG0279950.1	>EcCUZNSOD1	152	15.13 kb	5.65	-0.139
ELECO.r07.3BG0288910.1	>EcCUZNSOD2	163	16.48 kb	6.58	-0.075
ELECO.r07.8BG0669600.1	>EcCUZNSOD3	204	20.6 kb	5.49	0.111
ELECO.r07.8AG0641100.1	>EcCUZNSOD4	204	20.6 kb	5.34	0.104
ELECO.r07.7AG0580610.1	>EcCUZNSOD5	152	15.2 kb	5.76	0.303
ELECO.r07.3AG0248280.1	>EcCUZNSOD6	152	15.13 kb	5.65	0.139
ELECO.r07.3AG0239070.1	>EcCUZNSOD7	163	16.48 kb	6.58	0.075
ELECO.r07.7BG0611780.1	>EcCUZNSOD8	152	15.20 kb	5.93	0.272

Table 3: Ka/Ks values.

Gene 1	Gene 2	Ka	Ks	Ka_Ks
>EcCUZNSOD2	>EcCUZNSOD7	0	0.074958	0
>EcCUZNSOD3	>EcCUZNSOD4	0.013499	0.0638	0.211577
>EcCUZNSOD1	>EcCUZNSOD6	0	0.072895	0

Table 4: 2D structure analysis of EcSOD proteins.

Gene	**Alpha helix %**	**Extended stand %**	**Beta turn %**	**Random coil %**
>*EcFEMNSOD1*	50.85	12.29	0	36.86
>*EcFEMNSOD2*	48.73	12.29	0	38.98
>*EcCUZNSOD1*	3.29	31.58	0	65.13
>*EcCUZNSOD2*	3.68	28.83	0	67.48
>*EcCUZNSOD3*	7.35	26.96	0	65.69
>*EcCUZNSOD4*	5.88	25	0	69.1
>*EcCUZNSOD5*	4.61	30.26	0	65.13
>*EcCUZNSOD6*	3.29	30.26	0	66.45
>*EcCUZNSOD7*	3.07	30.06	0	66.87
>*EcCUZNSOD8*	3.95	30.26	0	65.79

Table 5: Gene ontology analysis results.

GENE	**BIOLOGICAL PROCESSES**	**MOLECULAR FUNCTION**	**CELLULAR COMPONENT**	**DESCRIPTION**
>EcFEMNSOD1	GO: 0019430	GO: 0004784	GO: 0005739	Superoxide dismutase
>EcFEMNSOD2	GO: 0019430	GO: 0004784	GO: 0005739	Superoxide dismutase
>EcCUZNSOD1	GO: 0006801	GO: 0004784	GO: 0005615	Superoxide dismutase (Cu-Zn)
>EcCUZNSOD2	GO: 0006801	GO: 0005507		Superoxide dismutase (Cu-Zn)
>EcCUZNSOD3	GO: 0006801	GO: 0004784	GO: 0005507	Superoxide dismutase (Cu-Zn)
>EcCUZNSOD4	GO: 0006801	GO: 0005507	GO: 0005507	Superoxide dismutase (Cu-Zn)
>EcCUZNSOD5	GO: 0006801	GO: 0004784	GO: 0005615	Superoxide dismutase (Cu-Zn)
>EcCUZNSOD6	GO: 0006801	GO: 0004784	GO: 0005615	Superoxide dismutase (Cu-Zn)
>EcCUZNSOD7	GO: 0006801	GO: 0005507		Superoxide dismutase (Cu-Zn)
>EcCUZNSOD8	GO: 0006801	GO: 0004784	GO: 0005615	Superoxide dismutase (Cu-Zn)

Table 6 – CpG islands predicted in promoter and gene body sequences.

Gene ID	No of islands	Region
EcCUZNSOD1	0	Promoter
	2	Gene body
EcCUZNSOD2	3	Promoter
	1	Gene body
EcCUZNSOD3	2	Promoter
	1	Gene body
EcCUZNSOD4	0	Promoter
	1	Gene body
EcCUZNSOD5	3	Promoter
	0	Gene body
EcCUZNSOD6	0	Promoter
	2	Gene body
EcCUZNSOD7	4	Promoter
	1	Gene body
EcCUZNSOD8	3	Promoter
	1	Gene body
EcFeMnSOD1	3	Promoter
	1	Gene body
EcFeMnSOD2	2	Promoter
	1	Gene body

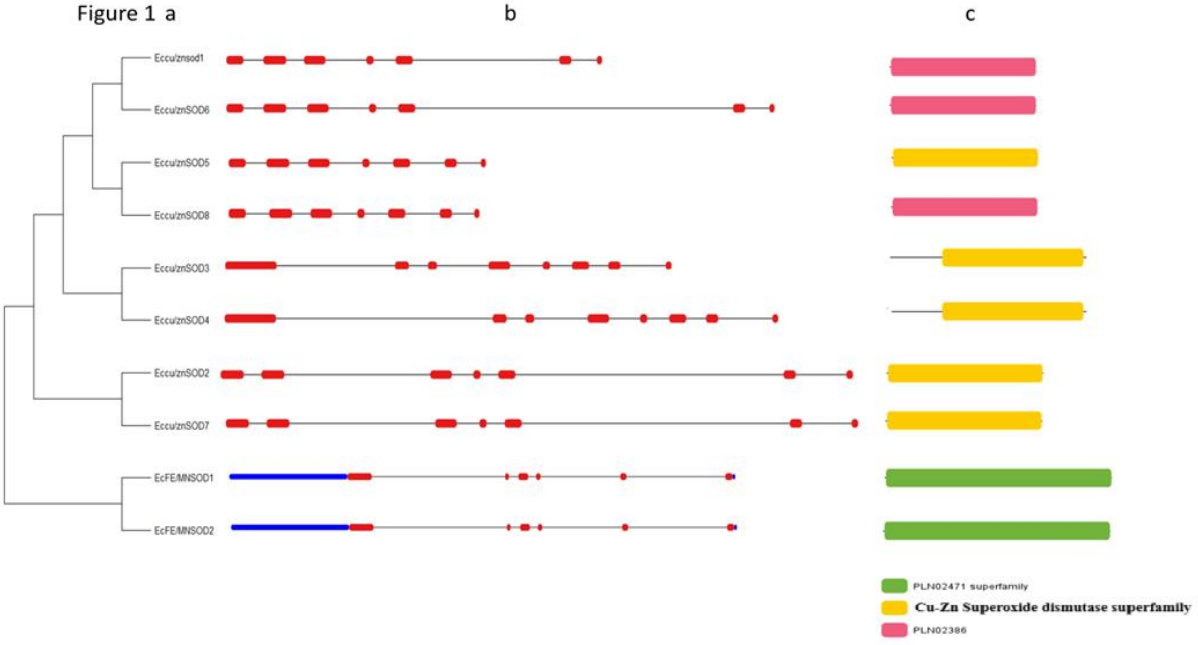


Fig.1- a) Phylogenetic analysis of EcSOD homologs in tree form. b) Distribution pattern of exon/intron of candidate

genes where red colour = exons, blue = UTRs and straight lines = introns. c) Domain analysis done by NCBI-CDD search.

Figure 2

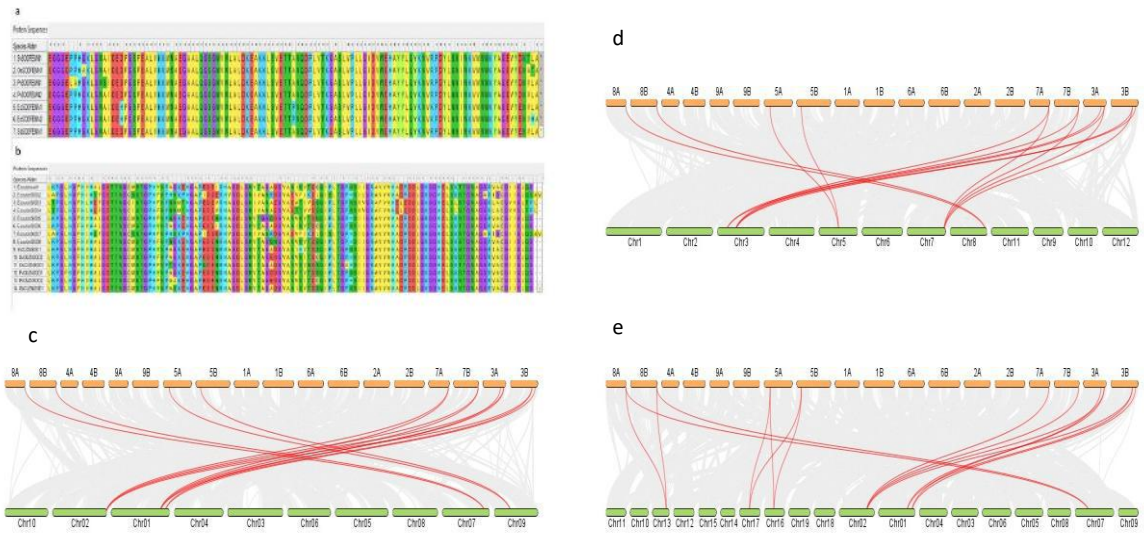


Fig.2- a) Multiple sequence analysis of Fe/Mn SODs among related species. b) Multiple sequence analysis of Cu/Zn SODs among related species. c) Synteny analysis of E. corcana vs S. bicolor. d) Synteny analysis of E. corcana vs O. sativa. e) Synteny analysis of E. corcana vs M. sinesis where pink lines indicate collinearity of candidate EcSOD genes and grey colour indicates collinearity among genomes.

Figure 3

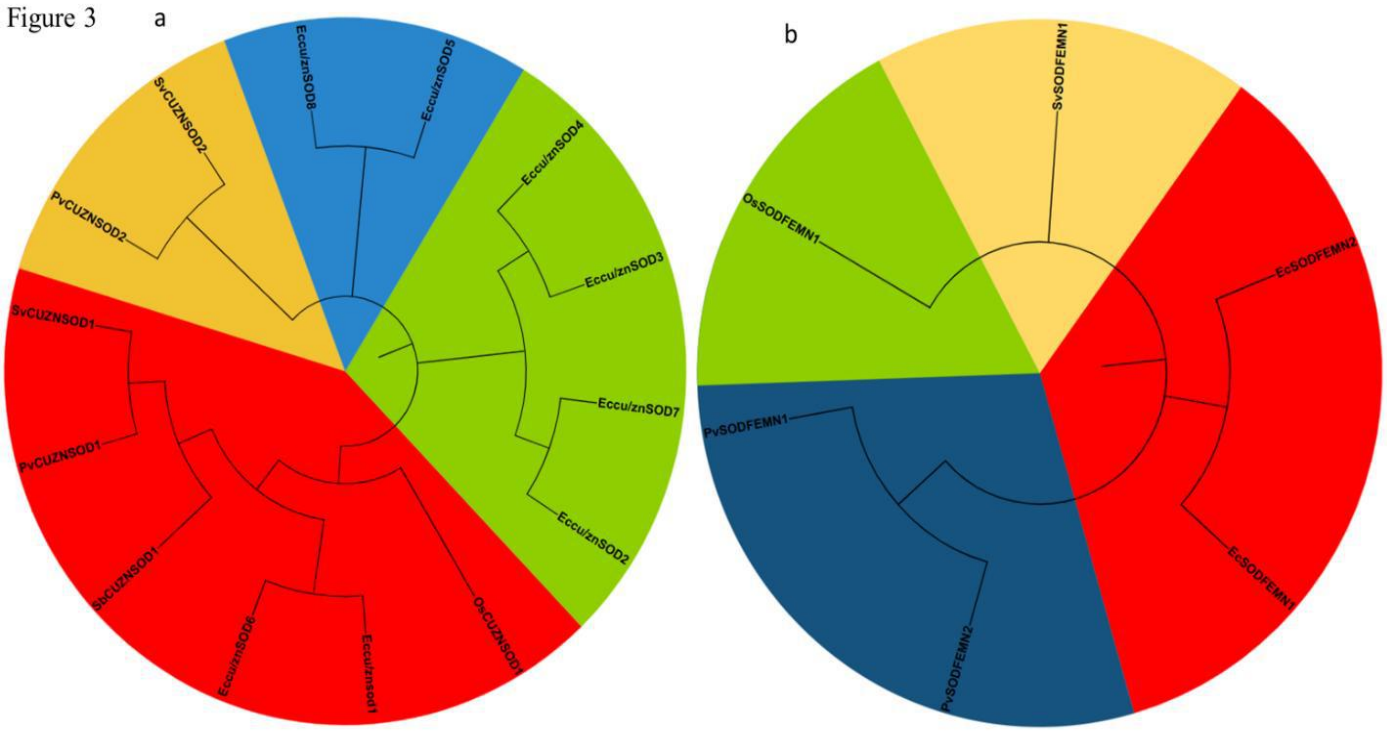


Fig.3 – a) Evolutionary relationship of EcCUZNSOD homolog genes with other species like Sorghum bicolor, Oryza sativa, Panicum virgatum, and Seteria viridis. b) Evolutionary relationship of EcFEMNSOD homolog genes with other species like Sorghum bicolor, Oryza sativa, Panicum virgatum, and Seteria viridis.

Figure 4

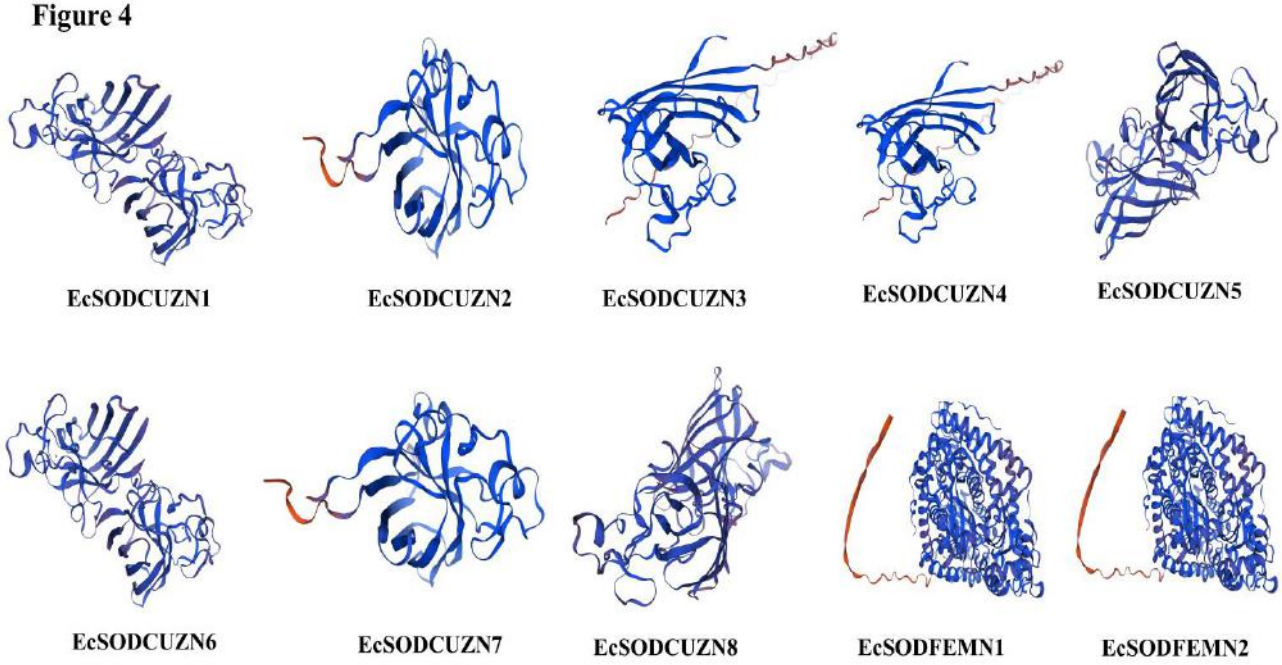


Fig.4 – 3D structure analysis of EcSODs proteins.

Figure 5

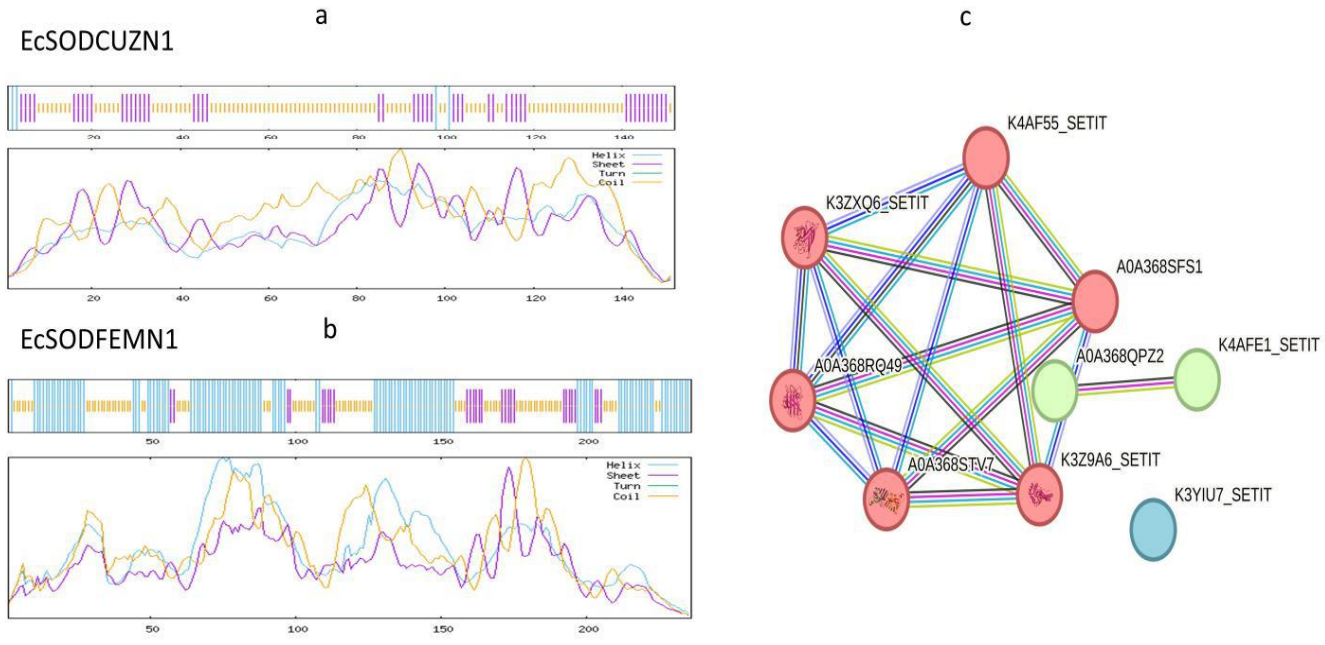


Fig.5 – a and b) secondary structure of EcSODCUZN1 and EcFEMNSOD1 proteins respectively. c) Protein -protein interactions among EcSOD candidate proteins selected.

Figure 6

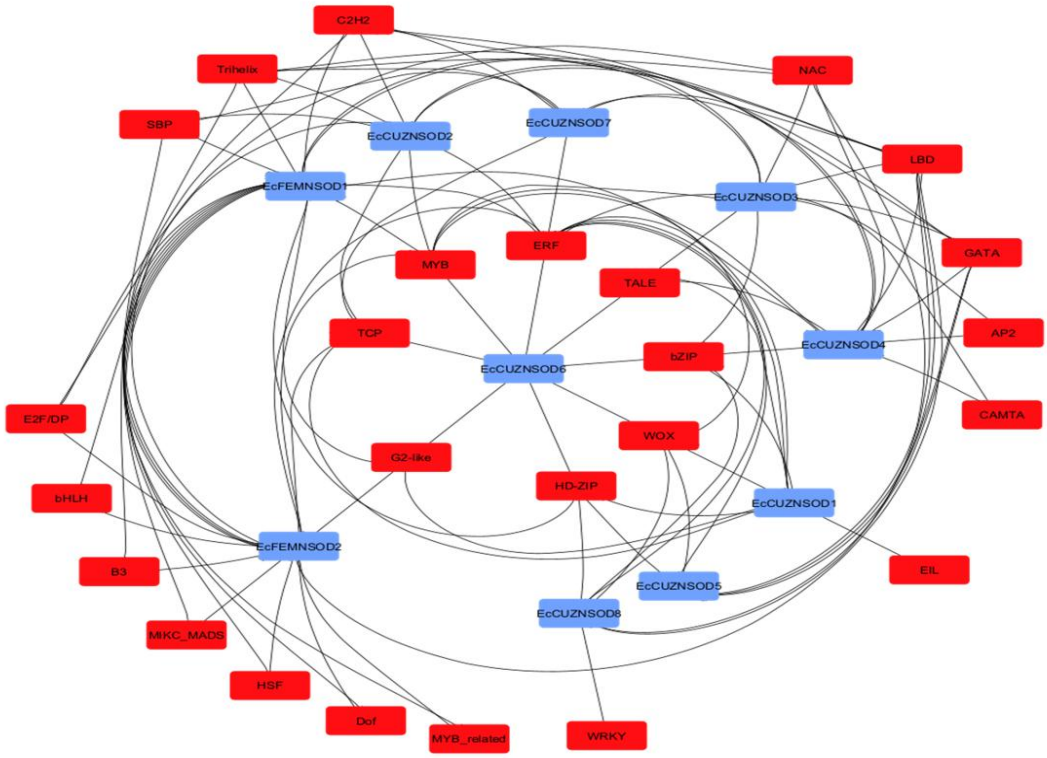


Fig.6 – Network created by Cytoscape tool shows predicted Transcriptional factors.

Figure 7

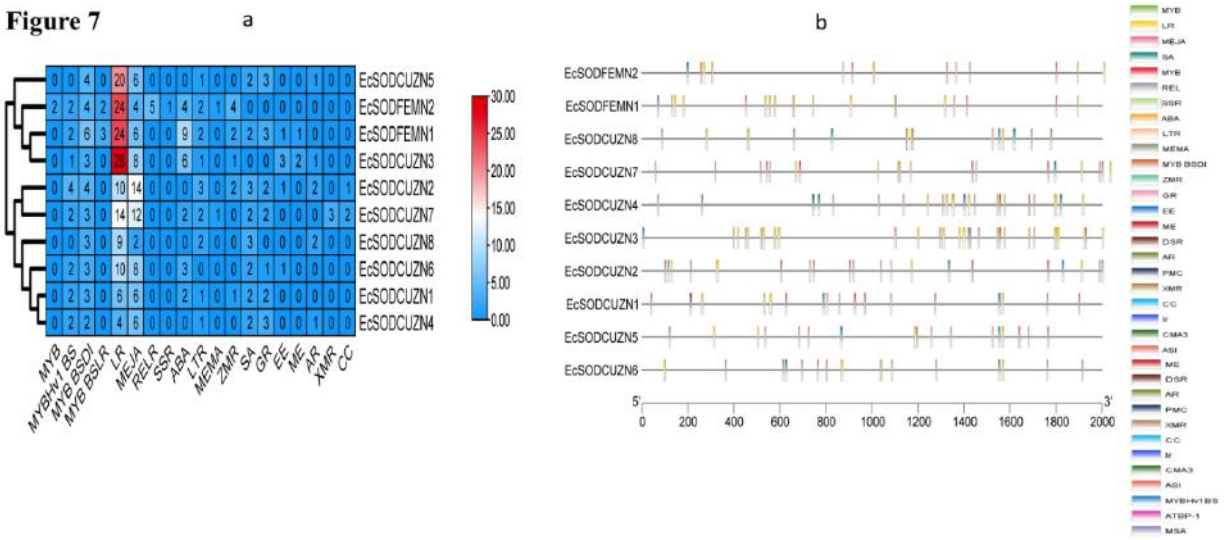


Fig.7- a) Heatmap representing distribution of cis regulatory elements among EcSOD homolog genes b) The distribution of promotor elements predicted.

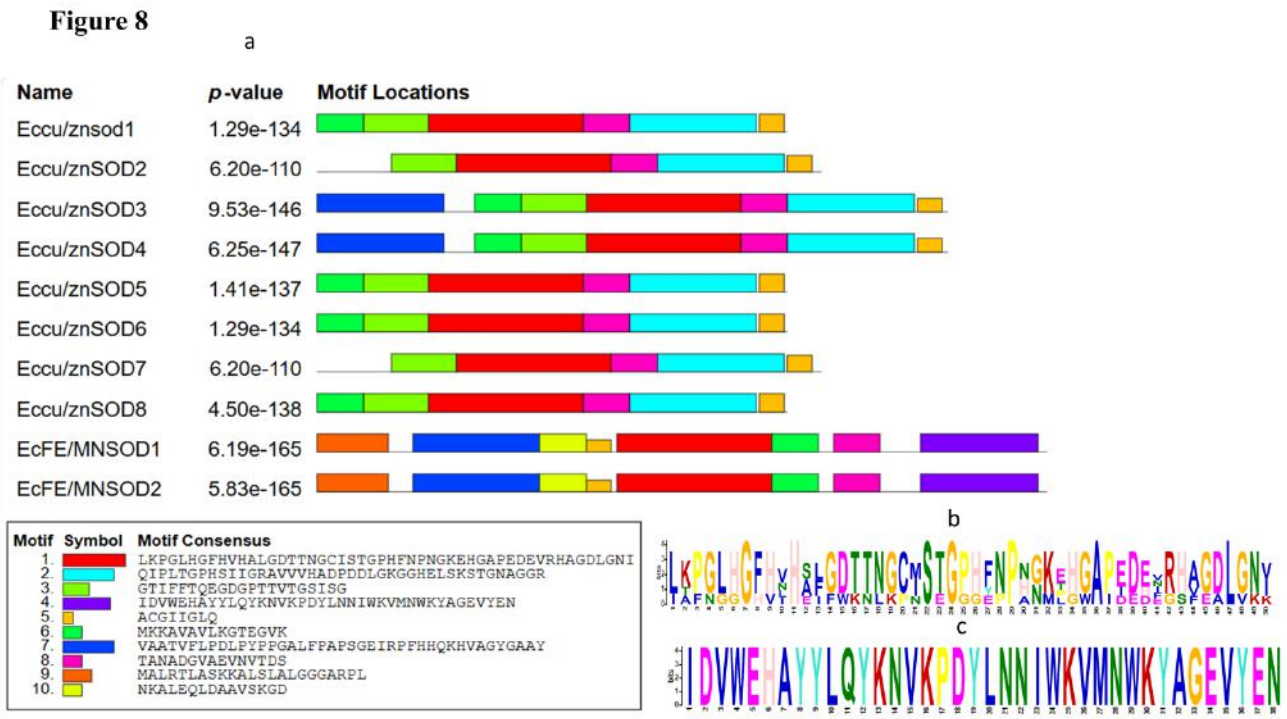


Fig.8- a) Motif structure analysis by MEME tool. Red colour motif is Superoxide dismutase activity. Blue colour motif is SOD with Cu/Zn ligands. Violet colour motif is SOD with Fe/Mn metal ligands. b) Motif pattern of SOD (red colour motif). c) Motif sequence pattern of Fe/Mn violet colour motif.

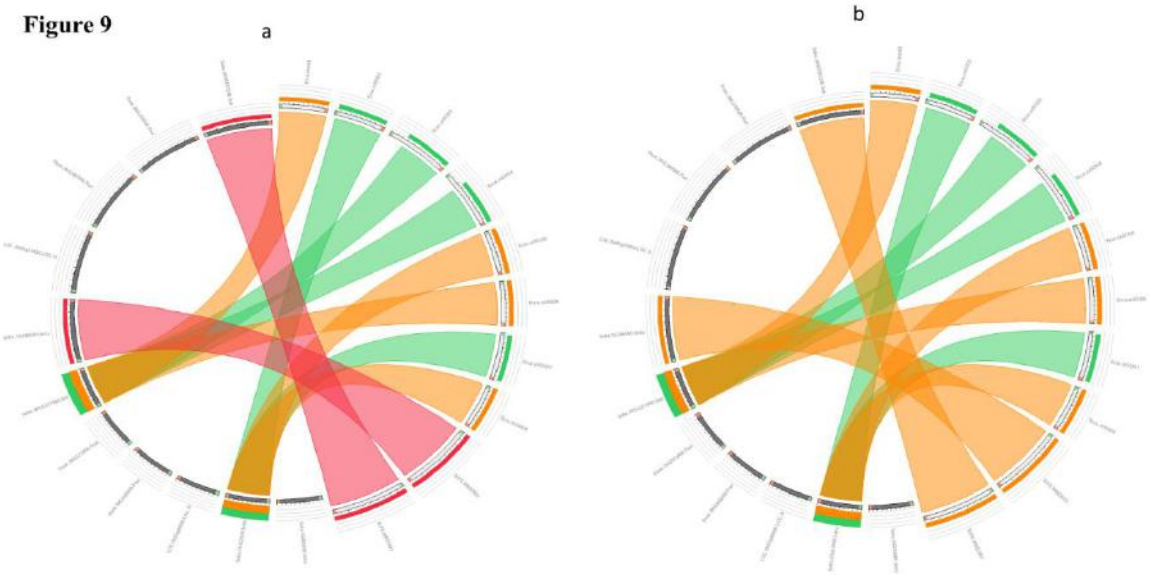


Fig.9- Multiple circos similarity analysis by Circoletto tool. a) analysis done through bitscore values where red colour > 0.75, orange = 0.75 and blue <0.75 value. b) Analysis done through %identity of protein sequences where orange colour means higher identity i.e. 99.999% and blue colour less than 99.9% similarity among species.

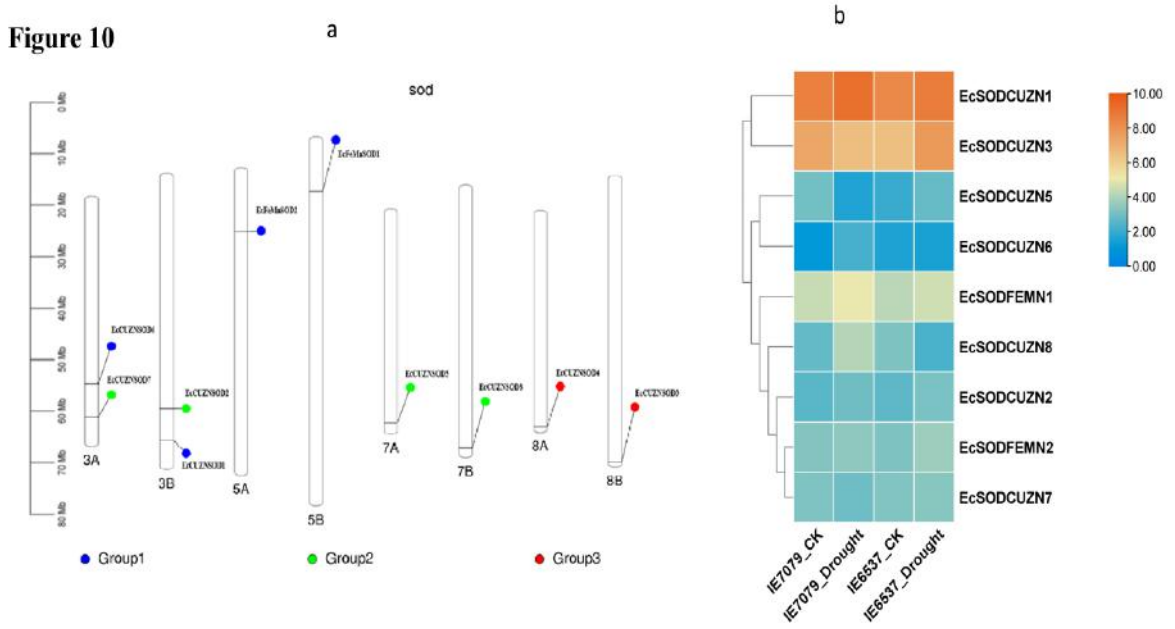


Fig.10- a) Phenogram of EcSODs genes on their respective chromosomes. b) Heatmap showing the expression levels of EcSODs genes based on TKM values.

V. CONCLUSION

Using a wide variety of bioinformatic methods, we have examined the finger millet genome in this work in order to discover and define the *SOD* gene family. *SOD* are the main antioxidants and are among those initial to take part in the ROS species scavenging process. It plays a key role in understanding how plants counter to stress. The findings of this work provide support for the functional characterization of *EcSOD* proteins and deepen our knowledge of the evolutionary links within the *SOD* family. To sum up, our research has yielded extensive details regarding the ten *SOD* genes found in finger millet, such as gene structures, phylogenetic relationships, chromosome locations as well as gene ontology. These important findings suggest that *EcSOD* genes play a significant role in controlling the development of plant tissue and are probably involved in the response to both abiotic and biotic stress. This methodical identification of the whole genome offers a foundation for further research on the role of *EcSOD* proteins in biological processes. It also offers a possible means of improving finger millet breeding under various biotic and abiotic stress, as well as for investigations including gene editing and manipulation.

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AUTHOR CONTRIBUTION STATEMENT

Viswanadha Naik. J and Srinivas Naik. K came up with the experiments and the article's framework. Viswanadha Naik. J and Anjana priyadarshani. K wrote the initial draft. All others have contributed lateral text to the manuscript and improved it. Prashanth B, Viswanadha Naik. J and Vikas Reddy has provided the figures. Srinivas Naik. K, Viswanadha Naik. J, Prashanth. B, Anjana priyadarshani. K, Vikas Reddy. O and Vijay Kumar. G edited the manuscript. All the Authors have given their approval.

STATEMENTS AND DECLARATIONS

Declaration of competing interest

The authors declare that they have no known conflicts of interest.

Data availability statement

The complete data is accessible with the corresponding author K. Srinivas Naik.

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A Review on the Role of Microbes in Degradation of Melanoidin from Distillery Wastewater

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Abstract— *Discharge of melanoidin containing distillery wastewater raises a serious environmental concern as it can pose severe health risks to aquatic bodies and soil due to absorption of sunlight, change in the alkalinity and inhibition of seed germination. Several physicochemical and biological based clean-up technologies have been investigated to combat this environmental pollution. Strategies to decolorize the distillery effluents using potential microbial communities are efficient and cost-effective. Distillery effluent treatment methods using fungi, algae and bacteria have demonstrated promising results. Microbial enzymes involved in the mechanism of decolorization have also been studied extensively. Current advances in melanoidin decolorization using nanoparticles show great promise for treating industrial effluents. The focus of the present review is to explore the current approaches of use of different groups of microbes and novel approaches such as use of nanoparticles in decolorization of melanoidin containing distillery wastewater.*

Keywords— ***Distillery wastewater, melanoidin, bacteria, fungi, algae, nanoparticles***

I. INTRODUCTION

Distilleries are among the major industries in India, generating significant volumes of wastewater, often referred to as spent wash or raw effluent, which can pose serious risks to soil and water quality. This effluent, produced during ethanol production, is a brown-colored liquid characterized by a high concentration of organic matter and nitrogen compounds, low pH, elevated temperature, and high salinity. The quality of the substrates and the unit activities employed to produce alcohol determine how polluted distillery effluent is, hence, effluent characteristics might vary from distillery to distillery. Molasses is frequently used as a raw material for alcohol manufacturing, due to its high carbohydrate content, along with its abundance of minerals and organic acids, making it an ideal fermentation substrate. The quality and composition of molasses can differ greatly based on factors such as the type of sugarcane, harvesting methods, and processing techniques employed [1,2].

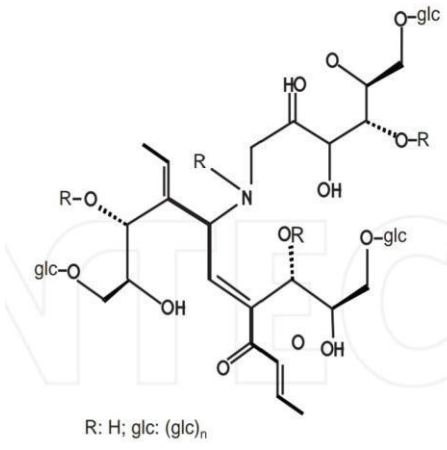

Fig: Basic structure of melanoidin [3]

Molasses based distilleries typically produce 8-15L of effluent, which is distinguished by high chemical oxygen demand (COD) and biochemical oxygen demand (BOD), for every litre of alcohol produced. The effluent's high COD

and BOD levels are caused by the presence of several organic components, including proteins, polysaccharides, polyphenols, waxes and melanoidin [4,5]. Because of this, spent wash needs to be pretreated before it can be properly disposed of in the environment.

Melanoidin emissions from alcohol distilleries present serious environmental problems, especially for aquatic environments. Because of its dark pigmentation, sunlight is absorbed by it, decreasing light penetration in water bodies and preventing aquatic plants and algae from photosynthesizing. The equilibrium of aquatic ecosystems is further impacted by this interruption in photosynthesis, which lowers oxygen production. Furthermore, because microorganisms use a lot of oxygen to break down melanoidin, its high organic content raises the biochemical oxygen demand (BOD) in water. As a result, dissolved oxygen levels are reduced, which frequently leads to anaerobic conditions that are detrimental to fish and other oxygen-dependent aquatic life. Since the effluent is high in nutrients like phosphorous and nitrogen, melanoidin in water also contributes to eutrophication [6]. These nutrients encourage algae blooms, which decompose and consume more oxygen, resulting in hypoxic or "dead zones" that are inhospitable to aquatic life. Additionally, melanoidin contains harmful substances that can directly impact aquatic plant and animal development, reproduction, and survival, hence decreasing biodiversity and upsetting ecosystems. Because of its resistance and persistence, it is difficult to break down and poses a long-term risk to groundwater and surface water. To lessen melanoidin's negative environmental effects and safeguard aquatic ecosystems, efficient wastewater treatment techniques like microbial degradation or improved oxidation processes are crucial.

Physical and chemical techniques such as ozonization, flocculation, chemical coagulation, precipitation, activated carbon adsorption, and advanced oxidation processes are examples of conventional methods for decolorizing melanoidins [7]. The rapid efficacy of these techniques in lowering color and organic load makes them popular. These strategies, however, have significant drawbacks that compromise their viability. For example, chemical processes like ozonization and coagulation can result in the production of enormous amounts of sludge, whereas physical techniques like activated carbon adsorption can be expensive because adsorbents must be replaced frequently. Additional treatment and disposal of this sludge will increase operating expenses and environmental concerns.

II. COMPOSITION OF DWW AND ITS ENVIRONMENTAL EFFECTS

In India, there are more than 325 distilleries that produce around 45 billion litres of wasted wash and 3 billion litres of alcohol yearly [8,9]. The complicated and highly contaminated character of distillery wastewater makes it one of the most difficult industrial effluents to handle. Among its many undesirable characteristics are its high temperature, low pH, dark brown colouring, substantial ash content, and large quantities of dissolved organic and inorganic materials. It also shows extremely high levels of chemical and biochemical oxygen demand (BOD and COD) which is a major environmental issue. The property of this effluent depends on the kind of feedstock and the procedures utilized to produce ethanol. During the anaerobic treatment process, the pH of spent wash rises from 4.5 to 8.5, and it is ultimately referred to as post-methanated distillery effluent (PMDE) [10]. The spent wash is a waste with extremely high levels of nitrogen (2,200 mgL^{-1}), phenolics (4.20 mgL^{-1}), sulphate (3,410 mgL^{-1}), total solids (TS; 82,480 mgL^{-1}), chemical oxygen demand (COD; 90,000-1,10,000 mgL^{-1}), and biochemical oxygen demand (BOD; 35,000-40,000 mgL^{-1}). Several heavy metals, including Cd, Mn, Fe, Zn, Ni, and Pb, are also found in addition to these pollutants. The anaerobic treatment procedure that the wasted wash predominantly goes through transforms a substantial amount (>50%) of the BOD and COD. Anaerobic digestion, however, causes distinct metabolic alterations in the spent wash. The reduction of oxidized sulphur compounds results in the creation of a sizable quantity of hydrogen sulphide (H_2S). Sulphide creates a colloidal solution of metal sulphide colorant by binding with the heavy metals in the effluent [11]. If left untreated, this enormous amount of wastewater, roughly 40 billion litres can put a great deal of strain on the waterways and harm aquatic species on a large scale.

The primary barrier to waste remediation is the color of the distillery spent wash (DS), which contains melanodin pig ment at a weight percentage of about 2% [12]. This dark brown complex biopolymer is formed because of Maillard amino-carbonyl reaction, a non-enzymatic browning process that occurs between the amino and carbonyl groups in organic materials [13,14,15]. The Maillard reaction between D-xylose and glycine produces colorful molecules [16]. These pigments were thought to be crucial intermediates in the production of melanoidins because they became brown when they broke down. A new blue pigment known as BlueM1 has been identified which has a methine proton between two pyrrole rings and are made up of four D-xylose and glycine molecules [17]. Disaccharides and amino groups from amino acids combine to generate a Schiff base in the first step of the Maillard reaction, after

which they undergo transformation via the Amadori rearrangement product [18,19].

In addition to melanoidins, DWW reports the presence of many other harmful substances, including 2-hydroxysocaproic acid, benzene propanoic acid, di-n-octyl phthalate, and di-butyl phthalate [20,21]. These harmful substances, especially phthalates, are known to be endocrine disrupting chemicals (ECDs), which lead to hormonal imbalances and several physiological and metabolic conditions that impair both human and animal reproductive fitness [22,23].

Melanoidins are harmful to agricultural crops, prevent seed germination, and result in a manganese shortage in the soil. Even at low concentrations of 5% (v/v), raw distillery effluent has a severely harmful effect on *Vigna radiata* seed development and germination [24]. Long-term usage of untreated or inadequately treated effluents can alter the pH, increase electrical conductivity (EC), and exchangeable salt levels, among other important soil characteristics. Crop production and soil health are negatively impacted by these alterations, which result in soil salinity and alkalinity. Distillery wastewater's organic content causes organic acids to develop during decomposition, momentarily immobilizing plant nutrients and preventing crops from accessing them. Additionally, microbial diversity is frequently decreased in effluent-irrigated soils, with fungi and actinomycetes increasing and nitrogen-fixing bacteria decreasing. Long-term soil fertility may be impacted by the disruption of nutrient cycles caused by this microbial imbalance. The buildup of salts and harmful substances, such heavy metals like copper, manganese, and zinc, further deteriorates soil quality and endangers crop safety. The possibility of long-term soil damage owing to salt buildup is still a major worry, even though post-methanation distillery effluents have higher pH values and lower organic loads, making them relatively safer for agricultural use. To reduce these negative impacts while utilizing the wastewater's nutritional potential, sustainable management techniques are crucial. These include dilution of effluents, periodic rest intervals for soil recovery, and regulated application rates.

It has been reported that several refractory components found in molasses effluent, such as xenobiotic substances, anthocyanins, caramel, sugar breakdown products, melanoidin, and tannins, are resistant to degradation and remain in the environment. Decomposable organics such skatole, indole, and sulphur compounds are the source of its disagreeable smell. It is extremely hazardous to the environment and has a foul stench when dumped into rivers or canals. since of the large organic and chemical load of the effluent, its untreated disposal poses a major pollution risk since it causes oxygen depletion, water contamination, and damage to aquatic ecosystems. For these effects to be lessened, proper therapy is essential.

III. CURRENT APPROACHES ON THE TREATMENT OF DISTILLERY EFFLUENT

The treatment of distillery effluent aims to eliminate undesirable substances present in the wastewater so that it can be safely released into the environment. Various treatment approaches have been done to reduce the pollution load from the spent wash, which includes treating the wastewater with physio-chemical methods (coagulation and flocculation, electrocoagulation, adsorption, advanced oxidation, membrane treatment), and biological methods (aerobic, anaerobic and enzymatic). Given that the presence of melanoidin is hazardous, efforts have been made to understand its chemical structure and lower its emissions through chemical and microbiological degradation. This will allow for the development of more effective solutions for decolorization and degradation.

3.1 Enzymatic mechanism of DWW decolorization

Numerous enzymes have been identified by various sources as being crucial to waste treatment procedures, including peroxidases, oxidoreductases, cellulolytic enzymes, cyanidase, proteases, amylases, etc [25,26]. The two primary categories of enzymes that make up the ligninolytic system are laccases and peroxidases, which include lignin and manganese peroxidases [27]. Because they oxidize both toxic and non-toxic substrates, bacterial laccases are crucial to the bioremediation of industrial waste. Laccases are a fascinating class of common oxidoreductase enzymes that have a lot of potential for use in biotechnological applications. Numerous studies have proposed the involvement of multiple enzymes with distinct processes in DWW decolorization. Thus, understanding enzymes in the bioremediation of different industrial pollutants will lead to numerous prospects for widespread use [28,29].

3.2 Microbial degradation of melanoidin

Microbial degradation and decolorization of distillery effluents are an economical and environmentally beneficial substitute for physiochemical approaches. Numerous microorganisms, including bacteria, fungus, and algae have been documented for their capacity to degrade and discolourise the distillery effluent. Melanoidin can be eliminated by microorganisms by enzymatic breakdown, flocculation by chemicals released by the microbes, adsorption onto the surface of living (resting) and dead (autoclaved) cells and using the pigment as a source of carbon and nitrogen [30,31]. It has been documented that a variety of intracellular and extracellular enzymes including

laccases, manganese peroxidases, lignin peroxidases and sugar oxidases like sorbose oxidase, exhibit melanoidin degrading activity [32,33].

Microorganisms, particularly white-rot fungi, produce a range of nonspecific extracellular enzymes, including lactase, H_2O_2, and oxidases, including lignin peroxidases and manganese peroxidase (MnP). Using H_2O_2, lignin peroxidase (LiP) oxidatively breaks down lignin. As substrates, lignin peroxidases (LiP) and manganese peroxidases (MnP) oxidize Mn^{2+}, phenolic and non-phenolic compounds, and different hues [34].

The catalytic mechanism of LiP and MnP differs in that the former catalyzes the one-electron oxidation of phenolic and non-phenolic compounds by H_2O_2, which induces the production of the corresponding free radicals, whereas the latter catalyzes the oxidation of Mn(II) to Mn(III) dependent on H_2O_2, after which the oxidized Mn(III) catalyzes the one-electron oxidation of phenolic and non-phenolic compounds by H_2O_2, which also produces the corresponding free radicals.

A wide range of contaminants, including melanoidins, are degraded by the free radicals produced by the microbes. Several fungal, bacterial, and algal species produce H_2O_2, laccase, manganese-dependent peroxidase (MnP), and lignin peroxidase (LiP), including *Bacillus licheniformis*, *Alcaligenes sp., Penicillium pinophilum*, *Alteraria gaisen*, *Coriolus hirsutus*, *Emericella nidulans*, *Flavodon lavus*, *Oscillatoria boryana* (BDU 92181) and *Neurospora intermedia*[35,36,37,38]. Thermophilic cutinase from *Thermobifida alba* demonstrated the best decolorization efficacy and removed 76.1-78.2% of colorants. *Thermobifida alba* cutinase was immobilized on a modified chitosan carrier that was both economical and effective, and it produced a decolorization yield of 79.3–81.2% [39].

3.2.1 Fungal degradation

Numerous fungal species, including *Aspergillus fumigatus* G-2-6, *Emericella nidulans var. lata, Geotrichum candidum, Trametes sp., Aspergillus niger, Citeromyces sp., Flavodon flavus*, and others, have been employed by different providers to treat DWW [40,41,42,43].

In addition to producing some important byproducts like protein-rich fungal biomass that can be utilized as animal feed or other fungal metabolites, fungal treatment is used to lower COD, BOD, and the breakdown of organic compounds. Filamentous fungi have less nucleic acid in their biomass and are less sensitive to changes in temperature, pH, nutrients, and aeration [44].

Under ideal conditions—5 g/L of fructose, 3 g/L of peptone, 5 pH, and 35˚C-*Cladosporium cladosporioides* was able to minimize 52.6% color and 62.5% chemical oxygen demand from DWW [45]. *Cladosporium cladosporioides* was also employed under various settings, including fructose concentration 7 g/L, peptone concentration 2 g L, pH 6, and 10% (w/v) inoculum concentration, where a decrease of 62.5% and 73.6% in color and COD were observed, respectively [46]. Furthermore, *Aspergillus niger* was used in conjunction with combined coagulants to demonstrate a 97.2% color reduction from DWW [47]. Some white rot fungi are also known to secrete ligninolytic enzymes (LiP, MnP, and Laccases) that can break down xenobiotics and organometallic contaminants. Some yeast strains such as *Candida glabrata, C. tropicalis* and various fungi such as *Aspergillus niger* and white rot fungi such as *Phanerochaete chrysosporium* have been reported to effectively decolourise melanoidin from distillery effluents [48,49,50].

3.2.2 Algal degradation

Researchers are interested in treating DWW using microalgae because of the waste's products and byproducts, which are highly sought after for social welfare [51]. The potential of DWW bioremediation was examined in conjunction with a novel *Chlorella sorokiniana* sp. grown in a high-density photo bioreactor in a semi-batch mode. Since the process is energy efficient and can meet its nutrient requirements from biomethanated spentwash and energy requirements from sunlight, micro algal treatment only becomes effective after the anaerobic treatment of spent wash [52].

A 52% color decrease was achieved when 10% DWW was anaerobically treated with the microalgae *Chlorella vulgaris* and *Lemna minuscula* [53]. Additionally, a marine cyanobacterium called *Oscillatoria boryana* (BDU 92181) was also studied that degraded 5% melanoidin. *Oscillatoria willei* also exhibited increased oxidative stress and a rise in ligninolytic and anti-oxidative enzymes like lignin peroxidase, laccase, polyphenol oxidase, superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase when grown with a lower nitrogen content but with optimal phenolic compounds. According to the study's findings, the Cyanobacterium *O. willei* decolorized the substrate phenol by up to 52% in just seven days thanks to these enzymes. Given the size of the wastewater market, combining the production of microalgae biomass with nutrient removal/pollutant degradation may thus signify a significant turning point in the bioenergy ambitions.

3.2.3 Bacterial degradation

An inexpensive and environmentally beneficial substitute for physico-chemical wastewater treatment methods is the bacterial breakdown and decolorization of industrial wastewaters. Many studies have recently employed pure culture and bacterial consortiums to effectively decolorize and degrade DWW. According to DWW, the bacterial

consortium made up of *Pseudomonas aeruginosa* PAO1, *Stenotrophomonas matophila*, and *Proteus mirabilis* reduced color and COD by 67% and 51%, respectively, in 24 and 72 hours at 37˚C, it was reported utilizing a combination of *Klebsiella oxytoca, Serratia marcescens*, and *Citrobacter sp*. to remove color from Viandox sauce (13.5% v/v), caramel (30% w/v), beet molasses wastewater (41% v/v), and sugarcane molasses wastewater (20% v/v) in 9.5, 1.13, 8.02, and 17.5% of cases in just two days [54]. Additionally, in 48 hours under aerobic conditions, they used a consortium of *Acinetobacter sp., Pseudomonas sp., Comamonas sp., Klebsiella oxytoca, Serratia marcescens,* and an unidentified bacterium to remove 26.5% of the color from DWW [55].

The capacity of bacterial cultures to break down these pigments was assessed. The melanoidins were recently decolorise using lactic acid bacteria (*Lactobacillus coryniformis, L. sakei, L. plantarum, Weisella soli, Pediococcus parvulus,* and *P. pentosaceus*). 44% of melanoidins are decouraged by the strain *Lactobacillus plantarum* [56]. An attempt was made to decolorize the melanodins by employing *Bacilli* consortia where the capacity to remove color was evaluated in two mixed bacterial cultures (C1 and C2) of the species *Bacillus* [57]. The consortiums *Proteus mirabilis* (IITRM5; FJ581028), *Bacillus sp.* (IITRM7; FJ581030), *Roullella planticola* (IITRM15; GU329705), and *Enterobacter sakazaki*i (IITRM16, FJ581031) were also created in a 4:3:2:1 ratio. Within ten days, this consortium oversaw 75% of the melanoidins' decolorization [58].

At the ideal pH of 7.5 and temperature of 37 °C, the isolate *Alcaligenes faecalis* strain SAG5 exhibited 72.6% melanoidin decolorization on the fifth day of incubation. The mung bean (*Vigna radiata*) toxicity study showed that the raw distillery effluent was extremely harmful to the environment in comparison to the biologically treated distillery effluent, indicating that the effluent following bacterial treatment is safe for the environment [59].

Many different bacterial cultures, including *Pseudomonas putida, P. aeruginosa, Lactobacillus plantarum, Bacillus circulans, B. megaterium, B. irmus, B. thuringiensis, B. cereus, Lactobacillus hilgardii, L. coryniformis,* and *Xanthomonas fragariae,* have been shown to be active in decolorization and degradation of distillery effluents [60,61,62,63].

From soil contaminated with distillery effluent, a thermotolerant bacterial culture consisting of *Bacillus subtilis, B. cereus*, and *Pseudomonas aeruginosa*. Of them, *B. subtilis* demonstrated the greatest degree of decolorization (85%) at 45°C using relatively low carbon (0.1%, w/v) and nitrogen sources (0.1%, w/v) throughout the course of a brief 24-hour incubation period [64]. Under ideal circumstances*, Pseudomonas sp*. and *B. cereus* had decolorization rates of 69% and 73%, respectively. *B. subtili*s demonstrated the strongest thermotolerance, withstanding temperatures between 35°C and 50°C without affecting the exponential development phase. The genus *Bacillus* demonstrated the best bioremediation efficiency when compared to other bacterial cultures, according to findings from several examinations.

Three thermotolerant bacterial isolates- *Bacillus nitratireducens* (B2), *B. paramycoides* (B1), and *Brucella tritici* (B3) were shown to be melanoidin-decolorizing agents in research. These isolates were further optimized for decolorization under a range of nutritional and physicochemical conditions. After 40 hours of incubation under static circumstances with 0.5% glucose (w/v), 0.5% peptone (w/v), 0.05% MgSO4, and 0.01% KH2PO4 at a pH of 6.0, *B. nitratireducens* (B2) showed the greatest degree of decolorization (86%) of the three species at 40°C [65].

Utilizing the axenic and mixed bacterial consortia [*Bacillus licheniformis* (RNBS1), *Bacillus sp.* (RNBS3), and *Alcaligenes sp.* (RNBS4)], the degradation of synthetic and natural melanoidins was investigated. The mixed consortium was more successful than axenic cultures in decolorizing synthetic and natural melanoidins by 73.7% and 69.8%, respectively, while axenic cultures RNBS1, RNBS3, and RNBS4 decolored synthetic melanoidins by 65.88%, 62.5%, and 66.1% and natural melanoidins by 52.6%, 48.9%, and 59.6%, respectively. In comparison to controls, the HPLC analysis of degraded samples revealed fewer peak regions, indicating that the breakdown of melanoidins by isolated bacteria may be primarily responsible for the color intensity drop.

Lactobacillus plantarum (No. PV71-1861) isolated from pickle samples exhibited greatest melanoidin pigment (MP) decolorization yield of 68.12% using an MP solution that included 2% glucose, 0.4% yeast extract, 0.1% KH2PO4, 0.05% MgSO47H2O, an initial pH of 6 and in a 7-day static condition at 30 °C [66].

Bacterial consortium containing three bacterial cultures showed the ability to decolorize and degrade wastewater quickly demonstrating 67 ± 2% decolorization in 24 hours and 51 ± 2% reduction in chemical oxygen demand in 72 hours when incubated at 37 °C under static conditions in wastewater supplemented with 0.5% glucose, 0.1% KH2PO4, 0.05% KCl, and 0.05% MgSO4.7H2O. Similar decolorization studies reported *Pseudomonas aeruginosa* PAO1, *Stenotrophomonas maltophila*, and *Proteus mirabilis* to be highly efficient in melanoidin decolorization. *Pseudomonas sp.* was able to accomplish maximum decolorization of up to 56% and a 63% reduction

in the COD of the wasted wash at pH 6.8 -7.2, temperature 30-35 ºC, and glucose 0.4% (w/v) after 72 hours. The isolate's biodegradation of melanoidin pigments was validated by spectrophotometric and HPLC examination of the treated effluent. This method might be utilized to create an environmentally acceptable and reasonably priced biotechnology package for the bioremediation of wasted wash prior to disposal. *Pseudomonas fluorescens*, which was isolated from molasses-contaminated soil samples, decolorized up to 76% of molasses wastewater (MWW) samples in four days at 30°C in non-sterile settings [67].

Three *Bacillus* isolates *B. thuringiensis* (MTCC 4714), *B. brevis* (MTCC 4716), and *Bacillus sp.* (MTCC 6506) were observed to decolorize synthetic melanoidins, such as GGA, GAA, SGA, and SAA. In addition to the decolorization of all four melanoidins (10%, v/v), a significant decrease in the values of physicochemical parameters was observed. *Bacillus sp.* (MTCC 6506) and *B. brevis* (MTCC 4716) induced the most decolorization, followed by *B. thuringiensis* (MTCC 4714). There was 15% higher decolorization in the medium with glucose as the only carbon source than in the one with both carbon and nitrogen sources. In the presence of glucose as the only energy source, melanoidin SGA underwent the greatest amount of decolorization (50%) whereas melanoidin GAA underwent the least amount. Melanoidins can be broken down oxidatively by acetogenic bacteria. Acetogenic bacterial strain, strain No. BP103 exhibited decolorization yield of 76.4 ± 3.2% after 5 days of cultivation at 30 °C in molasses pigments medium that contained 3.0% glucose, 0.5% yeast extract, 0.1% KH2PO4, and 0.05% MgSO4.7H2O, with the pH set to 6.0 [68].

Three bacterial strains obtained from the activated sludge of a distillery wastewater treatment plant *Xanthomonas fragariae*, *Bacillus megaterium*, and *Bacillus cereus* in both free and immobilized form were used in batch studies to investigate the degradation of anaerobically digested distillery wastewater. Up to 48 hours, the removal of COD and color with all three strains increased with time; beyond that, only a little rise in COD and color removal efficiency was seen for up to 72 hours. Removal efficiency was rather stable for the next 120 hours after this. The maximal COD and color removal efficiency for both free and immobilized cells of all three strains ranged from 66 to 81% and 65 to 75%, respectively.

The capacity of several microorganisms to decolorize molasses wastewater in both thermophilic and anaerobic settings was evaluated. The best strain was determined to be MD-32, which was recently obtained from a soil sample. According to taxonomical research, the strain most closely resembles *B. smithii* and is a member of the genus *Bacillus*. Under anaerobic circumstances, the strain decolorized 35.5% of the molasses pigment in 20 days at 55°C; however, when grown aerobically, no decolorization activity was seen [69].

Fifty isolates exhibited decolorization activity on solid medium (clear zone), according to the results. In the liquid medium containing molasses pigments, the strains No. BP103 and No.13A exhibited the best decolorization activity among them. When yeast extract was used as the nitrogen source, the decolorization activity of strains No. 13A and BP103 was 80.50% and 82.00% respectively. Under ideal circumstances and medium compositions, the strains No. 13 A and BP103 had decolorization activities of 90.54% and 96.75 % respectively. Strain No. 13A and No. BP103 were identified as *Acetobacter aceti* [70].

Three thermotolerant bacterial isolates - *Brucellatritici (B3), Bacillus nitratireducens (B2),* and *Bacillus paramycoides* (B1) were shown to be melanoidin-decolorizing agents. These isolates were further optimized for decolorization under a range of nutritional and physicochemical conditions. After 40 hours of incubation under static circumstances with 0.5% glucose (w/v), 0.5% peptone (w/v), 0.05% MgSO4, and 0.01% KH2PO4 at a pH of 6.0*, B. nitratireducens* (B2) showed the greatest degree of decolorization (86%) of the three species at 40°C. A consortium of *Staphylococcus aureus* and *Serratia odoriferae* demonstrated the highest decolorization efficiency, achieving 89% [71].

A thermotolerant bacterial culture comprising *Pseudomonas aeruginosa, Bacillus subtilis,* and *B. cereus* was recovered from soil polluted with distillery effluent. During a short 24-hour incubation period, *B. subtilis* showed the highest degree of decolorization (85%) at 45°C utilizing relatively modest carbon (0.1%, w/v) and nitrogen sources (0.1%, w/v). *B. cereus* and *Pseudomonas sp*. both exhibited decolorization rates of 73% and 69% under optimal conditions. Withstanding temperatures ranging from 35 to 50°C without compromising the exponential growth phase, *B. subtilis* showed the highest thermotolerance. According to results from many analyses, the species *Bacillus* had the highest bioremediation efficacy when compared to other bacterial cultures.

Thirteen bacterial isolates from a bioreactor treating a mixture of municipal and molasses wastewater were analysed for their ability to degrade and decolorize melanoidins. The isolates were initially screened for manganese peroxidase activity and their growth potential in four synthetic melanoidin solutions with concentrations ranging from 3 to 7 gL^{-1}. Among them, three isolates showed potential for manganese peroxidase production: two strains of *Klebsiella* sp. (B2 and B3), one strain of

Escherichia coli (B4), and one strain of *Lactobacillus kefiri* (B1). These isolates exhibited high tolerance to synthetic melanoidins. 16S rDNA sequencing confirmed their close relation to *E. coli* and *Klebsiella sp.* [72].

A strain of *Streptococcus sp.* from a distillery near a natural environment and optimized it for decolorizing distillery effluent at different physico-chemical and nutritional levels [73]. These bacteria demonstrated the highest degree of decolorization, 87%, at 40˚C using modified GYPE Medium, which is 1% molasses medium (1%, Grade-C molasses, 0.2%, Yeast extract, 0.3%, Peptone, 0.05%, MgSO4, 0.05%, K2HPO4 with 3.5 OD effluent) pH-6.0 in 30 hours.

Among the 19 bacterial isolates that were recovered from a distillery sludge, strain *Bacillus albus* showed greater capacity to remove the color of the effluent. The bacteria caused up to 83% of the effluent to become significantly decolored [74]. Manganese peroxidase (MnP) produced by *Pseudoduganella violacea* demonstrated significant decolorization of Maillard products, achieving up to 83.68% at a temperature of 37°C within 192 hours of incubation holding potential for effective bioremediation applications to degrade Maillard products [75].

IV. NOVEL APPROACHES USING BACTERIAL STRAINS

Although several bacterial strains have been found and used to decolorize melanoidin, these strains' effectiveness and versatility are frequently restricted. Effective breakdown of melanoidin requires the employment of highly specialized or diversified enzymatic pathways due to its complicated structure, which comprises a variety of chemical linkages and a large molecular weight. Furthermore, variables such as pH, temperature, salinity, and the presence of other contaminants in industrial effluents might affect the activity of already recognized bacterial strains. This restriction emphasizes the necessity of investigating novel bacterial strains that are hardy and more effective in a range of industrial and environmental settings.

Discovering new bacterial strains may help find microorganisms with potential enzymatic properties, improved metabolic processes, and cooperative relationships with other living organisms. These strains could enable more efficient and cost-effective bioremediation processes, reducing dependency on chemical treatments that are often less sustainable and environmentally harmful. Furthermore, investigating novel strains may result in the development of consortia or modified microorganisms specifically suited for industrial effluents, increasing the viability and scalability of melanoidin decolorization procedures. In conclusion, investigating novel bacterial strains is essential to overcoming the drawbacks of current bioremediation techniques and improving the sustainability and effectiveness of melanoidin removal, which helps to preserve the environment and ensure that industries adhere to pollution regulations.

The use of microorganisms for melanoidin decolourisation offers numerous advantages in wastewater treatment. Microbial decolourisation is eco-friendly, as it avoids harmful chemicals, thereby reducing environmental impact. It is also cost-effective, utilizing naturally occurring microorganisms instead of expensive chemical reagents [76]. Many microorganisms, such as bacteria and fungi, have the capability to biodegrade melanoidin into less toxic compounds, enhancing the safety of treated water. Furthermore, microorganisms exhibit adaptability to diverse environmental conditions, making them effective in various wastewater scenarios. This process is sustainable, as microorganisms are renewable resources that can be maintained with minimal inputs. Certain strains also demonstrate specificity in targeting melanoidin, ensuring efficient decolourisation without affecting other components in the wastewater. Additionally, microbial degradation pathways can produce valuable by-products, contributing to resource recovery. The scalability of microbial treatments, from laboratory to industrial applications, and their minimal energy requirements make them versatile and cost-saving. Finally, microorganisms can be integrated with other treatment methods for improved decolourisation efficiency.

Integrating bacterial decolorization into distillery effluent treatment systems aligns with the principles of green chemistry and promotes sustainable development. This approach provides an effective solution to the twin challenges of pollution mitigation and resource optimization, making it a critical area of scientific inquiry.

V. TREATMENT USING NANOPARTICLES

Melanoidin decolourisation by utilizing biosynthesized silver nanoparticles and bacterial extract (*Bacillus sp*.) in an immobilised state, where the bacterial extracellular supernatant showed over 65% melanoidin decolorization (in 12 hours) [77]. On the other hand, biosynthesized AgNPs demonstrated 82% clearance under comparable circumstances. The greatest melanoidin elimination of 92% in 12 hours is achieved by the cell free extract immobilized with manufactured AgNPs; this highlights nano-coupled biomaterial immobilization as an appropriate method for quick melanoidin decolorization. ZnO nanoparticles possess significant potential for melanoidin adsorption,

offering an alternative method for treating colored effluents [78].

VI. CONCLUSION

Environmental impacts on the ecosystem due to discharge of untreated distillery wastewater could be addressed through improvised microbial bioremediation methods. Release of microbial decolorized distillery effluent could reduce the damaging effects of the wastewaters and could overcome the detrimental effects of physical and other chemical methods of treatments. The novel techniques that include use of microbial enzyme systems, immobilized bacterial consortia and use of nanoparticles in treating the distillery wastewater could be a cost-effective method. Combination methods using microbial consortium containing mixed cultures of potential melanoidin decolorizing microbes and nanoparticles holds great promise in treating the distillery effluents.

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Contribution of green technologies on infrastructure performance in Rwanda, a case of green buildings constructed in the green Gicumbi project

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Abstract— *In Rwanda, integrating green technologies in building projects presents an opportunity to enhance infrastructure performance while minimizing environmental impact. However, the extent to which these technologies contribute to the overall effectiveness of these infrastructures remains underexplored, especially in rural contexts like the Gicumbi District. Therefore, this study aimed to assess the contribution of green technologies used in green building projects to infrastructure performance. To achieve this objective, the study used a descriptive research design and a census sampling technique to select the entire population of 107 people, including the residents and beneficiaries of the green buildings project. Primary data were collected through a combination of questionnaires, interviews, and direct observations, while secondary data were gathered through documentary analysis and published research studies. The findings revealed that all green technologies such as resources-efficient design, low-emission materials, and waste management have a significant positive influence on the performance of the Green Buildings project in the Gicumbi district where a increase of one unit in resources-efficient design, low-emission materials, and waste management would lead to increase of 0.152, 0.14, and 0.178 in performance of Green Buildings. The study concluded that the change of 65% in the performance of Green Buildings is due to green technologies in terms of resource-efficient design, low-emission materials, and waste management at a 95% confidence interval.*

Keywords— ***Gicumbi District, Green technologies, Infrastructure performance, Rwanda, Sustainable development.***

I. INTRODUCTION

In recent years, there has been an intensified global focus on sustainable development and environmental conservation, driving the widespread adoption of green technologies across various sectors. This movement, primarily aimed at mitigating the effects of climate change, has been supported by a broad array of international frameworks, governmental policies, and private-sector investments. The Global Green Economy Index (GGEI), which evaluates the green performance of 130 countries, consistently highlights nations such as Sweden, Denmark, and Switzerland for their exemplary integration of green technologies through robust policies and significant investments (Edenhofer et al., 2014). Globally, the development and deployment of green technologies have become integral to the sustainability agendas of leading nations. For instance, the United States has pioneered green innovation, with policies supporting renewable energy, energy efficiency, and clean transportation (U.S. Department of Energy, 2020). Similarly, China has made substantial strides in renewable energy infrastructure and electric vehicle adoption, driven by both government targets and increasing environmental awareness (International Energy Agency, 2020).

In Africa, nations are increasingly integrating green technologies into their development strategies, especially as climate change disproportionately impacts the continent. Programs like South Africa's Renewable Energy Independent Power Producer Procurement (REIPPP) have successfully attracted investments in solar and wind energy, significantly reducing the country's reliance on fossil fuels (Govender & Adam, 2021). Similarly**,** Uganda has made significant strides in expanding access to solar energy, particularly in rural areas (Naluwairo et al., 2020). For Rwanda, a country known for its ambitious sustainability goals, the adoption of green technologies has been pivotal in shaping national policies and infrastructure projects aimed at promoting environmental sustainability and climate resilience. Rwanda has been a leader in promoting eco-friendly infrastructure, notably through the Green Kigali Initiative, which seeks to transform the capital city into a sustainable urban environment through green spaces, sustainable waste management, and green building standards. This initiative is part of a broader vision to integrate green technologies into Rwanda's infrastructural landscape to foster long-term sustainability. Additionally, Rwanda has set a target to achieve 60% renewable energy generation by 2030 and is working to enhance electricity access and reduce carbon emissions (Government of Rwanda, 2020). These developments reflect Rwanda's growing role as a leader in green infrastructure across East Africa and the wider African continent.

The significance of green technologies in improving infrastructure performance is exemplified by global benchmarks, such as the Bullitt Center in the U.S. and The Edge in the Netherlands, which have set high standards for sustainability in commercial buildings. In Rwanda, the Green Gicumbi Project serves as a vital case study of how green technologies have been successfully integrated into building design, construction, and operation to improve sustainability. This project aligns with Rwanda's broader goal to reduce greenhouse gas emissions, enhance energy efficiency, and promote climate-resilient infrastructure. The study that forms the basis of this paper evaluates the impact of green technologies on the performance of infrastructures in Rwanda, with specific reference to the Green Gicumbi Project**.** The project serves as a model for integrating energy-efficient designs**,** renewable energy systems**,** and environmentally sustainable construction materials in Rwanda's infrastructure development. By examining the practical outcomes and operational benefits of these green technologies, the study offers insights into how such technologies can enhance both environmental and economic performance. This research contributes to the growing body of knowledge on sustainable infrastructure and green technologies, providing valuable recommendations for the future implementation of green building practices in Rwanda and beyond.

II. MATERIALS AND METHODS

2.1. Sampling techniques

The study employed a census sampling technique, which was deemed appropriate and reliable due to the limited number of respondents within the study area. This technique offers several advantages, including comprehensive coverage of the entire population under study, which eliminates sampling errors and ensures accurate and representative findings. The census approach allowed for precise estimates of various population characteristics, including demographics, attitudes, and behaviors, and produced highly accurate data, which were essential for decision-making, policy formulation, and resource allocation.

In addition to the census, semi-structured interviews were conducted with key stakeholders involved in the green building projects. These stakeholders included an engineer from the Rwanda Housing Authority, an infrastructure engineer and building inspector from Gicumbi District, an engineer from Rubaya Sector, and local leaders such as the executive secretaries of Rubaya Sector, Nyamiyaga Cell, and Kabeza Village. These individuals were selected using purposive sampling due to their direct involvement and expertise in the planning, execution, and oversight of the projects. The interviews were designed to explore the objectives, challenges, outcomes, and the role of local communities and leaders in the success of green building initiatives. The semi-structured format allowed for flexibility in responses while ensuring that key topics were addressed. The interviews were conducted in person to facilitate a detailed and contextual understanding of the green building projects.

2.2. Data collection tools

2.2.1. Questionnaire

The questionnaire is a suitable research instrument due to its structured format and convenience for collecting data within a short time. In addition, a questionnaire has the ability to accord a respondent adequate time to respond as well as a sense of anonymity to a respondent. Moreover, it's a cost-effective way of collecting data since a lot of respondents can be interviewed, covering a large geographical area (Walliman, 2011). The questionnaire was chosen as a suitable instrument for the above reasons. In this study, closed-ended questionnaires composed of 5-point Likert-scale questions were used. The first section of the questionnaire dealt with respondents' demographic details. The second section covered the variables used for the study.

107 questionnaires were distributed to the residents of the green buildings in Gicumbi District, with each questionnaire carefully designed to gather insights on their experiences and perspectives regarding green building initiatives. The questionnaires have been returned by all the respondents, achieving a 100% response rate. This full participation ensured a comprehensive and accurate representation of the residents' views, contributing valuable data to the research on the impact and effectiveness of green building projects.

2.2.2. Interview

The interview was employed as a vital data collection tool to gather detailed and qualitative information from individuals directly involved in the initiation, monitoring, and evaluation of the Green Gicumbi project. This method allowed for an in-depth exploration of specific insights, experiences, and expert opinions regarding the project's processes and outcomes. A semi-structured format has been used, combining pre-determined questions with the flexibility to delve deeper into emerging topics during discussions. To ensure a comprehensive understanding of the project, seven key informants were strategically selected based on their roles and expertise. These included an engineer in charge of construction at the Rwanda Housing Authority, who provided technical insights on the design and construction phases and their alignment with national housing policies, and an infrastructure engineer at Gicumbi District, who offered a district-level perspective on infrastructure planning, resource allocation, and supervision. Additionally, the building inspector at Gicumbi District shared observations on compliance with construction standards, environmental sustainability, and quality control during implementation. An engineer in charge of infrastructure in the Rubaya sector highlighted sector-specific challenges, opportunities, and contributions, while the executive secretary of the Rubaya sector provided administrative insights on governance, stakeholder coordination, and monitoring progress. At the grassroots level, the cell Executive secretary of Nyamiyaga discussed the project's impact on the community and integration of local needs, and the Kabeza village community leader offered perspectives on community engagement, project benefits, and feedback mechanisms. The interviews were conducted in a conversational format, ensuring participants could express their views freely while addressing critical aspects of project initiation, monitoring, and evaluation. The qualitative data obtained enriched the study by complementing other data collection methods and contributing to a holistic understanding of the Green Gicumbi project.

2.2.3. Observation

During the site visit conducted by the researcher to the green buildings in Gicumbi District, Rubaya Cell, Nyamiyaga Cell, and Kabeza village, several key observations were made regarding the green technologies implemented and the overall performance of the buildings. The observation tool used during the site visit was designed to assess the implementation and performance of various green technologies in the buildings. The researcher employed a structured observational checklist that focused on key aspects such as energy-saving measures, building orientation for natural lighting and ventilation, waste management practices, water management systems, and the use of local materials. Additionally, the condition of the buildings and the maintenance of green technologies were also observed. This observational method allowed for a comprehensive evaluation of how the green technologies were integrated into the buildings and how well they were functioning. The observations were recorded systematically, and the findings were later analyzed to assess the effectiveness of these green initiatives in promoting sustainability and operational efficiency.

2.2.4.Documentary analysis

This instrument was selected based on its relevance to sustainable practices, showcasing the implementation of green technologies in a real-world infrastructure project like Green Buildings Constructed in Green Gicumbi. The focus lies in evaluating how these technologies impact the building's design, construction, operational efficiency, and overall environmental sustainability. The researcher conducted a Contextual analysis to understand the broader landscape in which Green Buildings Constructed in Green Gicumbi were developed. This involves looking into Rwanda's environmental policies, sustainable development goals, economic factors influencing green technology adoption, and the roles played by various stakeholders such as government agencies, architects, engineers, and environmental experts. Understanding this context provides insights into the motivations, challenges, and opportunities associated with integrating green technologies into infrastructure projects in Rwanda.

III. RESULTS AND DISCUSSION

3.1. Assessment of green technologies employed in the green buildings project

The study sought to assess green technologies used by the developer for the Green Buildings Project. The respondents were asked whether they agreed or disagreed with the statement regarding green technologies used in the

project. The study used descriptive statistics such as mean, standard deviation, frequency, and percent.

3.1.1. Resources efficient design for the green buildings project.

The study sought to assess resource-efficient design for Green Buildings Constructed in the Green Gicumbi Project. The respondents were asked whether they agreed or disagreed with the statement regarding resource-efficient design. The study used descriptive statistics such as mean, standard deviation, frequency, and percent. The results are presented in Table 3.1.

Table 3.1: Resource-efficient design

Resources Efficient Design	SD		D		N		A		SA		Mean	SD
	fi	%	fi	%	fi	%	Fi	%	Fi	%		
Availability of energy-efficient LED lighting	4.0	3.7	3.0	2.8	5.0	4.7	26.0	24.3	69.0	64.5	4.4	0.977
Availability of high-efficiency HVAC systems	2.0	1.9	3.0	2.8	9.0	8.4	22.0	20.6	71.0	66.4	4.5	0.90
Availability of high-efficiency sound insulation	3.0	2.8	5.0	4.7	4.0	3.7	13.0	12.1	82.0	76.6	4.6	0.97
Availability of passive design strategies like orientation, shading, and natural ventilation	4.0	3.7	7.0	6.5	5.0	4.7	21.0	19.6	70.0	65.4	4.4	1.08
Use of water efficiency practices like utilizing low-flow fixtures, greywater recycling systems, and rainwater harvesting to reduce water consumption	5.0	4.7	5.0	4.7	2.0	1.9	19.0	17.8	76.0	71.0	4.5	1.06
					Overview						**4.45**	

Source: Primary data, 2024

The findings from Table 3.1 on resource-efficient design in the Green Buildings Project in Gicumbi District reveal a strong integration of sustainable practices across various key elements. Starting with energy-efficient LED lighting, this feature received high affirmation from respondents, with 64.5% strongly agreeing on its availability. LED lighting is a well-known energy-saving choice that significantly reduces electricity consumption, aligning with sustainable energy goals. This element's mean score of 4.4, with a standard deviation of 0.977, reflects a high level of satisfaction and acceptance among respondents, underlining its prominent role in enhancing energy efficiency within green buildings. The availability of high-efficiency HVAC (heating, ventilation, and air conditioning) systems also ranked highly, with 66.4% of respondents strongly agreeing to its presence, resulting in a mean score of 4.5 and a lower standard deviation of 0.90. These systems play a crucial role in maintaining optimal indoor air quality and climate control while minimizing energy use. The positive response suggests that such systems have been effectively incorporated, supporting the project's commitment to reducing energy waste and promoting a healthier indoor environment. Sound insulation emerged as the most positively received feature, with 76.6% of respondents strongly agreeing on its effectiveness. With a mean score of 4.6 and a standard deviation of 0.97, this element reflects a strong emphasis on comfort and noise reduction, which are vital for occupant well-being. High-efficiency sound insulation reduces noise from both internal and external sources, contributing to a more tranquil and conducive environment within the buildings. In terms of passive design strategies, such as building orientation, shading, and natural ventilation, these elements received 65.4% strong agreement from respondents, yielding a mean score of 4.4 and a slightly higher standard deviation of 1.08. The incorporation of passive design measures demonstrates a commitment to leveraging natural resources to enhance energy efficiency and occupant comfort. By reducing reliance on mechanical systems, these strategies contribute

to the overall sustainability of the project and highlight the use of thoughtful architectural design.

Lastly, water efficiency practices, including rainwater harvesting, showed strong positive feedback, with 71.0% of respondents strongly agreeing with their presence. This feature scored a mean of 4.5 with a standard deviation of 1.06, indicating a high level of satisfaction. Efficient water use is critical in green building design, as it addresses both resource conservation and the reduction of operational costs. The project's emphasis on water-saving measures underscores its alignment with environmental conservation objectives. Overall, the analysis reveals an average mean score of 4.45 across all design elements, which translates to an 89% agreement on the effectiveness of resource-efficient design within green buildings. This high level of consensus provides strong evidence of the successful integration of sustainable practices, demonstrating the project's commitment to enhancing infrastructure performance through green technologies.

3.1.2. Low-emission materials.

In sustainable construction, selecting low-emission materials is essential for minimizing environmental impact and improving indoor air quality. Low-emission materials help reduce the release of harmful pollutants, such as volatile organic compounds (VOCs), which can contribute to poor air quality and pose health risks to occupants. By using materials with reduced or zero emissions, green buildings can ensure a healthier indoor environment, contribute to lower carbon emissions, and support overall sustainability goals. Considering the importance of low-emission materials in achieving these objectives, this study examined the extent to which such materials have been incorporated into the Green Buildings Project in the Gicumbi District. This aspect was selected for analysis due to its direct impact on both environmental sustainability and the health and well-being of building occupants. Findings were collected on the types of low-emission materials used, their specific applications, and how frequently they were adopted within the project, with an emphasis on any patterns or trends observed. Through this evaluation, the study aims to provide insights into the project's commitment to reducing emissions and promoting a sustainable built environment. In the following sections, we will delve into the findings related to low-emission materials and explore how these materials have contributed to the project's environmental and health objectives.

The analysis of low-emission materials in the Green Buildings Project in Gicumbi District reveals significant incorporation of sustainable construction practices, particularly in terms of material selection aimed at minimizing environmental impact and enhancing indoor air quality. One of the main focus areas was the use of low-VOC (Volatile Organic Compounds) or zero-VOC materials, which are crucial for reducing pollutants that can affect respiratory health and overall air quality. The study found that 60.7% of respondents strongly agreed that low-VOC or zero-VOC materials were utilized, and an additional 29.3% agreed. This high approval rate resulted in a mean score of 4.36 and a standard deviation of 1.03, underscoring the project's commitment to healthy, sustainable indoor environments.

Another important area was adherence to formaldehyde content standards, an aspect that received strong agreement from 61.7% of respondents, with 28.3% also in agreement. The project's high compliance with formaldehyde standards led to a mean score of 4.40 and a standard deviation of 0.97, indicating a priority on reducing exposure to this harmful compound, which is commonly found in building materials and can cause eye, nose, and throat irritation. This commitment highlights the project's focus on occupant health and regulatory compliance.

The use of certified and labeled materials was also highly regarded, with 71.0% of respondents strongly affirming this practice, which yielded a mean score of 4.39 and a standard deviation of 1.16. Certification ensures that the materials used meet established low-emission standards and contributes to accountability and transparency in material sourcing. This level of assurance helps validate the project's dedication to environmentally responsible building practices, as certified materials are often tested and verified to have lower emission profiles.

Furthermore, the project prioritized the selection of building materials that are naturally low in emissions or have been treated to reduce emissions. This approach received strong agreement from 60.7% of respondents, resulting in a mean score of 4.28 and a standard deviation of 1.15. Using naturally low-emission materials not only aligns with sustainable construction principles but also reduces reliance on chemically intensive treatments, supporting both indoor air quality and overall project sustainability. Lastly, the use of sustainable materials that consider low embodied energy, recycled content, and recyclability was a significant focus of the project. This aspect received strong agreement from 68.2% of respondents, achieving a mean score of 4.33 and a standard deviation of 1.23. Selecting materials with low embodied energy helps reduce the total carbon footprint of the building while incorporating recycled content and recyclable options to support resource conservation and waste reduction. This lifecycle approach to material selection underscores the project's dedication to minimizing environmental impact not only during construction but also throughout the building's lifespan. In summary, the study

reveals an overall average mean score of 4.35, corresponding to 87% agreement among respondents on the effective use of low-emission materials. This high rating indicates a solid integration of sustainable practices within the project, demonstrating a proactive approach to enhancing indoor air quality, occupant health, and environmental performance. The careful selection and use of low-emission materials reflect a comprehensive commitment to sustainability, which positions the Green Buildings Project in the Gicumbi District as a model for environmentally responsible construction in Rwanda.

Table 3.2: Low-emission materials

	SD		D		N		A		SA		MEAN	STD
Low-emission materials												
	fi	**%**	**fi**	**%**	**fi**	**%**	**fi**	**%**	**fi**	**%**		
Construction materials are low-VOC or zero-VOC	5	5.1	3	3.03	5	5.1	29	29.3	65	60.7	4.36	1.03
Construction materials comply with Formaldehyde Content standards	4	4	2	2.02	7	7.1	28	28.3	66	61.7	4.4	0.97
Construction materials are labeled and certified	7	7.1	4	4.04	5	5.1	15	15.2	76	71	4.39	1.16
Building materials are naturally low in emissions or have undergone treatments to reduce emissions	6	6.1	6	6.06	5	5.1	25	25.3	65	60.7	4.28	1.15
Use of sustainable materials with low embodied energy, recycled content, and recyclability to minimize environmental footprint throughout their lifecycle	9	9.1	4	4.04	3	3	18	18.2	73	68.2	4.33	1.23
			Overview								**4.35**	

Source: Primary data, 2024

3.1.3. Waste management.

Effective waste management is a critical component of sustainable building practices, aimed at reducing the environmental footprint of construction projects. Waste generated during construction, if not properly managed, can lead to increased landfill usage, environmental degradation, and health hazards. Implementing waste management practices such as recycling, reusing materials, and minimizing construction waste helps conserve resources, reduce pollution, and lower disposal costs. For green building projects, sustainable waste management also aligns with broader environmental goals, promoting responsible resource use and contributing to a circular economy. In the context of the Green Buildings Project in Gicumbi District, waste management practices were evaluated to understand how effectively the project has managed waste during construction and operation.

This aspect was chosen for analysis because proper waste management is essential in ensuring that the project minimizes its ecological impact and aligns with the principles of green construction. By assessing practices like material recycling, waste segregation, and reduction in waste production, the study aimed to determine how well the project contributes to environmental sustainability and resource efficiency. The findings, presented in Table 3-3, shed light on the specific waste management practices

implemented in the project, including the extent to which recycling, reuse, and reduction efforts have been adopted and how they are perceived by stakeholders. Through this analysis, the study provides insight into the project's commitment to waste reduction and its effectiveness in meeting sustainability goals.

Table 3.3: Waste Management for Green Buildings

Waste Management	SD		D		N		A		SA		Mean	STD
	Fi	%	fi	%	fi	%	fi	%	fi	%		
Availability of Waste Reduction Goals	5	4.7	5	4.67	4	3.7	30	28.0	63	58.9	4.32	1.07
Availability of Waste Management Plan	4	3.7	2	1.87	12	11.2	25	23.4	64	59.8	4.34	1.01
Source Separation	7	6.5	4	3.74	4	3.7	17	15.9	75	70.1	4.39	1.16
Recycling and Reuse Programs	6	5.6	7	6.54	5	4.7	24	22.4	65	60.7	4.26	1.17
Certifications and Standards	8	7.5	10	9.35	2	1.9	20	18.7	67	62.6	4.20	1.29
Overall view											**4.30**	

Source: Primary data, 2024

The findings on waste management practices in the Green Buildings Project in Gicumbi District indicate substantial integration of sustainable waste management measures. Among the key aspects assessed, the availability of clear waste reduction goals was a notable factor. A majority, 58.9% of respondents, strongly agreed that waste reduction targets were established for the project, with an additional 28.0% agreeing. This strong alignment with waste reduction goals achieved a mean score of 4.32 and a standard deviation of 1.07, reflecting a consistent focus on minimizing construction waste from the outset. Another critical component was the presence of a waste management plan, which guides how waste should be managed throughout the construction process. Here, 59.8% of respondents strongly agreed, and 23.4% agreed, that such a plan was in place, leading to a mean score of 4.34 and a standard deviation of 1.01. This finding underscores the importance placed on structured waste management protocols, which help prevent unregulated waste disposal and promote organized handling of construction debris.

Source separation practices, aimed at sorting waste by type for more efficient recycling or disposal, were also highly rated. An impressive 70.1% of respondents strongly agreed that source separation was practiced, and another 15.9% agreed. The high level of support for this practice resulted in a mean score of 4.39 and a standard deviation of 1.16. Source separation is instrumental in enhancing recycling efforts and reducing contamination, thus facilitating more effective resource recovery. Recycling and reuse programs were similarly prioritized, as indicated by the 60.7% of respondents who strongly agreed and the 22.4% who agreed with their presence on the project. This emphasis on recycling and reuse resulted in a mean score of 4.26 and a standard deviation of 1.17, signaling a clear commitment to reducing raw material usage and limiting landfill contributions through responsible reuse of materials whenever possible. Lastly, adherence to certifications and standards in waste management received relatively strong support. A total of 62.6% of respondents strongly agreed that the project adhered to relevant waste management certifications, while 18.7% agreed, resulting in a mean score of 4.20 and a standard deviation of 1.29. Compliance with recognized certifications not only ensures high standards but also adds credibility to the project's environmental practices. Overall, the findings indicate an average mean score of 4.30, corresponding to an 86% level of agreement on the effective use of waste management practices. This high level of agreement across multiple dimensions, from waste reduction goals to recycling initiatives, highlights the project's dedication to sustainable waste management. These practices ensure that the Green Buildings Project in Gicumbi District aligns with environmental goals, reduces its ecological footprint, and promotes responsible resource management, setting an example for future green building initiatives in Rwanda.

3.2. Performance of green Buildings constructed in the Green Gicumbi Project

Evaluating the performance of green buildings is essential to understanding the effectiveness of sustainable building practices and their contribution to environmental

and social goals. Green building performance encompasses several critical aspects, including energy efficiency, indoor environmental quality, water conservation, and overall user satisfaction. By assessing performance, we can measure how well these buildings meet sustainability objectives, such as reducing resource consumption, minimizing waste, enhancing occupant health, and lowering greenhouse gas emissions. This evaluation is particularly important as it offers insights into whether the design and construction choices made in the Green Buildings Project in the Gicumbi District are achieving the intended positive impact. In the context of the Green Gicumbi Project, evaluating building performance is a way to gauge the success of the implemented green technologies and sustainable practices. Performance assessment allows stakeholders to identify areas of strength and any potential areas for improvement, which is invaluable for informing future projects and policy decisions. Moreover, understanding building performance helps validate the cost-effectiveness of green investments by analyzing the long-term operational savings and environmental benefits, reinforcing the economic and ecological rationale for green construction. Table 4-8 provides an overview of the performance indicators for the Green Buildings Project, offering data-driven insights into the project's outcomes. Through this analysis, the study aims to reveal the degree to which the green buildings in Gicumbi District have met their sustainability targets, benefiting both the environment and the local community.

Table 3.4 Level of Performance of Green Buildings

Performance of the Green building constructed in the Gicumbi project	**SD**		**D**		**N**		**A**		**SA**		**MEAN**	**STD**
	fi	**%**	**Fi**	**%**	**fi**	**%**	**fi**	**%**	**fi**	**%**		
The buildings have a low energy use intensity	5	4.7	5	4.67	4	3.7	29	27.1	64	59.8	4.33	1.07
The buildings have low Water Use Intensity	4	3.7	2	1.87	12	11.2	26	24.3	63	58.9	4.33	1.01
I am satisfied with the functionality of the building	7	6.5	4	3.74	4	3.7	12	11.2	80	74.8	4.44	1.16
The building is safe, adaptable, and Resilience	6	5.6	6	5.61	6	5.6	23	21.5	66	61.7	4.28	1.16
Adequate Indoor Environmental Quality (Air quality, Thermal Comfort, Lighting Quality, Acoustic Comfort)	9	8.4	9	8.41	3	2.8	19	17.8	67	62.6	4.18	1.32
				Overview							**4.31**	

Source: Primary data, 2024

The findings on the performance of green buildings in the Green Gicumbi Project highlight key aspects of sustainability and functionality achieved by the project. The assessment, covering metrics such as energy use, water use, functionality, safety, adaptability, resilience, and indoor environmental quality, provides a comprehensive view of how these buildings meet their intended green standards. A significant finding is that the buildings have low energy use intensity, with 59.8% of respondents strongly agreeing and 27.1% agreeing, resulting in a mean score of 4.33 and a standard deviation of 1.07. This suggests that energy-saving measures, possibly including efficient lighting and HVAC systems, have effectively reduced energy consumption in these green buildings, contributing to environmental goals and cost savings over time. The assessment of water use intensity also reflects positively on the project's performance. With 58.9% of respondents strongly agreeing and 24.3% agreeing, the mean score again stood at 4.33, with a standard deviation of 1.01. These results indicate the success of water-efficient systems, such as low-flow fixtures and potentially greywater recycling, in significantly reducing water usage. This is essential in enhancing

resource conservation and supporting sustainable water management practices in the district.

User satisfaction with the buildings' functionality is notably high, with 74.8% of respondents strongly agreeing and another 11.2% agreeing, leading to the highest mean score of 4.44 and a standard deviation of 1.16. This satisfaction suggests that the buildings meet users' needs effectively, demonstrating that sustainable designs can still fulfill functional requirements while adhering to green standards. This level of satisfaction is a positive indicator of the building's success in achieving both sustainability and usability. In terms of safety, adaptability, and resilience, 61.7% of respondents strongly agreed that the buildings offer these qualities, while 21.5% agreed, resulting in a mean score of 4.28 and a standard deviation of 1.16. This reflects the importance of resilient design in green buildings, as it enables these structures to better withstand environmental stresses and maintain safe conditions for users. Lastly, the indoor environmental quality, which includes air quality, thermal comfort, lighting, and acoustic comfort, was assessed. Here, 62.6% of respondents strongly agreed and 17.8% agreed on the adequacy of these aspects, resulting in a mean score of 4.18 and a slightly higher standard deviation of 1.32. These findings suggest a moderate level of success in meeting indoor environmental standards, although there may be areas for improvement, especially in consistently providing optimal thermal and acoustic comfort. Overall, the study found an average mean score of 4.31, corresponding to 86%, indicating a high level of performance for the green buildings in the Gicumbi Project. This assessment demonstrates that the project has successfully implemented green building practices that meet sustainability objectives while also delivering functionality and user satisfaction, establishing a strong foundation for future green initiatives in the region.

3.3. Relationship between the green technologies and infrastructure performance of the green buildings project in the Gicumbi district

This section describes the results of the relationship between the independent variables and the dependent variables and shows the influence of the independent variables on the dependent variable.

3.3.1. Multiple linear regression analysis

Multiple linear regression analysis was used to determine whether resource-efficient design, Low-emission materials, and waste management have a significant contribution to the performance of Green Buildings Constructed in the Green Gicumbi Project. The regression models were run to test whether the model was significant or not. The statistical significance was verified by the t-statistic. In addition, statistically significant relationships between the dependent variable and the independent variable from the model were accepted at a 5% significance level. Based on the model summary, the coefficient of determination (R-squared) shows the overall measure of the strength of association between independent and dependent variables.

Table 3.5: Model Summary

Summary output	Results
Multiple R	0.816867
R Square	0.667977
Adjusted R Square	0.650186
Standard Error	0.251995
Observations	107

a. Predictors: (Constant), resource-efficient design, Low-emission materials, waste management

Source: Primary data, 2024.

Findings in Table 3.5 indicate the overall contribution of the independent variables (resource-efficient design, low-emission materials, and waste management) on the dependent variables through the value of R^2 as well as the value of adjusted R^2. However, with the value of adjusted R^2, the study showed that 0.650186 (65.0%) of the performance of green buildings constructed in the green Gicumbi project is influenced by resource-efficient design, Low-emission materials, and waste management.

Table 3.6: ANOVA

	df	Sum of Squares	Mean Square	F	P-value
Regression	3	12.04535	2.408182	36.7915	0.000[b]
Residual	106	5.956724	0.062952		
Total	**109**	**18.00319**			

a. Dependent Variable: Performance of Green Buildings Constructed in the Green Gicumbi Project

b. Predictors: (Constant), Resource-efficient design, Low-emission materials, Waste management

Source: Primary data, 2024

The findings in Table 3.6 indicate that the overall model was significant with a p-value equivalent to 0.000, which is less than the critical p-value equal to 0.05 level of significance. Therefore, this implies that the combined effort of green technologies such as resource-efficient design, Low-emission materials, and waste management was statistically significant on the Performance of Green Buildings Constructed in the Green Gicumbi Project. This implies that there was a goodness of fit of the model fitted for this study. The study concluded that the null hypothesis stated that there is no significant relationship between the Green Technologies and the performance of Green Buildings Constructed in the Green Gicumbi Project, was rejected due to a p-value of 0.000, which is less than the acceptance critical value of 0.05. Hence, there is a significant relationship between the Green Technologies and the performance of Green Buildings constructed in the Green Gicumbi Project.

Table 3.7: Regression coefficients

	Coefficients	Standard Error	t Stat	P-value
Intercept	1.047	0.284	3.68	0
RED	0.152	0.083	8.041	0.007
LEM	0.14	0.105	4.558	0.019
WM	**0.178**	**0.131**	**4.674**	**0.018**

a. Dependent Variable: Performance of Green Buildings Constructed in the Green Gicumbi Project.

From the research findings, the following values were obtained: β_0 =1.047, β_1=0.152, β_2=0.14, β_3=0.178.

The regression model can therefore be expressed as follows:

PGCGP = 1.047+0.152RED +0.14LEM +0.178WM

The regression equation above has been established taking all factors into account (resource-efficient design, Low-emission materials, waste management), constant at zero, with a Performance of Green Buildings Project equal to 1.047

The regression results revealed that resources efficient design has a significant positive influence on the Performance of green buildings constructed in the green Gicumbi project as indicated by β_1= 0.152, p-value=0.007<0.05, This implies that taking all other independent variables at zero, a unit increase in resources efficient design would lead to 0.152 increase in the Performance of green buildings constructed in green Gicumbi project. Therefore, the study rejected the null hypothesis that stated that there is no significant influence of resource-efficient design on the performance of green buildings constructed in the green Gicumbi project. The regression results revealed that Low-emission materials have a significant positive influence on the performance of green buildings constructed in the green Gicumbi Project, as indicated by β_2= 0.14, p-value=0.019<0.05. This implies that taking all other independent variables at zero, a unit increase in Low-emission materials would lead to a 0.14 increase in the performance of green buildings constructed in the green Gicumbi project. Therefore, the study rejected the null hypothesis that stated that there is no significant influence of Low-emission materials on the Performance of Green Buildings Constructed in the Green Gicumbi Project.

The regression results revealed that waste management has a significant positive influence on the performance of green buildings constructed in the green Gicumbi project, as

indicated by β_3= 0.178, p-value=0.018<0.05. This implies that taking all other independent variables at zero, a unit increase in waste management would lead to a 0.178 increase in the performance of green buildings constructed in the green Gicumbi project. Therefore, the study rejected the null hypothesis that stated that there is no significant influence of waste management on the performance of green buildings constructed in the green Gicumbi project.

3.4. Discussion of findings

The findings of this study showed the significant positive influence of various green technologies on the performance of the green building project in the Gicumbi district. These results hold important implications for policymakers, urban planners, and building professionals in Rwanda and other developing nations. The observed relationship between resource-efficient design and enhanced building performance emphasizes the critical role that sustainable design principles play in improving the environmental and operational efficiency of infrastructure (Smith & Patel, 2018). By strategically incorporating resource-conserving strategies into the construction and operation of buildings, developers and architects can unlock tangible benefits in terms of reduced energy and water consumption, lower greenhouse gas emissions, and improved overall performance. These insights can inform the development of green building guidelines and standards to drive the widespread adoption of resource-efficient design practices.

Similarly, the positive influence of low-emission construction materials on building performance highlights the importance of material selection in sustainable construction (Thompson & Zhang, 2020). The use of environmentally friendly, low-impact materials not only reduces the carbon footprint of the building itself but also contributes to improved indoor air quality and occupant well-being. Policymakers and regulatory bodies should consider incentivizing or mandating the use of such materials in infrastructure projects to promote a more circular and resource-conscious built environment.

The findings regarding the significance of effective waste management practices on building performance underscore the need for a holistic approach to sustainable development (Building Council, 2021). Robust waste management systems, including on-site waste segregation, recycling, and disposal, are crucial for minimizing the environmental impact of construction activities and ensuring the long-term viability of green buildings. Urban planners and development authorities should prioritize the integration of comprehensive waste management strategies into infrastructure projects to support the overall sustainability of the built environment.

These research findings provide valuable insights that can inform policy decisions, urban planning initiatives, and building industry practices in Rwanda and other developing countries. By leveraging the synergistic benefits of resource-efficient design, low-emission materials, and effective waste management, stakeholders can drive the widespread adoption of green building technologies and unlock significant environmental, economic, and social benefits for local communities. Expanding the scope of this study to include additional case studies or a larger sample size could also provide valuable insights into the scalability and replicability of the observed findings across different regions and contexts.

IV. CONCLUSION

This study provided valuable insights into the role of green technologies in enhancing the performance of infrastructure projects in the context of Rwanda focusing on the green buildings project in Gicumbi district. The study adopted a descriptive research design considering 171 residents of the constructed green buildings. The findings reveal that the strategic integration of resource-efficient design, low-emission construction materials, and effective waste management practices into the Green Buildings Project has led to significant improvements in the infrastructure's environmental and operational performance. Specifically, the use of renewable energy, water conservation measures, and energy-efficient systems has resulted in substantial reductions in energy consumption and water usage, while also contributing to enhanced indoor environmental quality and occupant well-being.

The positive correlations observed between the implementation of these green technologies and the enhanced performance of the green buildings underscore the critical role that sustainability-focused interventions can play in addressing the challenges faced by the built environment in developing countries. By embracing holistic approaches to green building design and construction, the Green Buildings Project has demonstrated the potential for tangible benefits in terms of resource conservation, environmental impact reduction, and overall infrastructure performance. These research findings hold important implications for policymakers, urban planners, and building professionals in Rwanda and other developing nations. They emphasize the need to prioritize the integration of green technologies into infrastructure projects as a key strategy for driving sustainable development and promoting environmental stewardship within the built environment. The successful implementation of the green buildings project in the Gicumbi district can serve as a model for the

replication and scaling up of similar initiatives across the region.

As the global community continues to grapple with the pressing challenges of climate change, resource scarcity, and urban development, the insights gained from this study contribute to a growing body of knowledge on the transformative potential of green technologies in infrastructure projects. The findings presented here provide a strong foundation for future research and practical applications aimed at further advancing the sustainability and resilience of the built environment in developing countries.

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Teleconnection between Atmospheric Circulation and Meteorological Drought in Southwest China

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Abstract— *Meteorological drought represents one of the most prevalent and consequential climate disasters in China, exerting severe impacts on regional ecosystems and socioeconomic development. This study investigates the teleconnection between meteorological drought and atmospheric circulation patterns in Southwest China from 1960 to 2022. Employing the Standardized Precipitation Evapotranspiration Index (SPEI) across multiple temporal scales alongside atmospheric circulation data, we conducted a comprehensive analysis using statistical methodologies including run theory, Mann-Kendall trend tests, and Pearson correlation analysis. These approaches enabled an in-depth examination of the spatiotemporal characteristics of drought events and their atmospheric teleconnections in the region. The results demonstrate that (1) Since 1960, the seasonal drought occurrence probability in Southwest China follows the order: spring > winter > autumn > summer. Light and moderate droughts are the predominant types in the study area, while severe droughts occur relatively infrequently, with no extreme drought events recorded. (2) Significant spatial variations in meteorological drought exist at seasonal scales across Southwest China. Spring drought generally shows a mitigation trend, summer exhibits a northeast-wet/southwest-dry pattern, autumn demonstrates increasing aridity in most regions, and winter displays a distinct southeast-wet/ central-dry/ northwest-wet gradient. (3) Regional atmospheric circulation influences exhibit distinct patterns: ENSO demonstrates the most pronounced teleconnection impact across the entire region; PDO shows strong secondary influence, particularly in southeastern and northern areas; AO exhibits relatively weak effects, mainly affecting northern and northwestern sectors; and NAO displays minimal impact, with only 7.1% of southern and northern areas showing significant positive correlations. These findings provide valuable scientific references for drought research and integrated management in Southwest China, offering both theoretical foundations and empirical support for regional economic development planning. The results contribute to enhanced drought monitoring systems and informed policymaking for climate adaptation strategies in the region.*

Keywords— ***Southwest China; Meteorological drought; Atmospheric circulation; Standardized Precipitation Evapotranspiration Index (SPEI); Teleconnection***

I. INTRODUCTION

Drought, characterized by its cyclical recurrence, extensive spatial coverage, prolonged duration, and substantial economic impacts [1], represents one of the most economically consequential meteorological disasters among natural hazards [2]. This phenomenon typically induces cascading natural and socioeconomic consequences, including agricultural yield reduction, food security threats, ecosystem degradation, and regional gross domestic product (GDP) decline [3, 4]. A representative case is the 2022 extreme drought event in the Yangtze River Basin, which at its peak affected approximately 6.632 million hectares (≈66,320 km²) of arable land, caused critical water source depletion, and created drinking water shortages for approximately 4.99 million people and 920,000 livestock [5]. These events significantly impaired socioeconomic development across the Yangtze Economic Belt.

Atmospheric circulation constitutes a fundamental component of Earth's climate system, whose anomalies and long-term variations exert critical influences on drought genesis, persistence, and intensity. Large-scale circulation modes, including the El Niño-Southern Oscillation (ENSO), Pacific Decadal Oscillation (PDO), North Atlantic Oscillation (NAO), and Arctic Oscillation (AO), serve as dominant regulatory signals governing global climate variability. These circulation systems exhibit three defining characteristics: significant periodicity (ENSO has a period of 2 to 7 years, and PDO has a period of 20 to 30 years); prominent signal persistence, which can be maintained for months to decades through air-sea coupling; strong predictability; and based on quantifiable indicators such as sea surface temperature (SST) and the pressure field (such as the Southern Oscillation Index), the prediction window can reach 6 to 12 months (especially significant for ENSO) [6]. Contemporary research has significantly advanced our understanding of drought-driving mechanisms via circulation indices, spatiotemporal drought pattern forecasting, and cross-scale teleconnection effects. These investigations have been systematically conducted across multiple spatial domains, encompassing watershed, regional, national, and global scales [7-9].

In drought research, scholars have employed diverse methodological approaches to quantify drought characteristics from multiple perspectives. Meteorological station data-derived drought indices constitute the predominant analytical framework, with several widely utilized metrics including the Standardized Precipitation Index (SPI), Palmer Drought Severity Index (PDSI), Composite Meteorological Drought Index (CI), and Relative Moisture Index (MI). Notable applications include Wang et al., who investigated multi-scale relationships between meteorological drought and soil moisture across China using SPI and MI [10]; Zhang et al., who analyzed spatiotemporal variations of seasonal wet/dry patterns in Northwest China through PDSI [11]; and Zhang et al., who conducted spatiotemporal characterization of 50-year drought features in Gansu's Loess Plateau employing CI [12]. Recent years have witnessed increasing scholarly attention to the Standardized Precipitation Evapotranspiration Index (SPEI) for drought assessment [13-15]. This index demonstrates particular utility for arid/semi-arid regions through its integrative consideration of temperature, precipitation, and evapotranspiration parameters, thereby providing a more comprehensive framework for drought monitoring and evaluation.

The western region of Southwest China exhibits a natural predisposition to aridity, resulting from the synergistic interplay of the Tibetan Plateau's orographic barrier, monsoon system instability, karst-dominated permeable topography, and anthropogenic influences. Systematic monitoring and mechanistic investigation of these factors will facilitate evidence-based policymaking for drought mitigation strategies.

II. THE STUDY AREA AND DATA SOURCES

2.1 The Study Area

Southwest China is geographically situated in the southwestern part of the country (20°54'–34°19'N, 97°21'–112°04'E), encompassing five provincial-level administrative units: Sichuan, Yunnan, and Guizhou provinces; Guangxi Zhuang Autonomous Region; and Chongqing Municipality (Figure 1). The Tropic of Cancer traverses this region, which shares borders with Central China to the east, Northwest China to the north, Vietnam

and Laos to the south, and the Tibet Autonomous Region and Myanmar to the west. The region spans a total area of 1,376,300 square kilometers, accounting for approximately 14.33% of China's total land area.

The topography of Southwest China exhibits remarkable diversity, characterized predominantly by plateaus, mountains, and hills that extend across three distinct topographic terraces. A pronounced northwest-to-southeast elevation gradient is observed, with higher altitudes in the northwestern sectors gradually decreasing towards the southeast. Climatically, the region falls primarily within the subtropical monsoon zone, featuring synchronous patterns of temperature and precipitation. Summers are typically hot and humid with abundant rainfall, while winters remain relatively mild and dry. Significant altitudinal variations across the region contribute to substantial spatial heterogeneity in climatic conditions. Which annual mean temperatures range from -2.8°C to 23.9°C, while total annual precipitation varies considerably between 54.6 mm and 2,675.6 mm. The mean annual relative humidity exhibits a similarly wide range, fluctuating from 46.6% to 85.0% across different locations. These pronounced gradients in temperature and precipitation reflect the complex interplay between monsoonal circulation patterns and the region's diverse topographic features.

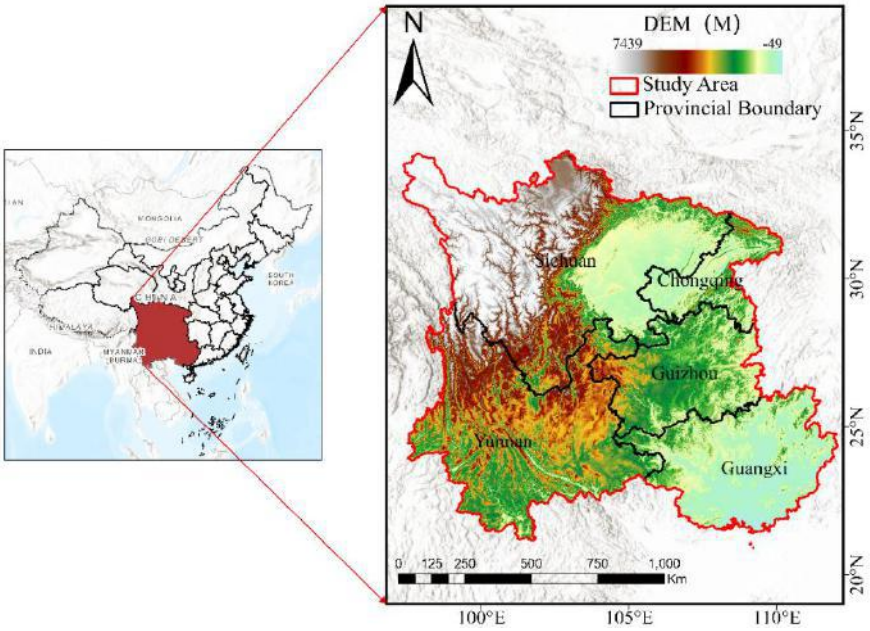

Fig.1 Map of the study area

2.2 Data Sources

SPEI data spanning 1960 to 2022 used in this study were obtained from the Software Engineering and Information Systems Institute (SEPI) created by the Consejo Superior de Investigaciones Ciengificas (Spanish National Research Council) (CSIC) (https://spei.csic.es/index.html). The atmospheric circulation indices employed in this research, including the North Atlantic Oscillation (NAO), Pacific-North American pattern (PNA), Arctic Oscillation (AO), Pacific Decadal Oscillation (PDO), and Southern Oscillation Index (SOI), were all acquired from the Earth System Research Laboratory (ESRL) of the National Oceanic and Atmospheric Administration (NOAA) (https://www.esrl.noaa.gov). These indices cover the same temporal period from 1960 to 2022 to ensure temporal consistency across all datasets.

III. METHODOLOGY

3.1 Material Processing Methods

3.1.1 The Standardized Precipitation-Evapotranspiration Index (SPEI)

SPEI, developed by Vicente-Serrano et al. [16], represents a multi-scalar drought index derived from the original SPI framework. This metric effectively captures regional drought conditions by incorporating both

precipitation and temperature influences while accounting for evapotranspiration effects. The computational procedure involves four sequential steps:

First, monthly potential evapotranspiration (PET) is calculated using the Thorn-thwaite method [17]:

$$PET = 16K(\frac{10T_i}{I})^m \quad (1)$$

$$m = 6.75 \times 10 - 7I3 - 7.71 \times 10 - 5I2 + 1.79 \times 10 - 2I + 0.492 \quad (2)$$

Here, PET denotes potential evapotranspiration (mm), Ti represents mean temperature (°C), K is a latitude and month dependent correction coefficient, I signifies the annual heat index (sum of 12 monthly heat indices), and m is a coefficient determined by I.

Second, the monthly climatic water balance (Di) is computed as the difference between precipitation (Pi) and PET:

$$D_i = P_i - PET_i \quad (3)$$

Third, the Di series undergoes probability distribution fitting using a three-parameter log-logistic distribution with the probability density function:

$$f(x) = \frac{\beta}{\alpha}\left(\frac{x-\gamma}{\alpha}\right)[1 + (\frac{x-\gamma}{\alpha})]^{-2} \quad (4)$$

where α, β, and γ represent scale, shape, and location parameters, respectively. The cumulative distribution function F(x) is derived as:

$$F(x) = [1 + (\frac{\alpha}{x-\gamma})]^{-1} \quad (5)$$

Fourth, SPEI values are obtained through normal standardization of F(x) [18]:

For $P \leq 0.5$:

$$w = \sqrt{-2\ln(P)} \quad (6)$$

$$SPEI = w - \frac{C_0+C_1W+C_2W^2}{1+d_1W+d_2W^2+d_3W^3} \quad (7)$$

For $P > 0.5$:

$$SPEI = -\left(w - \frac{C_0+C_1W+C_2W^2}{1+d_1W+d_2W^2+d_3W^3}\right) \quad (8)$$

where C0 = 2.5155, C1 = 0.8028, C2 = 0.0103, d1 = 0.4327, d2 = 0.1892, and d3 = 0.0013.

Three temporal scales are commonly employed: SPEI-1 captures monthly drought variability, reflecting fine-scale drought processes; SPEI-3 characterizes seasonal drought patterns (spring: March-May, summer: June-August, autumn: September-November, winter: December-February) [19]; while SPEI-12 reveals inter-annual drought variations, effectively representing long-term drought trends. This multiscalar approach enables comprehensive drought assessment across different temporal dimensions, with each scale providing unique insights into drought evolution mechanisms.

This study investigates the spatiotemporal characteristics of meteorological drought and its teleconnections with atmospheric circulation patterns in Southwest China using the SPEI at 1, 3, and 12-month timescales. The analysis employs SPEI-12 and SPEI-3 to characterize drought variability across temporal and spatial dimensions, while SPEI-1 is utilized for atmospheric circulation correlation analysis. Seasonal drought conditions are represented by specific monthly SPEI-3 values: April for spring, July for summer, October for autumn, and December for winter.

Drought severity is classified according to internationally recognized standards into five distinct categories based on SPEI threshold values (Table 1): no drought, mild drought, moderate drought, severe drought, and extreme drought. This classification system enables systematic quantification of drought intensity and facilitates comparative analysis of drought events across different temporal scales and geographical regions.

Table 1 SPEI drought grade

Grade	Drought Type	SPEI Value
1	No drought	-0.5≤SPEI
2	Mild drought	-1.0<SPEI≤-0.5
3	Moderate drought	-1.5<SPEI≤-1.0
4	Severe drought	-2.0<SPEI≤-1.5
5	Extreme drought	SPEI≤-2.0

The ENSO represents a naturally occurring ocean-atmosphere interaction phenomenon characterized by SST variations in the eastern and central equatorial Pacific. The SST anomalies in the tropical Pacific region (5°N-5°S, 170°W-120°W), known as the Niño 3.4 region, serve as one of the primary indicators for ENSO characterization. El Niño and the Southern Oscillation constitute distinct oceanic and atmospheric manifestations

of ENSO, respectively. As the most significant tropical air-sea interaction pattern, ENSO has become a crucial physical basis for short-term climate prediction.

The PDO indexes SST variations in the North Pacific poleward of 20°N, exhibiting periodicities of 5-30 years. The PDO's influence on precipitation distribution resembles that of ENSO patterns.

The NAO emerges as the dominant mode of winter surface pressure variability in the North Atlantic/European sector of the Northern Hemisphere, serving as the primary atmospheric circulation pattern governing Mediterranean climate. The NAO demonstrates peak energy at approximately 7.3-year intervals.

The AO constitutes the fundamental mode of internal atmospheric dynamics in the Northern Hemisphere's extratropical regions, displaying an equivalent barotropic structure extending from the surface to the lower stratosphere. This hemispheric-scale variability pattern [20] exhibits characteristic spatial and temporal coherence across atmospheric levels.

3.2 Analysis Methods

3.2.1 Drought Feature Recognition–Runs Theory

This study employs an integrated approach combining the SPEI index with runs theory for drought identification, adopting K = -0.5 as the drought threshold [21]. In the time series analysis, consecutive periods where drought indices remain above a specified threshold are classified as positive runs, while those below constitute negative runs. Monthly SPEI values serve as the basis for drought determination, with SPEI < -0.5 indicating drought occurrence.

The application of runs theory to SPEI time series enables the extraction of key drought characteristics, including Duration: The temporal span from drought initiation to termination; Severity: Cumulative SPEI deficit during a drought event; Frequency: Total count of drought occurrences within the study period; Intensity: Average drought severity per unit time (severity/duration).

Three characteristic drought variables were derived from SPEI time series at 1, 3, and 12-month timescales. Drought events separated by a single time unit were merged to account for intermittent recovery periods. The drought event identification process based on runs theory is illustrated in Figure 2, demonstrating the methodology for quantifying drought parameters across multiple temporal scales. This approach facilitates comprehensive characterization of drought events while maintaining temporal resolution appropriate for both short-term and prolonged drought analysis.

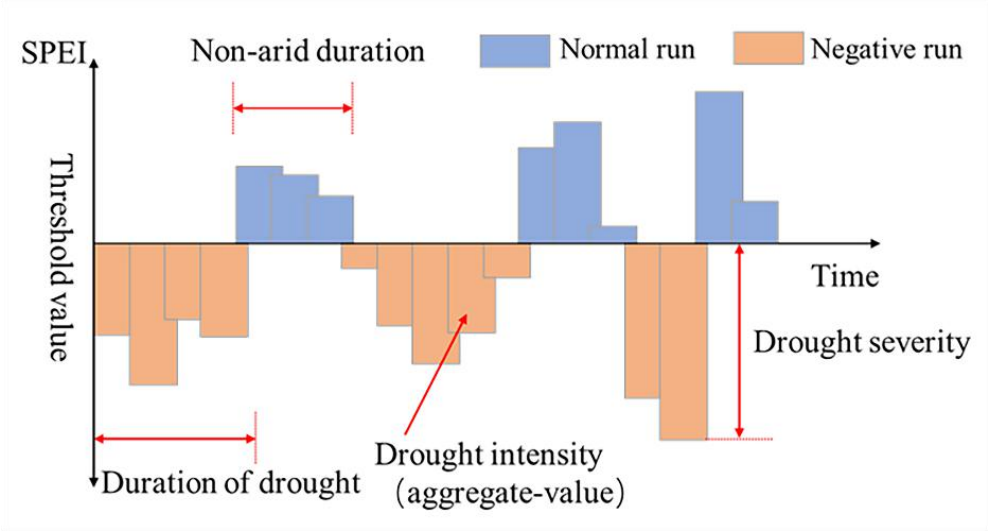

Fig.2 Identification of drought event process using runs theory

3.2.2 Trend Analysis and Detection

(1) Sen's Slope Estimator

The Theil-Sen Median method, commonly referred to as Sen's slope estimator, represents a robust non-parametric statistical approach for trend slope estimation, particularly advantageous when datasets

contain outliers or violate assumptions of conventional linear regression. This method requires no assumptions regarding underlying data distributions and derives the true slope as the median of all possible pairwise slopes within the time series, rendering it resistant to extreme values. Consequently, it has been widely adopted in meteorological, hydrological, and ecological studies involving long-term temporal analyses [22]. The computational formulation is expressed as:

$$\text{Slope} = Median(\frac{x_i - x_j}{i-j}) \quad (9)$$

where 1<j<i<n, with xi and xj denoting time series values at temporal points i and j, respectively, and Median representing the central tendency measure of the derived slopes.

Sen's slope estimator quantitatively characterizes the rate of temporal change within sequential data [23]. The interpretation criteria for slope magnitudes are presented in Table 2, facilitating standardized assessment of trend significance and directionality. This approach provides a distribution-free alternative to parametric trend analyses while maintaining robustness against non-Gaussian data distributions and measurement anomalies.

Table 2 Slope criteria

Slope value	Trend
Slope<0	Downward trend
Slope=0	Unchanged trend
Slope>0	Upward trend

(2) Mann-Kendall Trend Test

The Mann-Kendall (MK) trend test represents a non-parametric statistical method for detecting significant monotonic trends in time series data. This approach demonstrates particular robustness against outliers and requires no assumptions regarding data distribution, making it universally applicable to diverse datasets, including non-normally distributed and ordinal discrete variables. Widely adopted in climatological and hydrological studies [24], the method calculates test statistics through the following formulations: Test Statistic (S):

$$S = \sum_{k=1}^{n-1} \sum_{j=k+1}^{n} Sgn(X_j - X_k) \quad (10)$$

where: Xj denotes the j-th data point in the time series; n represents the sample size; The *sgn* function *sgn*(θ) is defined as:

$$sgn(\theta) = \begin{cases} +1 & (\theta > 0) \\ 0 & (\theta = 0) \\ -1 & (\theta < 0) \end{cases} \quad (11)$$

Standardized Test Statistic (Z):

$$Z = \begin{cases} \frac{(S-1)}{\sqrt{Var(S)}} & S > 0 \\ 0 & S = 0 \\ \frac{(S+1)}{\sqrt{Var(S)}} & S < 0 \end{cases} \quad (12)$$

where S follows a normal distribution with zero mean; Variance Var(S) = [n(n-1)(2n+5)]/18; Positive Z values indicate increasing trends, while negative values denote decreasing trends. Significance Thresholds: Trend significance is determined when |Z| exceeds critical values: 1.64 (90% confidence), 1.96 (95% confidence), and 2.58 (99% confidence) [25].

This study synergistically combines the Theil-Sen Median estimator for spatiotemporal drought characteristic analysis across Southwest China with the MK test to evaluate trend significance in SPEI data (1960–2022). The dual approach ensures robust quantification of trend magnitude (via Sen's slope) and statistical significance (via the MK test), providing comprehensive trend characterization. Detailed significance evaluation criteria are presented in Table 3.

Table 3 Significance detection criteria

Confidence standard Z	Significance level α	Significance
Z≤-2.58	α≤0.01	Extremely significant decline
-2.58≤Z≤-1.96	α≤0.05	Significant decline
-1.96≤Z≤1.64	α≤0.1	Unchanged
1.64≤Z≤1.96	α≤0.05	Significant rise
1.96≤Z≤2.58	α≤0.01	Extremely significant rise

3.2.3 Pearson Correlation Analysis

The Pearson correlation coefficient (PCC), also known as Pearson's product-moment correlation coefficient, is a statistical measure that quantifies the linear relationship between two variables. It is calculated as the covariance of the variables divided by the product of their standard deviations. The coefficient, denoted as r, ranges

between -1 and 1, where r < 0 indicates a negative correlation, r > 0 indicates a positive correlation, and values closer to |1| signify a stronger linear relationship [26]. In this study, Pearson correlation analysis was employed to quantitatively assess the relationship between SPEI and atmospheric circulation indices.

Given two samples, $X = (x_1, x_2, ..., x_n)$ and $Y = (y_1, y_2, ..., y_n)$, the Pearson correlation coefficient is computed as:

$$r = \frac{1}{n}\sum_{i=1}^{n}\left(\frac{x_i-\bar{x}}{\sigma_x}\right)\left(\frac{y_i-\bar{y}}{\sigma_y}\right) \quad (13)$$

where:

The significance of Pearson correlation coefficient is tested by t value test, in which the level of significance test is 0.05. When t is less than 0.05, it is significant, and t>=0.05 is not significant. The correlation formula is:

$$t = r\sqrt{\frac{n-2}{1-r^2}} \quad (14)$$

Where n is the number of samples, r is the correlation coefficient, and t is the test value. When the sample size n is larger, the correlation coefficient r reaching significant correlation is smaller, so when the sample size is larger, due to the difference of sample data, the correlation coefficient is generally not very high, but the significance test can consider that the correlation between the two groups of sample data is significant.

IV. RESULTS

4.1 Temporal Characteristics of Meteorological Drought

Analysis of the SPEI across multiple temporal scales provides critical insights into the dynamic evolution of hydrological processes, including precipitation and evapotranspiration patterns, over the study period. This study generated multi-scale SPEI time series (1-month, 3-month, and 12-month) for Southwest China from 1960 to 2022 (Figure 3), revealing distinct scale-dependent characteristics in drought variability.

The results show that there are significant differences in the sensitivity of the multi-time scale values of the SPEI in Southwest China to changes over time. The SPEI-1 time series exhibits a high degree of volatility, indicating that on a one-month time scale, the SPEI value is more sensitive to short-term climate changes. This high sensitivity suggests that SPEI-1 is more suitable to serve as a powerful tool for monitoring and assessing short-term climate anomaly events, such as extreme droughts or floods. Although the volatility of SPEI-3 remains relatively high, its changes are smoother compared to SPEI-1. This may be because on a three-month time scale, short-term meteorological changes are partially smoothed out, yet it can still reflect short-term or medium-term climate anomalies. The fluctuations in the SPEI-12 time series are significantly gentler, suggesting that on a 12-month time scale, the SPEI value responds more stably to climate changes. The long-term averaging effect reduces short-term fluctuations, making SPEI-12 more appropriate for evaluating long-term climate trends or predicting future climate changes.

It can be seen from the SPEI-12 time series graph that since 1960, there have been multiple obvious drought events in Southwest China, which demonstrates the frequent occurrence of droughts in this region. The obvious drought years in Southwest China include the period from spring 1960 to spring 1961, from autumn 1962 to spring 1964, from spring 1969 to spring 1970, from spring 1986 to spring 1990, from spring 2003 to spring 2008, and from spring 2009 to spring 2014. Some of these drought events had a relatively long duration, such as the periods from spring 1986 to spring 1990, from spring 2003 to spring 2008, and from spring 2009 to spring 2014. The results indicate that Southwest China experienced long-term droughts during these periods, which may have had long-term impacts on the local agriculture, water resources, and ecosystems.

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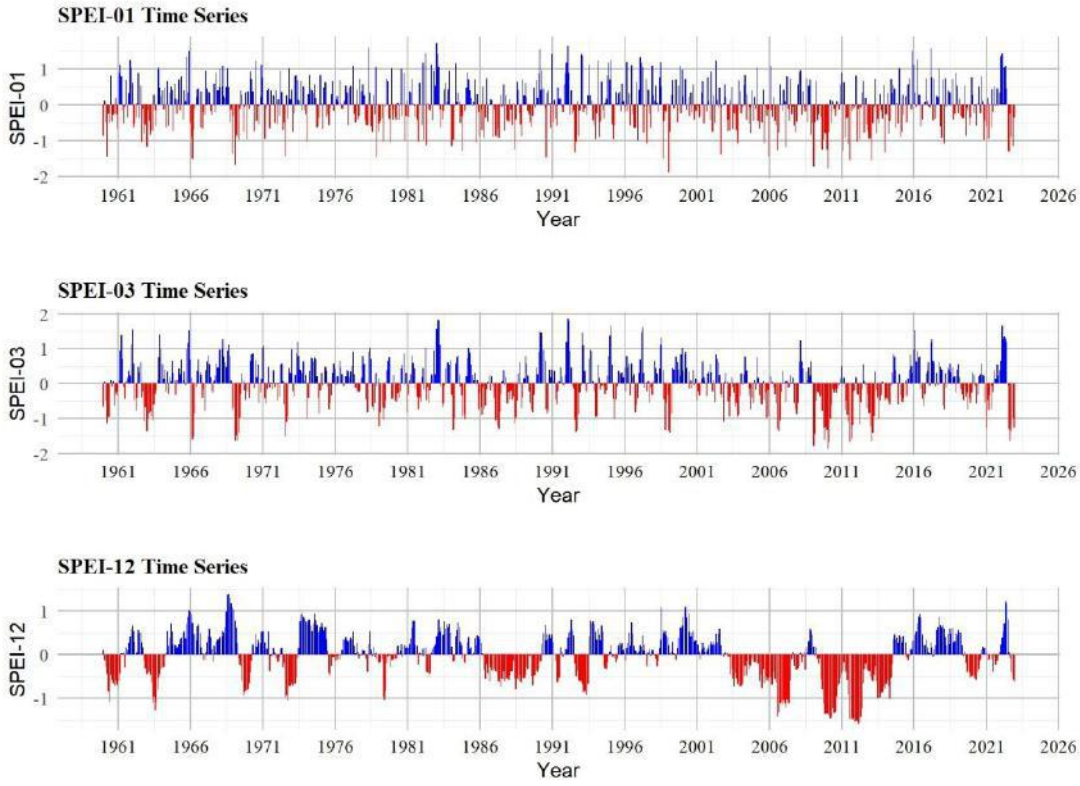

Fig.2 Multiscale time series from 1960-2022 in Southwest China

4.1.1 Temporal Characteristics of Drought Duration, Intensity, and Frequency

In assessing drought severity, both drought duration and intensity serve as critical evaluation criteria. This study employs runs theory to classify drought levels based on the SPEI, with a drought threshold set at k = −0.5. Statistical analyses were conducted on the annual and seasonal drought duration and intensity in Southwest China, with results presented in Figure 4.

As illustrated in Figure 4(a), the inter-annual analysis reveals that the maximum SPEI-based drought duration reached 21 months (2005–2007), with a minimum drought intensity of −1.31. Notably, a severe drought lasting five months (December 2011–April 2012) occurred in Southwest China, representing an extreme climatic event with profound environmental impacts. Additionally, frequent droughts were observed between 2002 and 2014, consistent with historical drought records in the region.

As shown in Figure 4(a), at the seasonal scale, the spring drought duration reached three months during the two consecutive years of 2009–2010. Similar three-month spring droughts were also recorded in 1960, 1963, 1966, 1969, 1979, and 1987. In summer, only the year 2011 experienced a drought lasting three months. Autumn droughts in 1992, 2003, 2009, and 2022 exhibited longer durations compared to other years, while winter droughts lasting three months occurred in 1962, 1968, 2010, and 2012.

Looking at Figure 4(b) reveals that the maximum SPEI-based drought intensity occurred in winter 2010, reaching −1.55. Additionally, the drought intensity in spring 1969 and winter 2010 fell below −1.50, classifying these years as severe drought events. Furthermore, the observational results from Figure 4 indicate a notable discrepancy between seasonal and inter-annual drought intensity, primarily attributable to differences in temporal scales and associated influencing factors. Seasonal drought intensity focuses on drought conditions within specific

seasons, closely linked to seasonal precipitation patterns, temperature fluctuations, and soil moisture levels. In contrast, inter-annual drought intensity encompasses drought conditions over an entire year, integrating drought severity across all seasons while accounting for the broader climatic influences. Consequently, inter-annual drought intensity tends to exhibit greater stability and is more susceptible to long-term climate change trends.

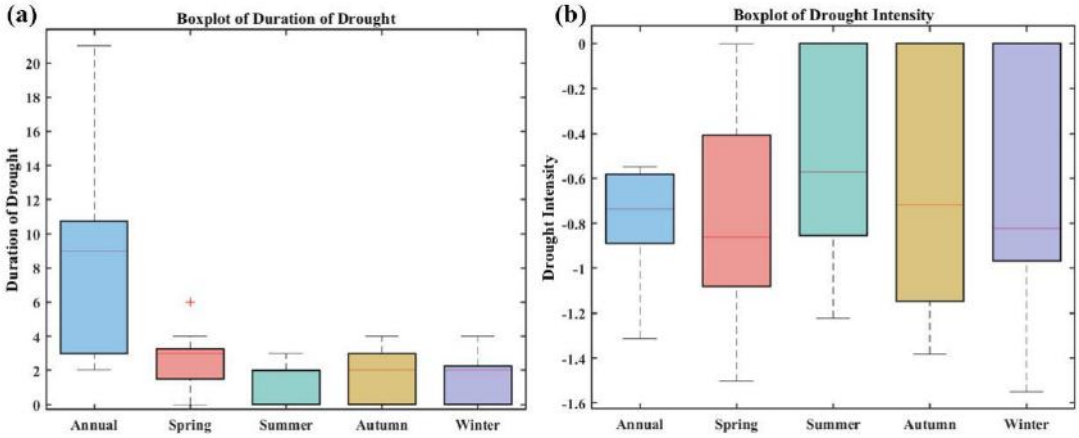

Fig.4 Seasonal characteristics of drought duration (a) and intensity (b) in Southwest China

Figure 5 presents the drought frequency statistics based on annual and seasonal SPEI drought classification standards, visualized through stacked bar charts. This representation enables observation of drought frequency distribution patterns in Southwest China at both inter-annual and seasonal scales. Comparative analysis of different drought severity categories reveals that mild and moderate droughts constitute the most prevalent drought types in the study region, whereas severe droughts occur relatively infrequently. Notably, extreme drought events were not observed at the inter-annual scale, as indicated by zero occurrence frequency.

Further examination of seasonal drought frequency demonstrates distinct patterns. Non-drought conditions exhibit their highest frequency during summer, likely attributable to increased precipitation in this season. Mild droughts emerge as the second most frequent category, peaking in spring with a frequency of 17.46% and reaching their lowest occurrence (11.11%) in summer. Moderate droughts show significant occurrence in autumn (7.41%) and winter (6.35%), with comparatively lower frequencies in other seasons. Severe droughts maintain consistently low frequencies across all seasons, though spring and winter share identical occurrence rates of 2.12%, exceeding those observed in summer and autumn.

The seasonal distribution of drought frequency demonstrates pronounced seasonal characteristics in Southwest China, following the hierarchical pattern: spring > winter > autumn > summer. This pattern suggests elevated drought risks during spring and winter, while summer presents the lowest drought risk due to abundant precipitation. These seasonal variations likely reflect regional climatic patterns and precipitation distribution, with higher drought frequencies in spring and winter potentially associated with reduced precipitation and specific climatic conditions characteristic of these seasons.

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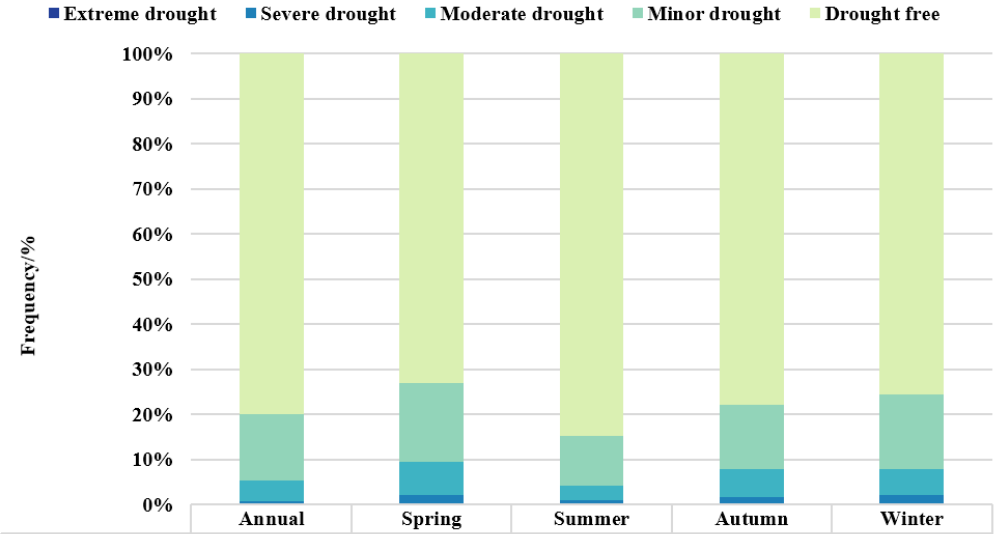

	Annual	Spring	Summer	Autumn	Winter
Drought free	79.89	73.02	84.66	77.78	75.66
Minor drought	14.68	17.46	11.11	14.29	16.4
Moderate drought	4.76	7.41	3.17	6.35	5.82
Severe drought	0.66	2.12	1.06	1.59	2.12
Extreme drought	0	0	0	0	0

Fig.5 Time variation of drought frequency at different levels in Southwest China

4.2 Spatial Variation Trends of Drought

This study conducted an in-depth analysis of the spatial characteristics of SPEI in Southwest China at the seasonal scale from 1960 to 2022 (Figure 6), yielding the following results:

Spring (Figure 6-a): Most regions in Southwest China exhibited a mitigation trend in meteorological drought. However, only northwestern Sichuan, Xishuangbanna in Yunnan, and southeastern Guizhou passed the significance level test. Specifically, the northwestern part of the Aba Tibetan and Qiang Autonomous Prefecture in Sichuan showed the most significant wetting trend (slope = 0.0047, $\alpha \leq 0.01$). Xishuangbanna in Yunnan reached a significance level of $\alpha \leq 0.05$, while southeastern Guizhou showed a weaker but still notable trend ($\alpha \leq 0.1$), indicating a more pronounced wetting tendency in these regions.

Summer (Figure 6-b): The drought-wetness trends in Southwest China displayed a distinct polarization. Most of the southwestern study area, particularly Lincang, Pu'er, Yuxi, and the Honghe Hani and Yi Autonomous Prefecture in Yunnan, exhibited a significant drying trend (minimum Sen slope = -0.0048, $\alpha \leq 0.01$). In contrast, the northeastern regions, especially Nanchong, Bazhong, Dazhou, and Guang'an in Sichuan, demonstrated a wetting trend (maximum Sen slope = 0.0042, $\alpha \leq 0.01$). This spatial divergence suggests contrasting climatic influences between the southwestern and northeastern parts of the study area during summer.

Autumn (Figure 6-c): Approximately 97.22% of the study area exhibited positive Sen's slope values, indicating a predominant drought intensification trend across Southwest China. Particularly significant drying trends ($\alpha \leq 0.01$) were observed in eastern Sichuan, Liupanshui, and southeastern Guizhou, as well as Baise City in Guangxi. The spatial pattern of significance levels revealed a concentric distribution centered around the tri-province border area, with decreasing significance levels (from 99% to 95% to 90%) extending toward the northern and eastern peripheries. The most pronounced drying occurred in Honghe Hani and Yi Autonomous Prefecture, Yunnan, with a minimum Sen's slope of -0.0052.

Winter (Figure 6-d): Meteorological drought trends displayed marked regional heterogeneity. Significant

drying trends ($\alpha \leq 0.01$) were evident in eastern Sichuan (Liangshan Yi Autonomous Prefecture, Leshan, and Yibin), with a minimum Sen's slope of -0.0069. Similar patterns were observed throughout most of Yunnan (western and northern regions) and Chongqing (except southeastern portions). Spatially, the winter drought trends followed a distinct southeast-central-northwest gradient, exhibiting a wet-dry-wet transitional pattern across the study area.

Spatiotemporal Synthesis: The analysis reveals pronounced seasonal and regional disparities in meteorological drought trends from 1960 to 2022. Spring was characterized by widespread drought mitigation across most regions. Summer exhibited a polarized northeast (wetting)-southwest (drying) dipole pattern. Autumn showed predominant drought intensification, particularly in eastern Yunnan ($\alpha \leq 0.01$). Winter displayed a tripartite spatial regime with alternating wet-dry-wet zones along the southeast-central-northwest axis. These patterns underscore the complex interplay of regional climate systems and topographic influences governing drought evolution in Southwest China.

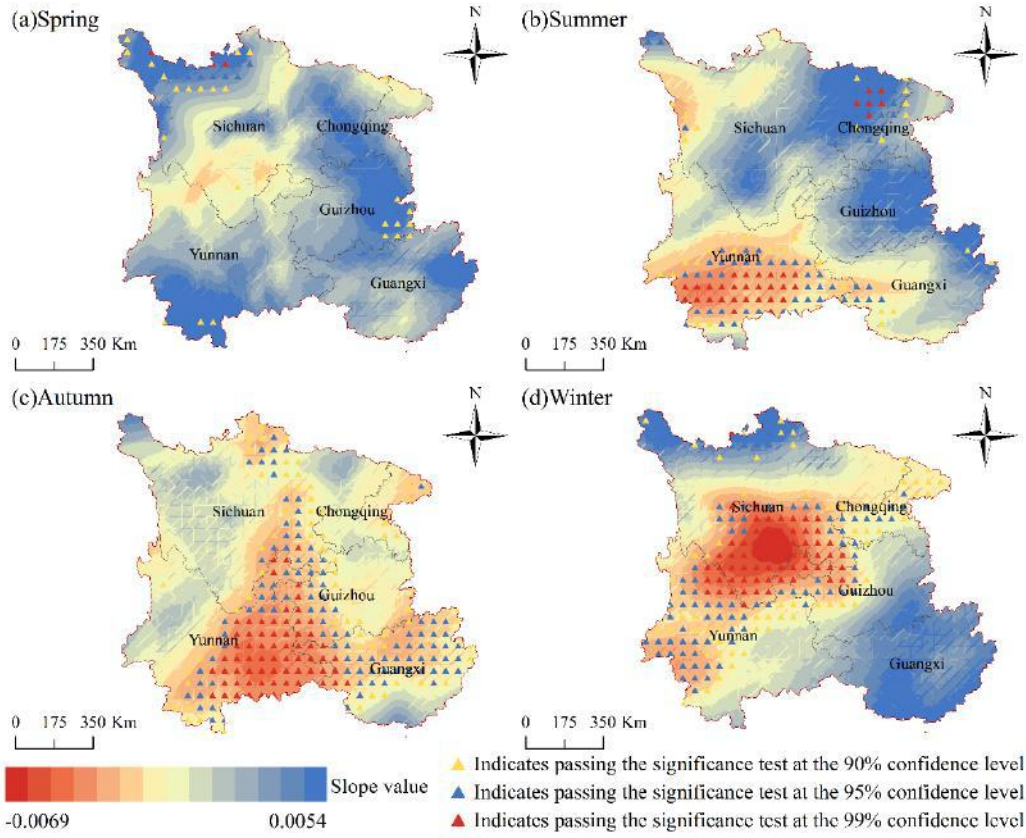

Fig.6 Spatial characteristics of seasonal scale meteorological drought in Southwest China from 1960-2022

4.3 Response of Meteorological Drought to Atmospheric Circulation

Using Pearson correlation analysis, we examined the relationship between drought indices and atmospheric circulation patterns, identifying key drought-influencing factors in Southwest China through significance testing (Table 4). The AO showed relatively weak correlations, with only 8.7% of the region exhibiting significant positive correlations and 1.1% showing significant negative correlations, primarily concentrated in the northwestern parts of Southwest China (Figure 7-a). The NAO demonstrated the lowest significance, with merely 7.1% positive correlations distributed along the northern and southern margins of the region (Figure 7-b). The ENSO exhibited the strongest statistical significance, indicating its dominant influence on drought patterns in Southwest China. Significant positive correlations covered 14.4% of the region, mainly in the southeastern areas, while negative

correlations accounted for 42.4%, predominantly distributed across northeastern and southwestern zones (Figure 7-c). The PDO showed the highest proportion of significant positive correlations (43.2%) among all indices, with negative correlations covering 10.1% of the region. Spatially, positive correlations clustered in eastern and partial northwestern areas, whereas negative correlations were concentrated in the northeastern sector (Figure 7-d).

Spatial analysis revealed distinct correlation patterns:

- AO-drought index correlations followed a north > central > south gradient (Figure 7-a).
- NAO's strongest influences appeared in southern and northern areas, particularly showing positive correlations in Aba Tibetan and Qiang Autonomous Prefecture (Sichuan) and Xishuangbanna Dai Autonomous Prefecture (Yunnan) and negative correlations in Laibin and Guigang (Guangxi) (Figure 7-b).
- ENSO correlations displayed a north > southeast > northwest > southwest hierarchy (Figure 7-c).
- PDO's strongest impacts occurred in southeastern and northern regions, with notable positive correlations in Guizhou and western Guangxi, and negative correlations in northern Aba Tibetan and Qiang Autonomous Prefecture (Sichuan).

These results demonstrate that AO, NAO, ENSO, and PDO primarily influence northern Southwest China, followed by southeastern and southern regions, revealing complex spatial variability in atmospheric circulation impacts on regional drought patterns.

Table 4 Significance of correlation between atmospheric circulation index and drought index (SPEI)

	AO	NAO	ENSO	PDO
Significant positive correlation	8.7%	7.1%	14.4%	43.2%
Significant negative correlation	1.1%	0	42.4%	10.1%
No significant correlation	90.2%	92.9%	43.2%	46.7%

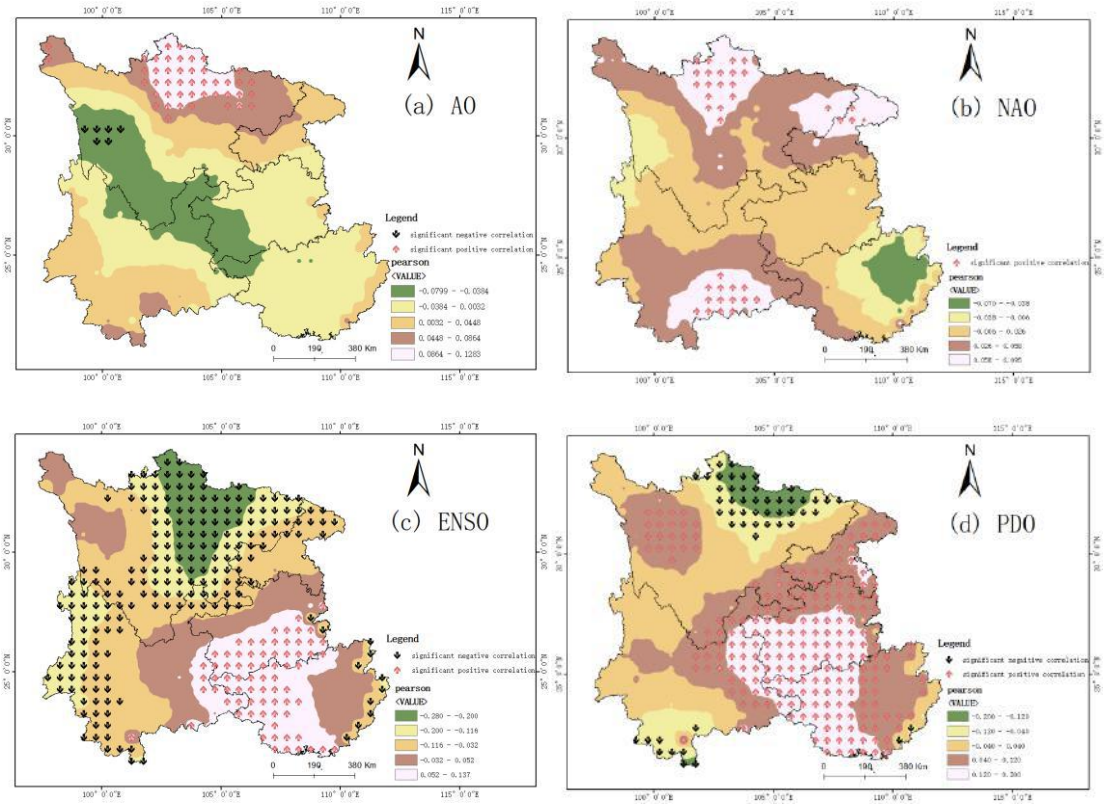

Fig.7 Spatial distribution of significant correlation between atmospheric circulation and drought index (SPEI) in Southwest China

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V. DISCUSSION

5.1 Comparative Analysis of Meteorological Drought in Southwest China and Other Regions

This study systematically investigated the spatiotemporal characteristics of meteorological drought in Southwest China and its teleconnections with atmospheric circulation patterns, yielding robust conclusions. The results reveal significant seasonal disparities in drought occurrence frequencies, with spring and winter exhibiting higher drought frequencies compared to summer and autumn. Notably, substantial variations in drought evolution patterns persist even among adjacent regions [18, 27], suggesting that drought drivers may be closely associated with region-specific topographic features, climatic regimes, and anthropogenic activities [28].

Through comparative analysis of the relationships between key atmospheric circulation indices (AO, NAO, PDO, ENSO) and the SPEI drought index, this study demonstrates distinct differential impacts among various teleconnection factors. ENSO emerges as the most influential factor on drought conditions in Southwest China, while NAO shows the weakest correlation. These findings align with Xu et al. [29], who identified ENSO as the dominant influence on drought patterns across China. Consequently, ENSO could be effectively incorporated as an input parameter in drought early warning systems to enhance forecasting accuracy.

This research elucidates both the spatiotemporal characteristics of drought in Southwest China and its atmospheric circulation linkages. The findings establish a novel theoretical framework for understanding regional drought events, with significant implications for water resource management, agricultural planning, and ecological conservation. Particularly, the strong drought response to ENSO variations provides a scientific basis for climate prediction and drought early warning systems in the region. The identification of ENSO's predominant influence offers valuable insights for developing targeted drought mitigation strategies in this ecologically sensitive area.

This study has made significant progress in elucidating the spatiotemporal characteristics of meteorological drought in Southwest China and its teleconnections with atmospheric circulation patterns. However, several limitations warrant consideration for future research improvements.

The complex climatic heterogeneity of Southwest China, characterized by substantial elevational gradients and diverse natural conditions [30], presents challenges in drought assessment. While this research primarily focused on atmospheric circulation indices as drought drivers, it did not fully account for other potential contributing factors such as land-use changes and anthropogenic activities [31]. Future investigations should adopt a more comprehensive approach by integrating multiple influencing factors to better understand drought formation mechanisms and evolutionary processes.

Methodologically, this study was limited to assessing meteorological drought within the region, without extending the analysis to hydrological, agricultural, or socioeconomic drought impacts and their progression cycles. Although the SPEI demonstrated satisfactory applicability in characterizing meteorological drought events—showing good consistency with historical drought records in terms of timing and severity—the regional suitability of drought indices requires further validation [32]. The question of whether SPEI represents the optimal drought indicator for this specific region remains open for investigation. Future research directions should focus on developing integrated assessment frameworks incorporating multiple drought types. Validating and optimizing drought indices for regional specificity. Employing advanced climate models and statistical approaches to enhance drought prediction capabilities. These methodological advancements would significantly improve drought management strategies and climate change adaptation measures, not only for Southwest China but also for other drought-prone regions globally. The integration of multidisciplinary approaches will be crucial for addressing the complex challenges posed by drought under changing climatic conditions.

VI. CONCLUSION

This study employed the SPEI index at various temporal scales from 1960 to 2022 to analyze meteorological drought characteristics in Southwest China. Using runs theory, we conducted a detailed assessment of drought duration, intensity, and frequency, investigating the spatiotemporal evolution and trends of drought over the past 63 years. Additionally, we examined the teleconnections between meteorological drought and atmospheric circulation patterns to identify key influencing factors. The main conclusions are as follows:

(1) **Temporal Characteristics of Drought:**

At the inter-annual scale, the longest drought duration in Southwest China reached 21 months (2005–2007). Seasonally, the maximum SPEI-based drought intensity occurred in winter 2010, with spring 1969 and winter 2010 classified as severe drought years. Seasonal drought frequency analysis revealed a consistent hierarchy: spring > winter > autumn > summer. Mild and moderate droughts were the most common types, while severe droughts occurred less frequently, with no extreme drought events recorded.

(2) Spatial Variability of Drought Trends:

Significant spatial heterogeneity was observed in seasonal drought patterns:

Spring: Overall drought mitigation trend.

Summer: A distinct northeast (wetting)-southwest (drying) dipole pattern.

Autumn: Predominant intensification of drought across most regions.

Winter: A tripartite spatial regime with wet-dry-wet transitions from southeast to central to northwest regions.

(3) **Influence of Atmospheric Circulation Patterns:**

Regional drought responses to atmospheric circulation exhibited marked variability: **ENSO** exerted the strongest influence, with significant correlations across 56.8% of the study area. **PDO** showed the second-highest impact (53.3% significant correlations), primarily affecting southeastern and northern regions. **AO** demonstrated weaker drought associations, limited to northern and northwestern areas. **NAO** had the least influence, with only 7.1% of the region (northern and southern margins) showing significant positive correlations.

These findings enhance our understanding of drought mechanisms in Southwest China, providing a scientific basis for drought prediction, water resource management, and climate adaptation strategies. Future research should further explore the combined effects of multiple climatic drivers and regional-scale anthropogenic influences on drought variability.

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Soil Pollution and its effects on Agriculture

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Abstract— *Soil pollution can lead to the emergence of new pests and diseases by changing the balance of ecosystems and causing the disappearance of predators or competing species that regulate their biomass. It also contributes to the spreading of antimicrobial resistant bacteria and genes, limiting humanity's ability to cope with pathogens. Pollution can also cause the quality of soil to dwindle over time, making it harder to grow crops. Currently, the degradation of land and soils is affecting 40 per cent of the world's population. Soil has a key role to play through its ecosystem functions as it affects water regulation, nutrient recycling, food production, climate change and the biodiversity of terrestrial ecosystems. Transitioning from soil degradation to practices that restore soil is critical to ensure the food security and wellbeing of generations to come has now become a call of time..*

Keywords— ***Land degradation, heavy metal pollutants, pesticides, sustainable agriculture, soil fertility.***

I. INTRODUCTION

The study of soil pollution is very important to many researchers and those interested in the environment, due to the great impact that the pollution of agricultural lands has on the lives of humans and animals alike. The chemical and physical changes in soil composition are caused by the entry of foreign bodies. Also, the use of pesticides and chemical fertilizers in large quantities, the fall of acid rain, as well as the dumping of solid and liquid waste from factories and others, contribute to the loss of soil fertility and organic materials. Also, volcanoes, fires and mining contribute significantly to soil pollution and losing its organic matter and fertility. Pesticides fungicides and chemical fertilizers affect soil and agricultural crops. The use of untreated wastewater to irrigate agricultural lands causes soil pollution through the growth of harmful insects and plants.

Soil pollution refers to the contamination of soil with anomalous concentrations of toxic substances. It is a serious environmental concern since it harbours many health hazards. For example, exposure to soil containing high concentrations of benzene increases the risk of contracting leukaemia. An image detailing the discolouration of soil due to soil pollution is provided.

It is important to understand that all soils contain compounds that are harmful/toxic to human beings and other living organisms. However, the concentration of such substances in unpolluted soil is low enough that they do not pose any threat to the surrounding ecosystem.

When the concentration of one or more such toxic substances is high enough to cause damage to living organisms, the soil is said to be contaminated. Environmental pollution is all the undesirable changes that occur in the environment, whether partial or total, due to the whole types of human activities. It is also known as the atmosphere that results from changes in the ecological environment created by humans, Environmental pollution can be considered as the cause of inconvenience, damage, disease, or death [1]. Soil pollution can be defined as the entry of foreign bodies into the soil that leads to a change in the chemical and physical composition. This often results from the use of pesticides and fertilizers, and acid rain that changes the pH of the soil, throwing off radioactive unions

and others [2]. Also, it can be defined as the destruction that affects the soil layers causing a change in the natural characteristics of the main environmental elements due to the leakage of complex chemical compounds or artificial radioactive materials that raise the radioactive level in the soil, and impede its analysis. Pollution of agricultural land is defined as the corruption that affects agricultural land, and changes its natural, chemical or biological characteristics and properties. It makes it negatively affect, directly or indirectly, on the person, animal or plant living on its surface. Agricultural soil pollutants include agricultural residues such as plant residues and their weeds, roots left over from burning the ground, vegetable residues, crop stems, tree leaves, and fallen fruits before they ripen [3].

The origin of soil pollution may be natural or anthropogenic and therefore may have happened long ago or recently. Certain contaminants are often associated with specific activities, such as pesticide use in agriculture or radionuclides from nuclear power plants, while many others could result from a variety of sources of pollution.

Agricultural soils can be contaminated with a wide range of compounds, from both direct inputs (point source pollution) such as the application of pesticides and fertilizers and indirect inputs (diffuse pollution) such as flooding and atmospheric deposition. Polluted soils also represent a secondary emission source of contaminants to surrounding air, surface waters, groundwater, and subsequently to oceans. The main anthropogenic sources of soil pollution are the chemicals used in or produced as byproducts of industrial activities; domestic, livestock and municipal wastes (including wastewater); agrochemicals; and petroleum-derived products.

These chemicals are released to the environment accidentally, for example from oil spills or leaching from landfills, or intentionally, through use of fertilizers and pesticides, irrigation with untreated wastewater, or land application of sewage sludge. soil pollution has an adverse impact on food security in two ways –it can reduce crop yields due to toxic levels of contaminants, and crops grown in polluted soils are unsafe for consumption by animals and humans. It urged governments to help reverse the damage and encouraged better soil management practices to limit agricultural pollution.

The main sources of soil pollution in agricultural areas can be grouped as:

i) pesticides ii) mineral fertilizers; iii) organic fertilizers (manure and sewage sludge); iv) wastewater for irrigation; v) plastic materials such as films for mulching and greenhouses, drip irrigation tubes and empty packaging; vi) and rural wastes.

II. HEAVY METALS

The contamination of agricultural soils by heavy metals is one of the most important methods of soil degradation (EU Soil Thematic Strategy). Soil contamination by heavy metals presents many problems for soil functions, the environment, agriculture production, food chains or even human health [Adriano,]. The maintenance of a suitable state of soil load by heavy metals should be the interest of every society. The evaluation of soil load by heavy metals must be supported by the knowledge of heavy metals' background values, their inputs into soils, their behaviour and fate in the soil environment and their transfer into the plants or groundwater [Kabata-Pendias].

Pikula and Stepien [Pikula,] deal with heavy metals mobility in the soil profile. The behaviour of Cd, Cu, Pb and Zn depending on selected soil conditions was studied in a long-term microplot experiment. The mobility of heavy metals was defined for light texture soil and medium texture soil.

The transfer of Cd from soils with different Cd contents caused by agricultural techniques in the Amazonian area into cocoa plants was observed in the article of Rosales-Huamani et al.. The increased Cd load in cocoa beans complicates the husbandry of farmers in the area and the study shows the main principles of the problem. The content of Cd in the leaves of maize (Zea mays) was studied by Franič et al. The authors compared different maize genotypes and the effect of Cd on photosynthesis through chlorophyll fluorescence in selected plants.

Skála et al. observed the contamination of soil and plant by zootoxic elements (As, Cd and Pb) loaded by increased heavy metals contents in fluvial zones. The main soil characteristics influencing the transfer of risky elements from soil into selected plants, barley (Hordeum vulgare) and triticale (Triticosecale) or individual parts of the plant, shoots and grain of oat (Avena sativa) were defined using statistical tools. The single correlation analysis compared risky elements uptake by plants with its mobile fractions in soil (extracts by NH4NO3, CaCl2 and Na2EDTA).

Kuziemska et al. present a study focused on gentle remediation techniques. The organic soil amendments available in agriculture (cattle manure, chicken manure and spent mushroom substrate) were applied into soil contaminated by increased content of Cu to decrease phytotoxic effect.

Jakubus and Graczyk studied the immobilisation effect of compost and fly ash on Pb uptake by narrow-leaved lupine (Lupinus angustifolius), camelina (Camelina sativa) and oat (Avena sativa). The Pb contents in the soil and plants were used to calculate the risk assessment code (RAC), individual contamination factor (ICF), bioconcentration

factor (BCF) and contamination coefficient level (CCL). The higher immobilisation effect of fly ash compared to compost was observed in the study.

III. PESTICIDES

The Pesticides are designed to kill bugs that are harmful to plants. Pesticides kill specific pests on plants such as slugs, beetles and flying insects. The chemicals used in most pesticides can kill more than just garden pests; they can kill the helpful organisms that live in the soil. Some of these chemicals can remain in the soil for years, effectively keeping necessary microorganisms from working the soil. Common chemical pesticides that are used in gardens and by large-scale crop producers include the following: • Basic Copper Sulfate • Silica Gel • Sodium Fluoride

The pesticides used in modern conditions allow not only to reduce crop losses from pests and maintain the resulting products quality [M.A. Daam, J. Gao, M. Hvězdová, E.M. John]. The soil-protective and minimal tillage is impossible without the pesticides use; it is possible to reduce the effectiveness of other measures, for example, the application of fertilizers and ameliorants [R. Kodešová, L. Zhichkina, S.-K. Lammoglia]. Insecticides and acaricides, nematicides, rodenticides, molluscicides, repellents, pheromones, fungicides, herbicides, desiccants, plant growth regulators - are pesticides [Q. Li, . A. Mudhoo, E. Pose-Juan, C. Qu, N. Rafique and V. Silva].

IV. CONCLUSION

The study of effects of pollution on agriculture is indeed an urgent issue to work upon since it leads to a number of environmental effects like soil pollution, air pollution, water pollution and land pollution. With the advent of modern agricultural practices like use of high yielding variety seeds and eventually huge amounts of fertilizers is leading to degradation of land and desertification all over the country. The only solution to the above environmental problems is shifting towards green energy sources like manures, vermicomposts, biogas slurry and composting practices which are not only beneficial for crops but also helps in regenerating the degraded land. These organic alternatives are purely biodegradable and eco friendly.

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Artificial Insemination and Embryo Transfer: Emerging Technologies in the Livestock Industry

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Abstract— *Artificial insemination and embryo transfer have emerged as revolutionary technologies in the livestock industry, offering remarkable opportunities for genetic improvement and efficient herd management. In modern agriculture, these assisted reproductive technologies are increasingly being utilized for a wide range of applications, including out-of-season estrus induction, enhancement of reproductive performance, and preservation of endangered species or breeds. Nonetheless, significant advancements have been made in embryo technologies, particularly in the areas of estrus synchronization, superovulation, and in vitro embryo production. Incorporating applied reproductive technologies continues to effect animal production systems by providing producers opportunities to enhance genetics, reduce transfer of disease, advance fertility, and ultimately increase offspring value. Improvements in fertility and technology, reductions in cost, and improvements in ease of application will ensure that more cattle producers will adopt applied reproductive technologies in future years. However, incorporation of applied reproductive technologies into production systems will vary worldwide depending on cattle markets, infrastructure, production systems, and climate.*

Keywords— ***Artificial insemination, embryo transfer, reproductive technologies, genetic improvement, estrus synchronization.***

I. INTRODUCTION

Reproductive biotechnology, such as artificial insemination and embryo transfer, is essential in animal husbandry for transferring desired traits between different types of farm animals. This is especially crucial for animals that do not produce milk. With the expected rise in demand for meat, the use of reproductive biotechnology is necessary to meet this demand and ensure food security in India and worldwide. Selective breeding of high-quality females with desirable males from specific breeds and crosses within the Indian genetic architecture is crucial to meet the demand for healthier A2 milk and to breed superior female calves with a long and productive life.

The use of reproductive biotechnologies in any given breeding system is determined by the natural behaviors of the species concerned, as well as by what is practically feasible in the way of reproduction. These new reproductive advances provide the potential to manipulate early embryonic development in some way to enhance reproduction or production. Research involves using embryonic technology to create embryos from the best pedigree cattle and supervise the reproduction and development of those embryos to maximize the number of cows and bulls born. The major goal of this paper is to provide an in-depth review on artificial insemination and embryo transfer in the livestock industry. India has a vast resource of livestock and poultry, which plays a vital role in improving the socio-economic conditions of rural masses. There are about 303.76 Mn bovines (Cattle, Buffalo, Mithun, and Yak), 74.26 Mn sheep, 148.88 Mn goats, 9.06 Mn pigs and about 851.81 Mn poultry as per 20th Livestock Census in the country. In the current scenario, India is the largest producer of Milk and Buffalo Meat, the 2nd largest producer of Goat meat, 3rd in Egg

production and the 8th largest in overall Meat Production in the world (Katoch, 2022) (Bankar *et al.*,) (Siripurapu *et al.*,2024) (Sharma & Shelly, 2023). Meat and milk from farmed animals including livestock (cattle, goat, and buffalo) and poultry are sources of high-quality protein and essential amino acids, minerals, fats and fatty acids, readily available vitamins, small quantities of carbohydrates and other bioactive components. Some poor countries may not be able to sustain these levels of meat and milk requirement, leading to malnutrition. (Rueda *et al.*,2024) (Tona, 2021) (Ponnampalam *et al.*,2022) (HE *et al.*,2021) Demand for meat and milk production is also expected to double in 2050 in developing countries, where population is expected to double (Latino *et al.*,2020) (Erdaw, 2023) (Van Dijk *et al.*,2021) (Humpenöder *et al.*,2022). Thus, to meet the requirement, increasing production, safe processing and marketing of meat and milk, and their products are big challenges for livestock producers. In that scenario Biotechnology is being an emerging field in various research and production field of livestock industry. It has the potential to improve the productivity of animals by increasing growth, carcass quality and reproduction, improving nutrition and feed utilization, improving quality and safety of food, improving health and welfare of animals, and reducing waste through more efficient utilization of resources. Therefore, Various biotechnology methods are being used in improving the breeding stock of animals. These include artificial insemination (AI), embryo transfer (ET), in-vitro fertilization (IVF), somatic cell nuclear transfer, and the emerging technology on somatic cell nuclear transfer.

Artificial insemination is by far the most widely used biotechnology in animal reproduction and has been reported to result in genetic progress that is four times better than natural mating. Artificial insemination (AI) and embryo transfer (ET) are probably the most popular methods that have been adopted in developed and developing livestock industries. Especially since the development of efficient semen freezing methods, Artificial insemination has become the most widespread biotechnology applied to livestock and especially cattle production. AI has allowed for the implementation of the progeny-testing scheme prevalent particularly in dairy cattle production, and which has had a major impact on the improvement of the herd by increasing the accuracy of selection despite the associated increase in generation interval. Supporting technologies that have increased the efficiency of AI and ET include micromanipulation of gametes and embryos for splitting, sexing, cloning, gene transfer, cryo-preservation of embryos, in-vitro maturation, fertilization, and culture (IVFMC) as well as genome analysis. The recent advances in biotechnology in reproduction also include production of transgenic animals and cloning (Said *et al.*,2020) (Arain *et al.*,2023) (Funahashi2020) (Das *et al.*,2022).

Although Embryo transfer technology presently not economically feasible for commercial use on small farms, moreover embryo technology can greatly contribute to research and genetic improvement of local breeds. Advances in this area are mainly applicable in cattle. There are two procedures presently available for production of embryos from donor females. One consists of superovulation, followed by AI and then flushing of the uterus to gather the embryos. The other, called in vitro fertilization (IVF) consists of recovery of eggs from the ovaries of the female then maturing and fertilizing them outside the body until they are ready for implantation into foster females. The principal benefit of embryo transfer is the possibility to produce several progenies from the female, just as AI produces many offspring from one male animal (Mueller & Van Eenennaam, 2022) (Daly *et al.*,2020) (Hansen, 2020) (Ferré *et al.*,2020) (Fesahat *et al.*,2020) (Baruselli *et al.*,2020).

1.1 Background and Significance

In the field of animal sciences, particularly in animal reproduction and breeding, artificial insemination and embryo transfer are considered to be significant technologies of the 20th century. Artificial insemination in domesticated farm animals was first demonstrated in the dog in 1780, and the first calf was born from embryo transfer in the rabbit (Sikka & Atheya, 2022) (Mukherjee *et al.*,2023). The successful application of these two biotechnologies in recent decades has had an unprecedented impact on genetic improvements of livestock and domesticated species. Increased reproductive performance has been observed in animals since then, particularly in relation to the amount of sperm and embryos produced per animal per year. (Verma *et al.*,2022)

Reproductive management naturally influences the genetic pool of domestic breeds and has a direct effect on animal productivity and meat and milk quality. Reports from various countries have shown that the influence of embryo transfer programs in cattle is rapidly reflected in their genetic gains. Artificial insemination and embryo transfer address livestock from the point of view of reproductive efficiency, which plays a decisive role in animal breeding. Sub fertile animals are responsible for serious economic losses due to reproductive failure and early culling. The organization on food and agriculture reported that a large amount of cattle genetic material was sent abroad by various regions. These areas have a large

market share of livestock, mainly sheep, goats, and bovines (Mebratu *et al.*,2020).

Artificial insemination (AI) and embryo transfer (ET) are pivotal technologies in modern livestock breeding, significantly enhancing genetic improvement and reproductive efficiency. These advanced reproductive technologies (ART) facilitate the rapid propagation of superior genetic traits across herds, thereby improving overall productivity.

Key Statistics and Trends

Conception Rates:

The conception rate after AI or ET is a critical metric for evaluating breeding methods. Studies indicate that combining AI with ET can yield higher pregnancy rates compared to AI alone, with some reports showing up to 61.9% pregnancy success when both methods are utilized together (Bortoluzzi, E. M. *et al*., 2024).

Production Data:

A comprehensive analysis from a large dataset involving over 2.5 million animal records revealed that: 95.68% of lactations were from AI, while only 0.23% were from IVF, indicating the predominance of AI in current practices. The use of MOET accounted for approximately 4.09% of lactations (Lafontaine, Simon *et al*., 2023).

Embryo Production Growth:

Since 2017, the number of embryos produced via IVF has surpassed those produced through traditional flushing techniques, highlighting a shift towards more efficient embryo production methods *(*Mikkola, M. *et al.*,2024).

Genetic Progress:

The integration of genomic assessments with ART allows for the identification of animals with high genetic potential at an early age, potentially achieving about seven years' worth of genetic progress in just one year through selective breeding practices (Mikkola, M. *et al.*,2024).

Economic Impact:

The economic advantages of these technologies are substantial, as they allow for the introduction of superior genetics into herds at a lower cost and with enhanced biosecurity measures (Mikkola, M. *et al*.,2024).

Advantages of ART

Increased Offspring Production: ART enables superior females to produce multiple offspring in a shorter timeframe compared to traditional breeding methods.

Genetic Diversity: Facilitates the introduction of diverse genetics into herds, which can enhance resilience and adaptability.

Improved Reproductive Efficiency: Technologies like ovum pickup and embryo freezing allow for flexible breeding schedules and improved synchronization among recipient animals (Mikkola, M. *et al.*, 2023).

The adoption of artificial insemination and embryo transfer technologies is revolutionizing livestock breeding by improving genetic quality and reproductive efficiency. As these technologies continue to evolve, they promise to play an even more significant role in shaping the future of livestock production globally.

II. HISTORY OF ARTIFICIAL INSEMINATION AND EMBRYO TRANSFER

The history of artificial insemination and embryo transfer dates back to the early 20th century, when scientists began experimenting with ways to improve breeding practices in livestock. Artificial insemination and embryo transfer have revolutionized the livestock industry by allowing for more controlled breeding practices. These emerging technologies have the potential to greatly improve genetic diversity and overall livestock health (Houston *et al.*, 2020) (Brito *et al.*, 2021) (Neethirajan & Kemp, 2021). Artificial insemination and embryo transfer have been significant advancements in the livestock industry, revolutionizing breeding practices and genetic selection. The history of artificial insemination is reported as with the first successful artificial insemination in livestock in 1949 with dairy cattle (Sharma *et al.*,2024) (Bruno, 2022) (Shanku, 2023).

III. TECHNOLOGICAL ADVANCEMENTS IN ARTIFICIAL INSEMINATION

Since the advent of genetics, IART has been an increasingly powerful tool in livestock industries. Technological advancements have since revolutionized the development of AI, facilitating the evolution of cryopreservation protocols to enhance sperm quality and potential cryoresistance at different developmental stages of livestock. Several researchers have continuously optimized the procedure of IART to improve associated productivity. Additionally, the application of air-dropping fresh and cryopreserved spermatozoa after uterine expulsion or transfer to the oviduct, or superovulatory flooding of sperm cells in the genital tract, has in many ways eliminated the critical limitations of reproductive technologies. Furthermore, numerous research findings

have facilitated the application of donor inseminated AI and unique short-term fertility tests of sires and males, blind sperm evaluation, and selection procedures because of the capability of oviductal sperm selection and removal of immotile or abnormal spermatozoa using in vitro or in vivo setups (Animal *et al.*,2020) (Mackenzie and Kyriazakis2021) (Bassey, 2021) (Stucki, 2023).

The development of different modern AI technologies has significantly enhanced the productivity of livestock industries by changing IART from a potential animal welfare concern to a less invasive AI procedure. These essential integrations of science and technology between reproduction specialists have drastically reshaped the mechanisms of AI and sperm cryostorage, the conventional AI technique used to improve the productivity of livestock. Additionally, more than 70 million livestock were artificially inseminated in the same year, in line with the assertion that artificial insemination is the safest way to improve the productivity of animals (Quelhas *et al.*,2023) (Panda *et al.*,2021) (Seidel Jr & DeJarnette, 2022). Art has revolutionized all aspects and transferred IART into an increasingly high-tech, high-impact AI.

IV. TECHNOLOGICAL ADVANCEMENTS IN EMBRYO TRANSFER

One of the key technological advancements in embryo transfer is the use of cryopreservation techniques to store embryos for future use. One of the key technological advancements in embryo transfer is the use of cryopreservation techniques to store embryos for future use. This method has revolutionized the livestock industry by allowing breeders to preserve genetic material and ensure the future of their herds. Technological advancements in embryo transfer have greatly improved the efficiency and success rates of breeding programs in the livestock industry. One of the key technological advancements in embryo transfer is the use of sexed semen to improve the gender selection process in breeding programs. Recent advancements in sexed semen technology have revolutionized the breeding industry by allowing for more precise gender selection in livestock. One of the key technological advancements in embryo transfer is the use of in vitro fertilization (IVF) techniques to improve success rates (Aljaser, 2022) (Kumar *et al.*,2022) (Khan *et al.*,2021) (Sharma *et al.*,2021) (Valente *et al.*, 2022) (Tharasanit & Thuwanut, 2021) (Sharafi *et al.*,2022).

V. BENEFITS OF ARTIFICIAL INSEMINATION AND EMBRYO TRANSFER IN LIVESTOCK INDUSTRY

Artificial insemination (AI) and embryo transfer (ET) have made significant strides in the livestock industry over the past two decades, with potential applications in producing recipients, the use of sexed semen, reproductive biotechnologies, and the creation of genetically modified animals. Several benefits exist in using AI and ET in the livestock industry. The use of AI and ET enables animal breeders, producers, and farmers to select and reproduce superior female animals and sires, which will have direct effects on genetic improvement. By selecting proven breeding stock through technological means such as AI and ET and superior male-female combinations, genetic improvement takes a giant step forward. The use of sexed semen also adds a bonus in terms of increased milk production and desirable female characteristics. The reduction of unwanted or less desired bull calves for the beef industry using sexed semen has also been reported. AI, in combination with induced estrous cycles in the dairy industry, permits flexible and improved herd management for ideal husbandry care, including scheduling of age and appropriate milk production and extended milk production patterns in early lactation cows (Varshney *et al.*,2021) (Razzaq *et al.*,2021). AI is also a practical and cost-effective method to introduce desirable traits into dairy herds when farmers or breeders have minimal farm facilities. Interestingly, the use of AI and ET in the livestock industry increases the financial wealth of farmers or breeders by reducing the costs in herd management, which are known to negatively impact their businesses (Singh *et al.*,2021) (Monteiro *et al.*, 2021) (Akhigbe *et al.*,2021) (Javaid *et al.*, 2023). Finally, animal welfare can be improved using breeding methods due to genetic selection based on reproductive biotechnologies such as AI and ET. Moreover, animal welfare is one of the critical purchasing factors in today's world. Sustainable animal breeding, together with technological improvements, is crucial in evolving methods to facilitate food security and reduce the impact of livestock rearing on the environment. Often, reduced maintenance costs, mainly herd-replacement costs due to genetic improvement, are expected to yield positive economic returns. In contrast, farmers and producers are encouraged by substantial economic gains as a result of optimal breeding strategies using AI and ET. Generally, AI and ET are of expanding importance in African countries and can make major contributions to livestock development.

VI. CHALLENGES AND LIMITATIONS

Despite several advantages to both technologies, both AI and ET are not without limitations or challenges. AI success rates are strongly affected by cryopreservation of semen, with variable results across different livestock species. Moreover, technical expertise and facilities are required even for AI in domestic animals. Embryo transfer also has variable success rates, with newer cryopreservation methods being far less successful than working with fresh or in vivo produced embryos, particularly in pigs. Further, the complexities enforce procedural costs, making it too expensive for some farmers to consider. Other technical issues with embryo transfer are that it requires labor and time, and, especially for international programs, comes with trade restrictions and quarantine regulations. More generally, both embryo transfer and artificial insemination require a certain level of knowledge, infrastructure, and technical expertise or training. This limits less developed countries in their take-up (Zuidema *et al.,* 2021) (Koch *et al.*,2022) (Pardede *et al.*,2020) (Boneya, 2021).

Financial and human resources are major limitations, particularly in developing countries. A second challenge, likely to affect societies with greater access to advanced breeding technologies more than the less developed ones, is a growing level of ethical and environmental concern about animal welfare and genetic manipulation. Regulation can also act to limit the use of such technologies, either explicitly or by mandating excessively high standards that are effectively unachievable. Other issues that are inherent to both AI and ET in farmed animal populations are the loss of genetic diversity, with its attendant risks to adaptability, and an increase in genetic or familial trends for common disease conditions which could provide an increased risk. Yet any of these traits can be reduced in frequency as genetic technologies mature (Jahanger *et al.*,2022) (Usman *et al.*,2022) (Khan & Ozturk, 2021) (Haakenstad *et al.*,2022) (Rahim *et al.*,2021). Overall, this section will not try to seek solutions to each of these concerns. Rather, it will highlight what might presently be seen as barriers to the increased use of advanced breeding technologies and allow consideration of ways to address the concerns in the future.

VII. ETHICAL AND LEGAL CONSIDERATIONS

Society's perception of AI and ET as ethically acceptable technologies is reflected in the growth of the AI and ET industries over the last century. However, as the population's belief focus changes, so do the ethical principles governing social behavior, including the ethics of these reproductive technologies. The use of genetic material from inbred and infertile animals, who cannot live a healthy life, raises ethical issues as it provides a "selection filter" for which animals are allowed to breed, raising the concern about the definition of an animal's right to live. As our understanding of AI and ET has grown, so have the concerns about the welfare of the animals who are part of these reproductive technologies. The alleviation of an animal's suffering is a moral obligation, and this should inform technological advancements that promote AI and ET. A breach of guidelines established by laws regulating ethical practice of AI and ET within animals may result in the removal of consent to use these ARTs and of laws that directly regulate AI and ET. (Quartuccio *et al.*,2020) (Seidel2020) (Engdawork *et al.,* 2024) (Hart-Johnson & Mankelow, 2022)

Comparison of legal documents across the globe found no country adheres to the same AI and ET guidelines. Enforcement of the policy is crucial for implementing the AI and ET ethical principles. They must therefore follow ethics set by international and national government law as well as those developed by industries, researchers, veterinarians, and animal breeders. There is also a growing market of breeders who are environmentally ideologizing their AI and ET services, showing a clear stakeholder concern with environmental ethics. (Daly *et al.,* 2022) (ÓhÉigeartaigh *et al.*,2020).

VIII. COMPARISON WITH NATURAL BREEDING METHODS

When comparing artificial insemination and embryo transfer with natural breeding methods in the livestock industry, it is evident that these emerging technologies offer numerous advantages in terms of genetic improvement, disease prevention, and overall production efficiency. For example, artificial insemination allows for the use of superior genetics from a few elite animals to be spread more widely throughout a herd, leading to significant improvements in overall herd quality. In contrast, natural breeding methods rely on the mating of animals within the same herd, which may limit the genetic diversity and overall quality of the offspring. In contrast, emerging technologies in the livestock industry such as artificial insemination and embryo transfer offer a wider range of genetic diversity and can improve the overall quality of the offspring. When comparing artificial insemination and embryo transfer with natural breeding methods in the livestock industry, it is evident that these emerging technologies provide a wider range of genetic

diversity, leading to an overall improvement in the quality of the offspring. Additionally, they offer increased control over breeding outcomes and can help in the selection of desirable traits. (Brito *et al.*,2021) (Salgotra & Chauhan, 2023) (Houston *et al.*,2020) (Vaintrub *et al.*,2021).

IX. APPLICATIONS IN DIFFERENT LIVESTOCK SPECIES

The use of artificial reproduction techniques in domestic animals such as cattle, sheep, and goats has increased over the past few decades. It has now become an essential tool in the cattle industry because of the increased genetic improvement, selective breeding of species, and the potential to enhance the introduction of superior germplasm from different regions for genetic improvement of depleted populations worldwide. These assisted reproductive techniques are suitable for use in a variety of farming systems, including smallholder and commercial. To achieve optimum results with the different techniques, such as artificial insemination and embryo transfer, it is essential to have a good understanding of the reproductive characteristics of the animal, particularly the female germplasm, such as estrous patterns.

Each livestock species has specific reproductive characteristics, which influence the applicability of different reproductive techniques. A few examples of artificial insemination and embryo transfer of some selected mammalian species will be discussed, including cattle, sheep, goats, pigs, deer, and Cape buffalo. In sheep, long-term storage of oocytes and their in-vitro applications as a valuable reproductive tool in many facets of biology and medicine, such as studies about storage of tissues and organs for the possible future generation of animals with valuable alleles and genotypes, are noted. Artificial insemination and embryo transfer are applied worldwide in cattle. Both techniques have gained a lot of knowledge in the last few decades, resulting in increased technical knowledge and success in the development of both artificial insemination and embryo transfer. Furthermore, artificial insemination in cattle has been commercialized, and a large number of companies worldwide produce semen and provide technical expertise related to the procedures. These new techniques have given livestock producers an opportunity to access superior genetics from livestock breeding programs, increasing the genetic pool and allowing for genetic gains to be made in some countries (Mebratu *et al.*,2020) (Hansen, 2020) (Baruselli *et al.*,2020).

X. ECONOMIC IMPACT IN THE LIVESTOCK INDUSTRY

Artificial insemination (AI) and embryo transfer technologies have made significant headway in genetic improvements and will continue to play important roles in the future. Substantial financial benefits have steadily attracted many reproductive physiologists to strategically focus on this area of research, as profit potential is a major driving force in commercial livestock production and genetic advancements. Clearly, the adoption and rate of utilization of such technologies decreased the overall production costs, thus increasing profits. However, one of the only ways to influence an industry's reproductive extent can be through economic means (Singh & Singh, 2022) (DeCherney *et al.*,2022) (Singer *et al.*,2021).

It has long been established that in both AI and ET, financial benefits are possible via improved pregnancy rates. In the case of AI, elevated pregnancy rates using superior sires are made possible, presenting the potential to increase financial revenue by as much as twenty times the original seedstock bank. Moreover, the progeny is generally able to achieve much higher annual profit margins of more than ten times per seedstock. Upon commercial company entry, such trends become even more predominantly obvious, with the further potential to increase annual profit margins by as much as 10–15% through the elimination of problematic genetic traits and gene carriers. Differing market trends can have significant financial implications for a company, in the success of producing higher profitability or, by logical analysis, negative consequences. Though a quick cash injection it may be, AI and ET must be seen as long-term investments. This is because the initially high semen prices, for instance, will, through a systematic deployment strategy, decrease, thus increasing the gene pool, which can also further benefit commercial producers. Additionally, profit margins can increase dramatically when a company also takes advantage of reducing disease prevalence by using reproductive technologies. Over the long term, in addition to the economic incentives, other long-term benefits in utilizing one of the technologies are the decreased costs associated with increasing productivity by eradicating animal disease, efficiency, parasitism, and other animal welfare issues, in turn ultimately contributing to the reduction of industry disease-related expenditure and improvement of national stock. Therefore, this must be developed as a cohesive strategic plan including a multi-faceted approach between government, commercial companies, and breeding companies alike to have the most impact at a national level in production animals (Davidson & Boland,

2021) (Davidson and Boland2020) (Medenica *et al.*,2022).

XI. FUTURE TRENDS AND INNOVATIONS

The livestock industry is on the brink of a major transformation, thanks to new developments in genetic selection, reproductive technologies, and data analytics. Artificial insemination and embryo transfer technologies are set to revolutionize the industry, leading to increased efficiency and genetic improvement. One potential future trend in artificial insemination and embryo transfer technologies in the livestock industry is the use of advanced genetic selection techniques to further enhance desirable traits in livestock populations. One example of advanced genetic selection techniques in the livestock industry is the use of marker-assisted selection to improve disease resistance and overall health in livestock populations. In the future, artificial insemination and embryo transfer technologies are expected to become even more advanced, allowing for greater precision and efficiency in breeding programs. Some potential future advancements in artificial insemination and embryo transfer technologies include the use of gene editing techniques to enhance desired traits in livestock.

XII. CONCLUSION AND FUTURE DIRECTIONS

To sum up, the progress made in artificial insemination and embryo transfer technologies has completely transformed the livestock industry, resulting in better breeding effectiveness and a wider range of genetic characteristics. Moving forward, further advancements in artificial insemination and embryo transfer technologies are expected to revolutionize the livestock industry even more. This includes the potential for increased efficiency, genetic diversity, and overall productivity. In conclusion, the implementation of artificial insemination and embryo transfer technologies in the livestock industry is expected to continue advancing, leading to improved efficiency, genetic diversity, and overall productivity. Moving forward, further research and development in this area will be crucial for maximizing the potential benefits of these emerging technologies.

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Development of one step spot detection method for hydrogen peroxide in raw milk as preservative and adulterant

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Abstract— *There was an incident in our locality that one-ton litre raw milk was detained in check post alleging adulteration of milk with Hydrogen peroxide. The news was reported in media and widely discussed by public. In the view of incident five under graduate students under my guidance conducted study of effect of hydrogen peroxide in raw milk. The study includes the preservative and adulterant characteristics of hydrogen peroxide in raw milk. They developed a simple method for quick detection of hydrogen peroxide in the raw milk. The investigation proved that optimum amount of hydrogen peroxide can act as preservative particularly in the occasion when quick cooling of milk is not possible. Addition of hydrogen peroxide increases shelf-life anti-bacterial properties of the row milk. The study developed a one step spot detection method for hydrogen peroxide in row milk. The test gives positive result above*

Keywords— ***Raw milk, adulterant, hydrogen peroxide, Methylene blue, Anti-microbial, Lactoperoxidase, Clot on boiling***

I. INTRODUCTION

Milk is one of the most complete foods for humans, containing nutrients including proteins, minerals, fats, carbohydrates, and vitamins, and is widely marketed and consumed by the population across the globe. The composition of milk is the combination of several solid components (12–13%) in water. These components, their distributions, and interactions determine the structure and functional properties of the milk, together with its suitability for processing and consumption. This rich composition makes milk an excellent substrate for the growth of various groups of microorganisms, hence storage and transportation of milk is always challenging [1]. Currently refrigeration is the only permitted method of preservation of milk. But it is not a viable method in rural, underdeveloped and conflict region due to the lack of electricity and high cost. It is observed that raw milk is deteriorated within two or three days even after storing in refrigerator [2]. Hence the practice of a relatively harmless preservative method is advisable. The components added for the extension of shelf life shall be nontoxic, non-reactive with components of milk and inexpensive.

Milk has limited inherent ability to inhibit microbial growth due to the presence of molecules Lactoperoxidase(LP), Hydrogen Peroxidise H_2O_2 in the milk. The average concentration of LP in row milk is 39mg/L. This enzyme molecule with Hydrogen Peroxide together activates the peroxidation of thiocyanate to hypothiocyanite ion, which is supposed as the main antimicrobial component of milk. The challenging factor of this lactoperoxidase system of protection is insufficient concentration of Hydrogen Peroxide. Hence the moderate addition of Hydrogen peroxide in the milk will enhance the antimicrobial property of the row milk [3].

Hydrogen peroxide is one of the most versatile chemicals. It is an environmentally-friendly oxidant that

is widely employed in foods, textiles, and personal care products. United States accepted hydrogen peroxide for the production of cheeses up to maximum 0.05% of the weight of the milk. It has been recommended as a dairy preservative in tropical countries [4]. The side effect of hydrogen peroxide will be suppressed by the presence lactoperoxidase and thiocyante ions present in the milk [5]. The use of liquid and vapor phase H_20_2 is common in the food, pharmaceutical and medical industries to reduce or eliminate bacterial contamination. Hydrogen Peroxide control of microbial deterioration of fruits and vegetables are common. The wide application of Hydrogen Peroxide in food industry is due to its broad-spectrum activity as well as its nontoxic nature following degradation [6]. The addition of hydrogen peroxide beyond the optimum level has been strictly banned. Over concertation of hydrogen peroxide in row milk decreases its nutritional value. It may lead to the oxidative destruction of vitamins and other components.

There may chances of addition of hydrogen peroxide to halt the microbial activity in raw milk close to the expiry date or already unsuitable for the consumption. Hence the monitoring of concentration of hydrogen peroxide in the milk is essential. There are a few complicated analytical techniques to detect and quantify presence of hydrogen peroxide in the milk. Most of them are highly sensitive, requires prior sample preparations. It required trained operators and costly instruments. Hence simple and quick methode of detection of hydrogen peroxide workable with un educated, rural people are vital [7-9]. This paper discusses a simple method of detection of hydrogen peroxide in the raw milk. It also studies optimum concentrations of hydrogen peroxide in the milk for better shelf life without compromising the quality of milk.

II. MATERIALS AND METHODS

Fresh Cow milk samples were collected directly from farmers in clean, sterilized bottle from the rural areas of Kozhikode, Kerala, India. All samples were collected in the morning, the day experiments started. Collected samples kept in refrigerator till experiments begin. Hydrogen Peroxide (diluted in the ratio 1: 2.5 with distilled water), Methylene Blue, Sodium stearate, KI were purchased from Merck (Germany). Mineral salt broth and nutrient agar are obtained from Himedia Chemicals (India). All solution were prepared in sterilized water.

2.1 Preparation of milk sample:

Five different milk samples were prepared by mixing 100ml of raw milk with different concentrations of hydrogen peroxide. Sample A, without hydrogen peroxide was considered as blank sample. All samples were kept in room temperature and all test were carried out in room temperature.

Table 1: Milk sample

SAMPLE	% of H_2O_2 (V/V)
A	0
B	0.05
C	0.1
D	0.15
E	0.2

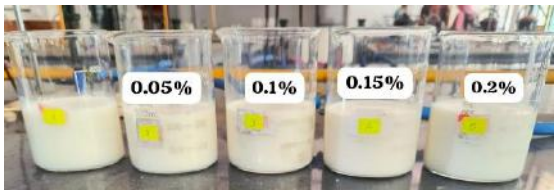


Image-1: Milk samples

2.2 Changes in flavour:

Appearance of unpleasant (slight sour or bitter) flavour was detected by organoleptic test periodically and tabulated.

2.3 Clot on boiling (COB):

5mLof milk taken in clean test tube from each sample and boiled over Bunsen burner in different time intervals. Clotting or coagulation of the milk samples was tested and tabulated.

2.4 Acidity test:

10mL from each milk sample was taken and diluted with equal amount of water. 3 to 4 drops phenolphthalein was added and shaken well. The mixture was titrated against 0.1N NaOH solution till the colour changed to pink. Noted the burette reading and percentage of acidity was calculated.

% acidity =(Volume of NaOH × 0.1 × 90 × 100)/ (V×1000)

2.5 Methylene blue reduction (MBR) test:

Previously prepared 1 ml of 1ppm Methylene Blue solution added to 10ml of milk taken in separate beakers corresponding to each sample and kept in room temperature and noted the colour change.

2.6 Anti-Bacterial analysis

Anti-bacterial analysis was carried out using Colony forming Unit (CFU) method. Each milk sample was

serially diluted to seven times using diluent phosphate Buffer. 1 ml of diluted samples were added to Petry plates contain Agar media at 45^0C. All plates were incubated for three days and bacterial count was recorded.

2.6 One step spot detection of H_2O_2

Previously prepared 4% Potassium iodide solution and 10ml of sodium stearate solution taken in dropper. The mixture added to the milk samples containing hydrogen peroxide and noted the changes.

III. RESULT AND DISCUSSIONS

3.1 Changes in flavour:

The trend of change in flavour of different samples of milk when kept in room temperature given in table 2. The milk sample without hydrogen peroxide exhibited change in flavour at earliest. The sample containing 0.2% hydrogen peroxide can withstand in room temperature over night without changing the flavour. It results explicitly proved that hydrogen peroxide (H_2O_2) has significant role in preserving flavour of milk [10]. Flavour of milk depends upon stability of milk constituents such as lactose, lipids, citrate and proteins, the stability of milk constituents and flavour depend temperature, p^H, water content etc [11]. Change in flavour of milk is attributed to the formation of various undesirable compounds in the milk. Lactose will be converted to furans, pyrones, cyclopentanes, carbonyl compounds etc. similarly, lipids will be converted to methyl ketones, lactones, and aldehydes [12]. The formation of undesirable compounds depends upon physico- chemical conditions of the milk sample. The presence of hydrogen peroxide in the milk has been arrested all changes in the milk and preserved the physio chemical balance of the milk in the room temperature. It is due to the presence of reactive oxygen generated by hydrogen peroxide, which inhibit microbial growth and prevent oxidative changes of milk sample.

Table 2: Changes in flavour

Hour	A	B	C	D	E
1	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
2	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
3	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
4	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
5	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
6	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
16	Sour	Sour	Slightly sour	Pleasing	Pleasing
17	Sour	Sour	Sour	Pleasing	Pleasing
18	Sour	Sour	Sour	Slightly sour	Pleasing
19	Bitter	Bitter	Sour	Sour	Pleasing
20	Bitter	Bitter	Bitter	Sour	Pleasing

3.2 Clot on boiling:

The clotting behaviour of milk samples given table3. The sample without hydrogen peroxide exhibited clot on boiling at 9.5hrs whereas sample E didn't exhibit clot on boiling even after keeping overnight at room temperature. The result is matching with the observations of flavour change. The result of clot on boiling showed the ability of hydrogen peroxide to preserve the milk sample at room temperature. Clotting of milk taking place due to the enzymatic actions of various bacteria and fungus present in the milk [13]. The clotting of milk produces annoying chemicals and render milk unfit for use [14]. The presence of hydrogen peroxide inhibits the growth microorganisms in the milk hence prevent from clotting of milk while storing in room temperature. The reactive oxygen generated by hydrogen peroxide prevent the microbial growth the extend the shelf life of the sample. Clotting of milk is largely associated with flavour change, the presence of hydrogen peroxide has the collective effect on flavour change and clotting.

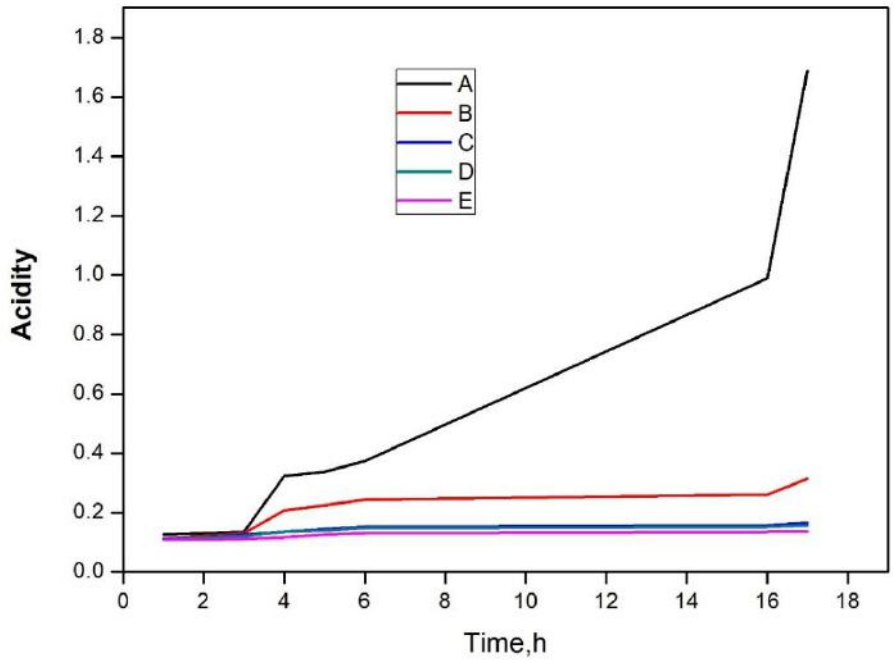
3.3Acidity

Titratable Acidity of the milk is its capacity of neutralisation with the base [15]. It is a very important parameter for the technical evaluation of the quality of milk. The titratable acidity of samples in different time intervals were recorded and given in table 4. The different components of the milk are acidic and contribute to normal acidity value. These components are carbon dioxide,

protein, phosphate and citrate. When bacterial count increases, lactose converted to lactic acid and increased the titratable acidity of the sample. the titratable acidity of fresh milk typically varies from 0.15 to 0.20 depending on the composition of milk [16]. The table shows that sample without hydrogen peroxide showed significant change in acidity within in 20hrs. it has exhibited gradual increase of acidity from 0.126 to 1.6875 where as such a gradual change was not observed in other samples containing hydrogen peroxide. The sample E containing maximum hydrogen peroxide showed lowest change in acidity. It clearly correlates the bacterial growth in the milk samples. When hydrogen peroxide is added to milk, it can potentially lower the overall acidity of the milk due to its antimicrobial property. Hydrogen peroxide added in the either kill or inhibiting the growth of bacteria that contribute to milk acidity through fermentation. By reducing the population of bacteria, the production of lactic acid through fermentation is decreased, leading to lower acidity in the milk. Hydrogen peroxide would also react with proteins in milk, potentially stabilizing them and preventing their degradation over time. Since proteins can act as buffers, helping to regulate the pH of the milk. By stabilizing proteins, hydrogen peroxide may indirectly contribute to maintaining a lower acidity level in the milk.

Table 3- clotting behaviour of milk

Hour	A	B	C	D	E
1	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
2	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
3	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
4	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
5	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
6	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
16	Clotted	Slightly Clotted	Free flowing	Free flowing	Free flowing
17	Clotted	Slightly Clotted	Free flowing	Free flowing	Free flowing
18	Clotted	Clotted	Free flowing	Free flowing	Free flowing
19	Clotted	Clotted	Slightly Clotted	Free flowing	Free flowing
20	Clotted	Clotted	Clotted	Slightly clotted	Free flowing



Graph -1: Titratable Acidity

3.4 Methylene blue Reduction

The Methylene Blue Dye Reduction Test is widely used in dairy industry to determine the microbial load in the milk. This test involves the addition of methylene blue into a milk sample and measuring the time required for decolourisation. The disappearance of the colour indicates a high microbial load [17]. Methylene Blue act as a redox indicator that loses its colour under the absence of oxygen. Microbial load causes low oxygen concentration and fast decolourization of methylene blue. The decolourisation of samples in the presence of methylene blue exhibited in fig-2. The sample without hydrogen peroxide changed colour within 6 hours of keeping room temperature whereas sample with 0.2% hydrogen peroxide exhibited delayed decolourization. The sample E decolourised keeping 32hrs in room temperature. MBR test clearly verified the inhibiting properties of H_2O_2 towards the bacterial growth in treated milk and slow microbial load in milk samples containing hydrogen peroxide.

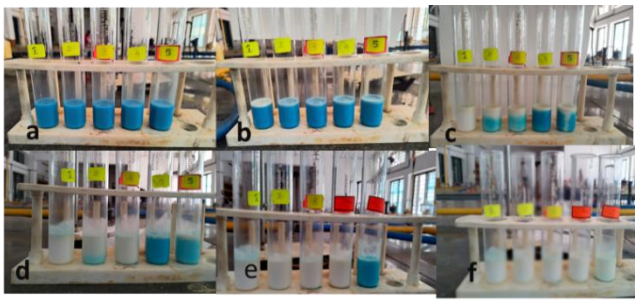


Image 2: Methylene Blue Dye Reduction Test

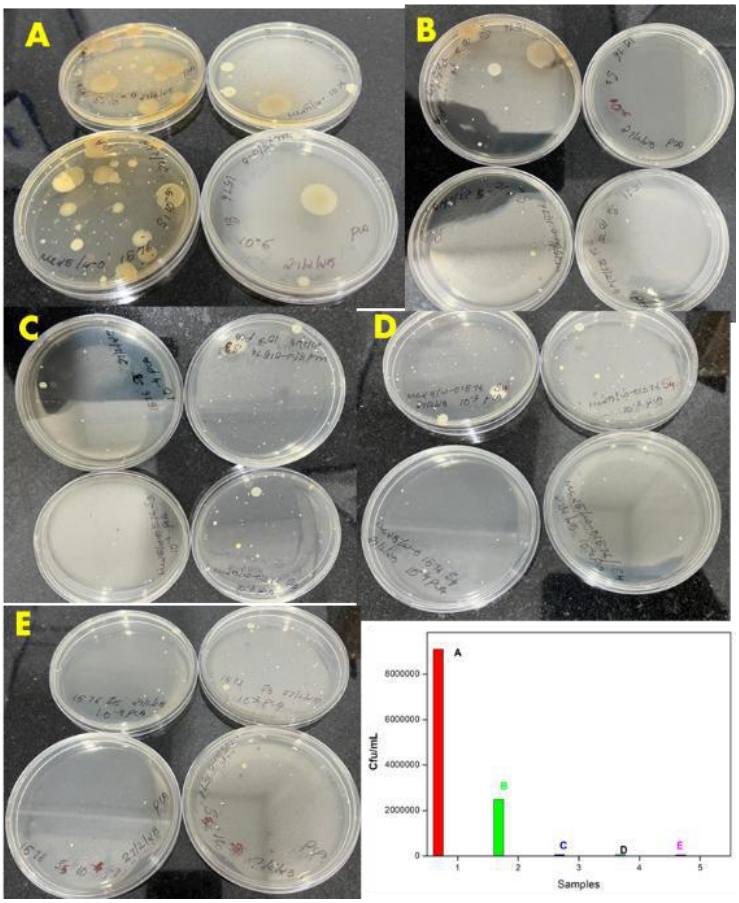


Image 3: Antimicrobial analysis

3.4 Anti-Microbial Analysis

Anti-microbial analysis of milk samples was carried out using Colony forming Unit (CFU) Methode. The result showed in Fig3. As shown in figure, sample without hydrogen hydrogen peroxide (A) exhibited maximum microbial growth. This result is matching with all other observations. When the amount of hydrogen peroxide increased bacterial growth gradually decreased. The enhanced antimicrobial activity of milk samples is attributed to improved activity of lactoperoxidase system in the milk. Lactoperoxidase is a natural enzyme present in the milk, which has inherent ability to protect milk from pathogens. The raw milk which containing greater amount

of lactoperoxidase will have longer shelf life. The amount of lactoperoxidase vary with a species, cow milk conatin an average of 1.4UAmL^{-1} lactoperoxidase. The antimicrobial activity is the combined effect of LP, SCN^{-1} produced by hepatic metabolism and hydrogen peroxide.the SCN^{-1} ion is oxidised by H_2O_2, the reaction is catalysed by LP. The two oxidation products hypothiocyanic acid (HOSCN) and hypothiocyanite ion (OSCN^{-1}) inhibits the growth of microorganisms in the milk. These compounds destroy or modify microbial cell walls leading to death or inhibition of growth of microorganisms[18-20]. The lactoperoxidase system has broad spectrum antimicrobial activity, kill or inhibit bacteria, fungi and virusus. Mamelian cells are not affected by oxidation products of SCN^{-1} and it is suggested that the addition of H_2O_2 is safe to some extent. This fact has greater relevance in places which have no facilities of quick cooling to preserve raw milk.

3.5 Detection of H2O2:

A one step spot detection method for H_2O_2 in raw milk was developed. Four drops hydrogen peroxide testing mixture added to all samples of milk. The sample without hydrogen peroxide didn't show any significant colour change when testing mixture was added dropwise. All other samples containing hydrogen peroxide exhibited colour changes and foams. More the hydrogen peroxides more the quantity of foams generated. All experiments were repeated three times. The KI in the testing mixtures was oxidised to I_2 in the presence of H_2O_2 present in the milk. Meantime H_2O_2 get reduced and oxygen gas released. This oxygen gas leads to foams in the presence lather forming content of testing mixture. The yellow colour is attributed to the presence of I_2. The method is simple and can be practiced anyone. When the amount of hydrogen peroxides increased intensity of yellow colour and hight of foam will also increase.

IV. CONCLUSION

There are situations when milk cannot be preserved in refrigerator, Hydrogen peroxide can act as effective chemical preservative. Raw milk naturally contains low concentration of H_2O_2, which has active role in the shelf life of the raw milk. The addition of optimum amount H_2O_2 in to raw milk has excellent preservative power boosting the activity of LP system naturally present in the raw milk. In the present study, we prepared four different samples with different concentrations of hydrogen peroxide. It was observed that, at the room temperature the increased level of H_2O_2 leads to delay in sour flavour development, positive COB test, decolorization of methylene blue and acidity development. The bacterial count study clearly proved the inhibition effect of hydrogen peroxide in treated sample. The study developed simple qualitative method for the determination of hydrogen peroxide in the raw milk**.**

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Collection and Quantification of Infectious Medical Waste at the University Hospital Center (CHU) of Abidjan / Cocody, Health and Environmental Risks, Ivory Coast

Collecte et Quantification des dechets Medicaux Infectieux AU Centre Hospitalier Universitaire (Chu) D'abidjan / Cocody, Risques Sanitaires et Environnementaux, Côte D'ivoire

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Abstract— *A questionnaire, an interview guide and observation were used to collect data for this action research. The CHU of Cocody has a modern technical platform and a wide range of treatment options for various diseases. Thus, the vastness and diversity of the CHU's care activities inevitably lead to the production of enormous medical waste of various types. Among these wastes, infectious medical waste and sharp objects (OPCT) are the subject of this research. In fact, the CHU produced 4267.12 kg of these infectious medical waste and sharp objects (OPCT) from 01/01/2024 to 31/03/2024. Quantification by weighing of this infectious waste, made it possible to note that 40.04% of this waste, or 1708.66 kg are OPCT and 59.96% or 2558.46 kg, are infectious medical waste. 90% of this waste is collected, however the route of the remaining 10% is unknown according to our surveys. 66.66% of the administrative and technical staff of the CHU. 72.72% of the PCI and PGOP staff are aware of the existence of the ministerial decree on the regulation of the management of health waste. The waste management process requires the establishment of a program for the management of said waste. This program, although existing at the PCI, requires training of the various actors in the medical waste management process as a whole. Sorting is abnormally done because pre-collection is and collection can only undergo. Only 90% of infectious medical waste is collected. The three-quarter (¾) filling of safety boxes (BS) and the method of introducing OPCTs into the BS are not respected by a large part of the medical staff. In fact, 45.46% of this staff is not even aware of the existence of decree 131 of June 3, 2009 relating to the management of medical waste and the lack of training is the cause.*

Keywords*— *Safety box, collection, sharps, infectious medical waste, weighing, sharps, pre-collection, quantification, sharps and sorting.

***Resume*—** *Un questionnaire, un guide d'entretien et l'observation ont permis de collecter les données pour cette recherche-action. Le CHU de Cocody, possède un plateau technique moderne et une large proposition de traitement de diverses maladies. Ainsi, l'immensité et la diversité des activités de soins du CHU, conduisent inéluctablement à la production de déchets médicaux énormes et de diverse nature. Parmi ces déchets, les déchets médicaux infectieux et les objets piquants, coupants et tranchants (OPCT) font l'objet de cette recherche. En effet, le CHU a produit du 01/01/2024 au 31/03/2024 4267,12 kg de ces déchets médicaux infectieux et objets piquants, coupants et tranchants (OPCT). La quantification par pesage de ces déchets infectieux, a permis de noter que 40,04% de ces déchets, soit 1708,66 kg sont des OPCT et les 59,96 % soit 2558,46 kg, sont des déchets médicaux infectieux. 90% de ces déchets sont collectés, par contre l'itinéraire des 10% restants, est inconnu selon nos enquêtes. 66,66% du personnel administratif et technique du CHU. 72,72 % du personnel de PCI et PGOP ont connaissance de l'existence de l'arrêté ministériel sur la réglementation de la gestion des déchets sanitaires. Le processus de gestion des déchets, nécessite l'établissement d'un programme de gestion desdits déchets. Ce programme bien qu'existant au PCI, nécessite une formation des différents acteurs du processus de gestion des déchets médicaux dans leur ensemble. Le tri est anormalement fait car la pré collecte l'est et la collecte ne peut que subir. Seuls 90% des déchets médicaux infectieux sont collectés. Le remplissage au trois-quarts (¾) des boîtes de sécurité (BS) et le mode d'introduction des OPCT dans les BS ne sont pas respectés par une large partie du personnel médical. A l'effet, 45,46% de ce personnel n'a même pas connaissance de l'existence de l'arrête 131 du 03 Juin 2009 relatif à la gestion des déchets sanitaires et le manque de formation en est la cause.*

Mots clés*— *Boîte de sécurité, collecte, coupants, déchets médicaux infectieux, pesage, piquants, précollecte, quantification, tranchants et tri.

I. INTRODUCTION

La croissance démographique, le développement industriel et le développement de la technologie médicale entraînent une augmentation de la production des différents types de déchets responsables d'une menace pour l'homme et l'environnement. La gestion de ces déchets sanitaires ; si elle n'est pas correctement organisée peut entraîner des risques de maladies chez le personnel de santé, le personnel chargé de l'élimination des déchets, les patients, la population et même provoquer des problèmes environnementaux (OMS, 2000). En effet, de nombreux accidents se produisent lors de la manipulation des déchets sanitaires infectieux occasionnant des blessures du fait des aiguilles de seringues ou autres objets coupants ou tranchants, qui n'ont pas été collectés dans des boîtes sécurisées (OMS, op.cit). Une étude menée par l'Organisation Mondiale de la Santé (OMS, 2002) auprès de 22 pays en développement a montré que 18 à 64% des établissements sanitaires n'éliminent pas correctement leurs déchets issus des soins. La Côte d'Ivoire n'étant pas en marge de ce schéma classique de traitement des déchets rencontre les mêmes difficultés dans cadre du traitement de ces déchets. En effet, une étude de caractérisation réalisée en 2016 par le PRSSE[1] avec l'appui financier de la Banque mondiale, révèle que : "ce sont au total 25,55 tonnes de déchets solides, qui sont produits chaque jour soit environ 9 325,09 tonnes par an. 62% de ces déchets sanitaires sont infectieux, 2% sont des déchets chimiques et pharmaceutiques et 36% sont assimilables aux déchets ménagers". Nous retenons que : "malgré les risques auxquels les acteurs sont exposés, le système de gestion des déchets sanitaires en Côte d'Ivoire souffre de certaines insuffisances malgré les efforts consentis par le pays et ses PTF [2] ". Le Ministère de la santé a intégré dans ses attributions et ce depuis 2005, l'hygiène publique incluant l'hygiène hospitalière et la gestion des déchets sanitaires. Dans chaque établissement sanitaire, un Comité d'Hygiène a été créé pour assurer au quotidien, la gestion des questions liées aux déchets sanitaires. Selon le Plan de Gestion des Déchets Sanitaires (PNDS)[3], malgré les initiatives menées pour renforcer le cadre institutionnel et réglementaire, toutes les structures créées sont restées peu opérantes voire inefficaces, la coordination des activités de gestion des déchets sanitaires est insuffisante. "De plus, au niveau

[1] Projet de Renforcement du Système de Santé et de Réponse aux urgences Épidémiques

[2] Partenaire Technique et Financier

[3] Plan de Gestion des Déchets Sanitaires (PGDS), Version finale, 19 juin 2023

réglementaire, même avec l'existence des différents arrêtés relatifs[4] au tri, à l'usage de contenants spécifiques, à la polarisation et la contractualisation, toutes ces dispositions ne sont pas toujours respectées par les établissements sanitaires et l'intervention du secteur privé n'est pas encore réglementée par des textes[5]".

Les constats soulevés ici, relatifs à la gestion des déchets sanitaires en Côte d'Ivoire, sont entre autres des facteurs limitant du processus de gestion desdits déchets sur toute l'étendue du territoire national. Le centre hospitalier universitaire de Cocody (CHU), une des références du système sanitaire ivoirien, nous amène à réfléchir sur son système de gestion des déchets dans cette étude. Le CHU est, en effet une des vitrines du système de santé ivoirien et avec ses nombrables services et le niveau de plateau technique offre les multiples facettes de difficultés dans la gestion des déchets produits. La question centrale que nous nous posons à travers cet article est : Comment se fait la gestion des déchets au CHU ? ou encore quelles sont les difficultés rencontrées dans la gestion des déchets sanitaires du CHU ? L'objectif que nous, nous fixons est de contribuer à l'amélioration de la gestion des déchets sanitaires dans leur ensemble au CHU d'une part et d'autre part d'étendre nos propositions à l'ensemble des structures de santé et assimilées en Côte d'Ivoire. Il s'agira par conséquent, de ressortir les réponses aux différentes questions posées.

II. MATERIELS ET METHODE

2.1 Site de l'étude

Le centre hospitalier universitaire de Cocody (CHU) créé par décret[6], est un hôpital public de troisième niveau de référence inauguré en juin 1970 et situé à Abidjan, Côte d'Ivoire. Il est érigé en Etablissement Public à Caractère Industriel et Commercial (EPIC) le 6 juin 1984. Le Service de Prévention et Contrôle des Infections (SPCI) est animé par un Chef de service, un Chef Adjoint, et cinq (05) Techniciens en Hygiène et Assainissement et cinq (05) Auxiliaires de santé. Il est localisé au sein de la S/DSIO[7] qui dépend de la DMS. Le SPCI a entre autres pour mission de "suivre et d'organiser les activités de Gestion des déchets hospitaliers et de gestion de la Buanderie". Le centre hospitalier est subdivisé. Pour la collecte et l'enlèvement des déchets hospitaliers ; les déchets produits au CHU, trois (03) lots ont été constitués et la gestion de chacun de ces lots, est confié à deux (02) sociétés privées.

Le lot 1, c'est exclusivement le Grand bâtiment du deuxième au treizième étages. L'entreprise prestataire, en charge de la collecte et de l'enlèvement des déchets, est SEQUOIA. Cette entreprise compte soixante-sept (67) agents.

Le lot 2 les services constituant ce lot sont : les urgences médicales, le bâtiment des consultations externes, l'administration, le laboratoire d'immuno- hématologie, les services techniques et l'internat. Et le lot 3, c'est le lot du PGOP. Il s'agit des services de la gynéco-obstétrique et de la pédiatrie. La gestion des déchets produits au niveau des lots 2 et 3, est confiée à la société NETSI. Celle-ci (NETSI), avec soixante-trois (63) agents pour le lot 2 et trente (30) agents pour le lot 3, est par conséquent chargée de collecter et d'acheminer les déchets à l'incinérateur.

Au total, ce sont cent-soixante (160) agents, qui, comme techniciens/techniciennes de surface collectent, enlèvent les déchets. Les enquêtes menées pour cette étude portent sur le premier trimestre de 2024, c'est-à-dire du 1er /01/2024 au 31/03/2024.

2.2 Population

La population d'étude est constituée du personnel médical du CHU, du personnel administratif, du personnel technique de collecte, d'enlèvement des déchets. Pour cette étude, nous avons tenu compte des critères d'inclusion et d'exclusion.

2.3 Critères d'inclusion

Toute personne travaillant au CHU et faisant partie du personnel médical ou administratif de l'hôpital ou des sociétés SEQUOIA et NETSI. Toutes ces personnes sont en service au CHU depuis le début jusqu'à la fin de notre enquête. Ces hommes et femmes, enquêtés, sont des acteurs du système de gestion des déchets.

2.4 Critères d'exclusion

Toute personne travaillant au CHU et faisant partie du personnel médical, administratif de l'hôpital ou des sociétés SEQUOIA et NETSI et participant à la gestion des déchets issus des activités de soins et autres, mais absente au moment du recueil des données, de l'enquête.

2.5 Type d'étude

Notre étude est de type exploratoire et descriptif. Il s'agit d'identifier et d'analyser les problèmes de gestion des déchets au CHU et faire ensuite des propositions. Les variables étudiées sont de type quantitatif et qualitatif.

[4] l'arrêté n°131/MSHP/DGHP/DRHP du 03 juin 2009 portant réglementation de la gestion des déchets sanitaires en Côte d'Ivoire.

[5] Plan de Gestion des Déchets Sanitaires (PGDS), Version finale, 19 juin 2023

[6] Décret (Côte d'Ivoire) nº 59-188 du 9 octobre 1959, portant création d'un centre hospitalier à Abidjan, J.O. 1959, p. 943.

[7] Sous-Direction des Soins Infirmiers et Obstétricaux

2.6 Matériel

Pour la collecte des données, un questionnaire a été administré au personnel médical, administratif de l'hôpital et des sociétés SEQUOIA et NETSI. Un guide d'entretien a aussi permis de recueillir des informations qualitatives auprès des enquêté(e)s. Enfin, une grille d'observation a permis d'observer nos enquêtés pendant le service.

2.7 Echantillon

Pour la collecte des données, l'échantillonnage accidentel a été utilisé. Ainsi, il a consisté à interroger les enquêtés au fur et à mesure qu'ils se présentaient dans leur service ou quand nous les rencontrions ou arrivions à les joindre par appel téléphonique. Notre échantillon se compose au total de soixante-onze (71) personnes. Les deux sexes ; hommes et femmes, ont participé à l'étude. Le Microsoft Word a servi pour le traitement des textes, et l'analyse des donné a occasionné la création de tableaux et des graphiques.

Personnel du CHU						**Personnel technique d'entretien**			
Administratif		**Médical(A)[8]**		**Médical(B)[9]**		**SEQUOIA**		**NETSI**	
V.A	**V.R (%)**	**V.A**	**V.R(%)**	**V.A**	**V.R(%)**	**V.A**	**V.R(%)**	**V.A**	**V.R(%)**
03	4,22	11	15,49	7	09,86	20	28,17	30	42,25

(V.A) Valeur absolue ** (V.R) Valeur Relative

Tableau I : Répartition des enquêté(e)s par service Source : Données d'enquête

III. MOYENS DE GESTION DES DECHETS MEDICAUX DU CHU

3.1 Précollecte des objets piquants, coupants et tranchants (OPCT)

Photographie 1 : Boîtes de sécurité (BS) stockées au Service de Prévention et de Contrôle des Infections (SPCI). Source : PCI/PGOP, 2024

Photographie 2 : Collecte de seringues et autres objets piquants coupants tranchants (OPCT) dans les boîtes de sécurité stockées. Source : PCI/PGOP ;2024

Image 1 : Différentes dispositions de boîtes de sécurité ; Source : Données d'enquête, janvier 2024

Sur La photographie 1, nous voyons des boîtes de sécurité (BS). Les boîtes de sécurité constituent les contenants recommandés le CICR pour recueillir les déchets médicaux infectieux piquants/coupants. Les boîtes de sécurité sont en

[8] Personnel du Service de prévention et du contrôle des infections (SPCI) et du PGOP ayant répondu à nos questions

[9] Personnel médical en fonction et n'appartenant au SPCI ou au PGOP.

carton. Il faut donc les protéger de toute humidité, d'où l'usage du sachet (sachet noir). Cette précaution à s'en tenir aux propos d'un auxiliaire d'hygiène et assainissement du PCI :

> « *l'usage du sac poubelle, c'est pour garder, protéger les quelques boîtes de sécurité disponibles, de l'humidité due aux eaux d'entretien et de lavage des sols. On peine à nous approvisionner à temps et en quantité ces contenants nécessaires pour une gestion adéquate des déchets hospitaliers infectieux surtout les déchets piquants, coupants et tranchants* ».

En effet, parmi les critères de choix du conteneur à déchets piquants/ tranchants édictés par le (CICR,2016) nous notons le critère-ci : la « *Résistance à la perforation et étanchéité aux liquides* ». Le critère d'étanchéité n'est pas observé ou respecté avec ces boîtes de sécurité en carton. C'est un manquement à relever tant l'humidité est quasiment permanente. Cette humidité est liée au lavage régulier des sols par les agents d'entretien des locaux et aux déchets de tout genre jetés dans les poubelles de récupération.

Sur La photographie 2, deux boîtes de sécurité sont en phase d'utilisation. On peut noter que les déchets ne sont pas entièrement introduits dans les boîtes de sécurité. Ce comportement fait courir plusieurs dont le risque de blessure et celui d'éparpillement. L'infirmier major de ce service s'indigne en ces termes :

> *« Ce comportement ne nous honore pas, nous même, qui sommes personnel médical. Je rattrape beaucoup de fois quand je suis dans la salle et quand c'est le premier acte. Il m'arrive par moment de rappeler à l'ordre ces fauteurs de trouble, cet écart de comportement peut provoquer une piqure ou une déchirure d'un tenant du bureau voire de la personne qui vient récupérer ou précollecter les déchets* ».

M. T. A G, technicien d'hygiène :

> *« Certains de nos infirmiers et médecins du CHU, ne nous écoutent pas. Pour eux c'est l'acte médical, qui soigne le malade et non la façon dont les déchets sont introduits dans les boîtes de sécurité. Ils nous retorquent qu'ils sont formés à soigner et non à gérer des déchets .je ne sais pas si ces quelques personnes, qui se comportent ainsi, si elles savent le risque qu'elles courent elles-mêmes. Beaucoup de nos collègues professionnels de santé, les médecins les infirmiers, les sage-femmes et les techniciens de santé, etc. ne savent que la boîte de sécurité et même les sacs poubelle doivent être remplis au trois quart (3/4). Une fois ce niveau atteint, il faut alerter les collecteurs pour la vidange et remettre en place un autre sac poubelle ou des boîtes de sécurité selon le besoin ».*

Pour le CICR, 2011 « *Une gestion appropriée des déchets médicaux repose sur une bonne organisation, un financement adéquat et la participation active d'un personnel informé et formé. Ce sont là, en effet, les conditions pour que les mesures soient appliquées d'une manière constante tout au long de la filière du déchet (du point de production jusqu'à l'élimination finale)* ». Le personnel dans son ensemble : du producteur (le CHU, personne morale et physique) au personnel d'enlèvement, doit être formé et informé des risques liés aux déchets hospitaliers de façon globale et singulièrement les risques liés aux déchets médicaux infectieux.

L'approvisionnement en équipements de collecte des déchets : sachets-poubelle et en boîtes de sécurité

Au PCI, M. B.K. *« il faut déjà dire que le marché est attribué par appel d'offre ou de gré à gré. Le premier cas de figure est traité par le Ministère des marchés publics, le Ministère en charge du portefeuille de l'Etat et le Ministère de la santé, qui est représenté pour l'occasion par le principal intéressé le CHU. Le deuxième cas de figure, la marché gré à gré, l'entreprise en question rentre directement en contact avec la Direction du CHU pour signer le contrat. Nous avons les deux cas de figure actuellement au CHU ».*

Un autre tenant du bureau répond en ces termes :

> « *les sacs-poubelles ou si vous voulez les sachets sont fournis par les entreprises en charge de la collecte des déchets hospitaliers. Nous (le service d'hygiène subdivisé en deux branches : le PCI et le PGOP) des sachets de couleur jaune pour les déchets infectieux et le sachet noir pour les déchets hospitaliers assimilables aux déchets hospitaliers. Toutefois, vous pouvez retrouver parfois dans les paniers des sachets, d'autres couleurs que les deux couleurs jaune et noir, il s'agit notamment du sac bleu et du blanc. Ce sont les sacs disponibles sur le marché que les entreprises livrent au CHU. Pour ce qui est des boîtes de sécurité (B.S comme nous aimons appeler entre nous agents techniques), c'est le CHU, qui approvisionne le PCI et le PGOP »*

M. Y. un des techniciens d'hygiène et assainissement va continuer pour nous éclairer un peu plus *:*

« le CHU doit puiser dans son budget, doit inscrire dans ses dépenses, l'achat des B.S. Il ne

> *faut donc pas s'étonner quant au manque fréquent en boîtes de sécurité ou encore au retard constant observé dans l'approvisionnement desdites boîtes. Le marché gré à gré soulage un tant soit peu le CHU mais ce type de marché est instable car beaucoup de cas c'est un accord, qui se lisse entre la direction du CHU et les acquéreurs. Quand la direction change, les acquéreurs de ce type de marché, courent le risque de se voir retirer le marché ou bien les termes d'octroi sont revisités et revus à l'avantage de la nouvelle direction. Je vous dis actuellement nous vivons ce cas puisque le Directeur est affecté ailleurs. On va encore observer un manque de boîte de sécurité. Le retard pris pour nous fournir les boîtes de sécurité, complique notre tâche auprès des majors, des Surveillantes et Surveillants d'Unité de Soins (SUS) pour la récupération des déchets médicaux infectieux et les OPCT ».*

L'article 10 de l'arrêté 131 MSHP/CAB/DGHP/DRHP du 03 Juin 2009 relatif à la gestion des déchets indique que : « la personne physique ou morale qui produit des déchets issus des activités du secteur de la santé peut, par convention écrite, confier en tout ou partie à une autre personne physique ou morale. Les modalités de ces conventions sont fixées par voie réglementaire par le Ministère de la Santé et de l'Hygiène Publique ». Le mode d'attribution du marché est bien formel pour ce qui est des appels d'offre. Pour le marché ou accord gré à gré, c'est plus la sympathie, le favoritisme, qui prévalent.

Le mode de distribution des équipements de collecte des déchets

Monsieur A.O répond :

> *«Les sacs poubelles et les boîtes de sécurité sont disponibles aussi bien au PCI qu'au PGOP. Ces deux services sont les services en charge de la gestion de l'hygiène et de la biosécurité au CHU ».*

Madame D.S.T répond à notre question en ces termes :

> « *Pour ce qui est du mode de distribution, nous suivons une méthodologie bien claire. Pour les sacs poubelles, nous avons des fiches de traçabilité (si vous voulez un registre) et ici ce sont les techniciennes et techniciens de surface de NETSI et SEQUOIA, qui passent récupérer les sacs poubelles. Nous mentionnons sur la fiche de traçabilité ou dans le registre la quantité et le type de sacs poubelle transmis à l'agent d'entretien. Concernant les boîtes de sécurité, celles-ci sont récupérées exclusivement par les Chefs de Service (SUS et Majors des services) produisant les déchets infectieux et les OPCT. Toutefois, il faut dire que les Chefs de Service ne viennent pas toujours à nos bureaux ils confient la tâche des tiers ou tierces* ».

3.2 Collecte des déchets médicaux infectieux

La littérature scientifique en matière de gestion des déchets sanitaires nous enseigne que : *"La façon la plus simple d'identifier les différentes catégories de déchets et d'encourager le tri est de séparer les déchets dans des conteneurs ou des sacs en plastique de différentes couleurs et/ou marqués d'un symbole. Les recommandations internationales sont les suivant*[10]*".*

Les photographies ci-dessous regroupées en une seule image, montrent des conteneurs avec des sachets de différentes. Ces conteneurs sont posés à différents endroits dans les services pour recueillir les déchets.

[10] Manuel de gestion des déchets médicaux, CICR, mai 2011

Image2: Boîte de sécurité (B.S) Source : Service de Prévention et de Contrôle des Infections/Pôle de la gynéco-obstétrique et de la pédiatrie

Une technicienne de surface d'un des services répond :

> « *Notre travail, est pénible, nous qui passons récupérer les déchets, nous qui passons vider ces panier d posés ci et là. Nous trouvons tout genre de déchets dans les services. Des peaux de banane aux seringues en passant par les poches de sang, vous en trouver de tout genre dans les services. Des déchets assimilables aux déchets infectieux c'est le même contenant* ».

Un des vidangeurs ou technicien de surface M. K. O continue :

> « *Nous ne sommes pas allés à l'école pour bon nombre d'entre nous. On a l'impression que les travaux salissants et moins payés, nous sont réservés. Et ce qu'il faut craindre à notre niveau c'est que nous n'avons pas d'information ou assez sur les dangers que nous courons quand nous vidons les poubelles. Quand on demande à nos supérieurs, ils nous disent que notre tâche, c'est de nettoyer les locaux et vider les poubelles, quand celles-ci sont remplies, le reste ne nous concerne pas. C'est quelquefois, quand je cause par exemple avec M. Yéo, qui est technicien, qu'il m'explique les dangers liés aux déchets médicaux et cela me permet de m'éloigner un peu des poubelles quand mon travail le permet »,*

Photographie 7 : Un panier comme poubelle avec des déchets souillés de sang

Source : Archive, Coulibaly Foundéré, 24/05/2012 à 09 h17 mn

Cette pratique est encore visible dans certains services. Il, est vrai que le service où cette capture d'image a été réalisée s'est améliorée en matière de précollecte des déchets qu'il produit mis l'usage des paniers demeure et ceci n'est pas un cas isolé. Répondant à notre question pourquoi un panier sans utiliser un sachet-poubelle ? Une professionnelle de santé répond

> « *Les sacs poubelles ne sont pas toujours disponibles au service d'hygiène. On fait donc avec les moyens à bord. Sinon je sais qu'il faut mettre un sac, pour éviter les écoulements d'une part et d'autre part pour faciliter la récupération une fois la poubelle est remplie ou atteint niveau habituel de vidange* ».

M. O. M, infirmier spécialiste va prendre la parole pour dire :

« *Les déchets dans leur ensemble et surtout les déchets médicaux sont gênants. Ils sont gênants parce qu'ils sont encombrants et à cette occupation de l'espace quand vous avez ajouté les odeurs des poches de sang bien que vides ou vides de moitié, des sérums et des bandages de pansement, vous voyez que l'air est irrespirable. Nous autres, nous sommes habitués mais certains de nos collègues développent des allergies en voyant le sang humain coagulé* ».

L'image 2 fait ressortir les erreurs voire le manque de tri. Nous convenons avec Khelladi (2015, 33) qu'il y a des erreurs de tri. Ainsi, nous retrouvons le coton souillé par le sang dans les boites de sécurité, « cette opération ne devrait pas être possible car la boite qui reçoit les déchets coupants et piquants est fermée hermétiquement » Khelladi (2015, 33).

3.2 QUANTIFICATION OUPESAGE DES DECHETS

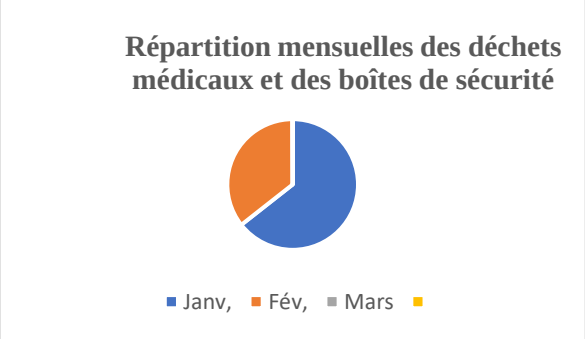
3.2-1 Quantification des déchets

La quantification des déchets médicaux infectieux porte sur la période d'enquête du 1er/01/2024 au 31/03/2024. La quantification ou le pesage est réalisé par les onze (11) agents techniques du PCI et du PGOP. Cette quantification fait suite à la collecte réalisée en amont.

Activités	Janv.	Fév.	Mars	Total (kg)
Boîtes de Sécurité	855.41	410.04	443.21	1708.66
Déchets Médicaux Infectieux	1152.73	698.27	707.46	2558.46
Total (kg)	2008,14	1108,31	1150,67	4267,12

Tableau 2 : Typologie et quantification des déchets médicaux infectieux et autre OPCT du CHU Source : Données d'enquête ; 2024

Les activités de soins des différents services du CHU ont dans leur ensemble, produit 1708.66 kilogrammes de déchets infectieux composés de boîte de sécurité soit 40,04% de la production trimestrielle, qui est de 4267,12 kg. Les 2558.46 kg de déchets infectieux médicaux autre que les boîtes de seringues, soit 59,96 % de la production trimestrielle, constituent le reste de ces déchets médicaux infectieux.



Graphique 1 : Cambiaire de la répartition mensuelle des déchets médicaux infectieux et autres OPCT du CHU Source : Données d'enquête ; (premier trimestre 2024)

A l'analyse des données de ce graphique, il est à noter que le mois de janvier est le plus prolifique en déchets toxiques ar dangereux pour l'Homme et son environnement.

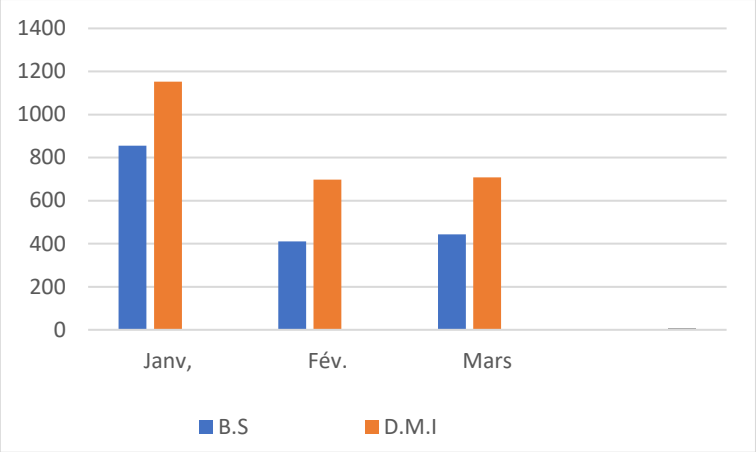
En effet, avec les différentes activités, les différents déplacements, fait remarquer Monsieur Y.,

> *« Les Abidjanais sont excités, ils bougent comme-ci, à part ces événements, ils ne vont plus fêter. Les accidents sont signalés par-ci, par-là quand ce ne sont des renversements de voiture, ce sont des bagarres entre amis, soulards sous l'effet de l'alcool ou autre excitant. Les petits voleurs aux armes blanches, ne sont pas en reste. Les causes à cette augmentation visible des déchets ne peuvent être énumérées de façon exhaustive »,*

Activités	Janv.		Fév.		Mars		Total (kg)	
Production mensuelle des déchets	V.A	V.R (°/°)	V.A	V.R (°/°)	V.A	V.R (°/°)	V.A	V.R (°/°)
	2008,14	47,06	1108,31	25,97	1150,67	26,97	4267,12	100

Tableau 2 : Répartition mensuelle des déchets médicaux infectieux et OPCT Source : Données d'enquête, 2024

47,06% des déchets infectieux et les OPCT du premier trimestre de 2024, ont été produits en janvier contre 25,97°/° pour le mois de février et 26,97°/° pour le mois de mars.



Graphique 2 : Histogramme des déchets médicaux infectieux et OPCT par type et par mois

Source : Données d'enquête ; (premier trimestre 2024)

« Nous recevons beaucoup de personnes en janvier et cela s'explique du fait des fêtes. Les ivoiriens aiment les fêtes, les jeunes aiment s'amuser et cela ne va pas sans accrochage entre véhicules, engin roulant. A ces accidents de la circulation, il faut ajouter les agressions physiques, les palabres entre jeunes. Ces différents événements occasionnent des consultations pour coups et blessures d'une part et d'autre part pour vol, accidents », nous fait savoir Mme. S.T. personnel médical du CHU.

M.K.Y du service de PCI, nous dira :

« en plus des accidents liés aux fêtes de fin d'année et du nouvel, il faut que vers fin décembre, les gens paressent en peu dans l'exécution des leurs tâches. Cet état de fait impacte un retard dans l'enlèvement des déchets. Ainsi, il arrive que déjà dès mi-décembre que certains services se trouvent un personnel d'entretien réduit. Et quand le personnel n'est pas réduit, c'est la motivation, qui n'y est pas. Chaque personne pense à comment s'employer à offrir un cadeau à son enfant ou à ses enfants en bas âge ».

Une des techniciennes de surface approchée et qui a souhaité garder l'anonymat, même l'usage des initiales n'a pas été souhaité dit ceci :

« ce n'est pas que l'effectif est seulement réduit mais c'est notre argent, notre intéressement, qui traîne et cela n'est pas fait pour encourager, qui que ce soit. Vous voyez nous souffrons d'injustice pour le même travail, nous ne bénéficions pas du même traitement et pire il y a des privilégiés parmi nous. On regarde les services où il y a moins à faire et on affecte certaines et d'autres comme moi par exemple, nous sommes affectés toujours dans les services où le travail est colossal d'une part et d'autre où les déchets ont de nature dangereuse. Nous avons la remarque quelquefois, à nos responsables ici mais rien n'a changé et rien ne va changer. Il arrive par moments que certains des responsables, ceux-là sur qui nous comptons, disent qu'ils n'y peuvent rien. C'est la consigne qu'on nous a donnée ».

3.1-1 Enlèvement des déchets et analyse

L'enlèvement des déchets est fait en fonction du type de déchets produits. Il s'agit des déchets issus des activités de soins (DAS), lesquels déchets peuvent être infectieux ou à risques infectieux (DASRI/) ou non-infectieux (DASNI). Les déchets non infectieux ne contenant pas d'éléments biologiques (un organe humain, du sang, les selles, les urines, etc.) peuvent être mêlés à la paperasse de bureau, aux produits alimentaires pour constituer ainsi les déchets ménagers.

Janvier-Mars	Fréquence	Taux de satisfaction	Taux de non-satisfaction
Déchets ménagers	Irrégulière	75%	25%
Déchets Infectieux	régulière	90%	10%

Tableau II : Taux d'enlèvement des déchets médicaux infectieux et autres OPCT du CHU

Source : Données d'enquête (premier trimestre 2024)

Le taux d'enlèvement des déchets médicaux infectieux et autres objets piquants coupants et tranchant est de 90%. Ce sont par conséquent 10% de ces déchets à risque infectieux ou comportant des risques de blessure, qui ne sont pas enlevés soit des lieux de production soit des lieux de collecte.

IV. ANALYSE DE LA GESTION DES DECHETS

L'état des lieux de la gestion des déchets sanitaires au centre hospitalier universitaire de Cocody voire en Côte d'Ivoire réalisé fait ressortir :

Points Forts

- **Aperçu réglementaire et législatif**

Le nouveau code de l'environnement, la loi 2023-900 du 23 novembre 2023, est un cadre législatif, qui vise à améliorer la gestion environnementale en Côte d'Ivoire. il intègre les réalités nationales et les meilleures pratiques internationales pour une gestion rationnelle de l'environnement. En effet, ledit code au paragraphe 3 en ses articles 154 « *les établissements hospitaliers et vétérinaires adoptent des mesures pour la gestion écologiquement rationnelle des déchets sanitaires* » et 155 : « *l'élimination des déchets sanitaires est faite sous la protection des services d'inspection des installations classées pour la protection de l'environnement en collaboration avec les administrations compétentes* « , invite à la prise en compte de la santé de l'environnement dans l'élimination des déchets sanitaires. Par déchets sanitaires, il faut entendre déchets sanitaires infectieux ou déchets hospitaliers infectieux ou encore déchets infectieux dangereux.

L'arrêté 131 MSHP/CAB/DGHP/DRHP du 03 Juin 2009 relatif à la gestion des déchets sanitaires, à l'article 9, stipule entre autre que « *toute personne physique ou morale qui produit des déchets médicaux dans le secteur de la santé est tenue de les gérer conformément aux dispositions du présent arrêté…* ». L'article 10 dudit arrêté, indique que cette responsabilité peut être cédée à travers une convention écrite à une personne physique et morale. Quant à l'article 11, nous retenons qu'il faut au préalable une autorisation du Ministre de la Santé et de l'Hygiène Publique pour la gestion des déchets sanitaires. Cette autorisation peut être octroyée à une personne physique ou morale.

C'est le premier cadre réglementaire en matière de déchets sanitaires en Côte d'Ivoire. Il précise les types de déchets sanitaires, les modalités d'autorisation, les modes de traitements et d'élimination et les acteurs qui entrent en jeu.

Nous notons au CHU que dix-neuf (19) soit 90,48% du personnel impliqué dans cette recherche ont connaissance du document du CICR sur la gestion des déchets médicaux et disent s'y rapporter beaucoup. Pour ces personnes c'est un guide idéal pour la gestion quand le pays ne dispose de programme de gestion des déchets médicaux et dangereux.

- **Les acteurs du système de gestion des déchets**

Le CHU, en référence à l'article 9 de l'arrêté 131 du 03 Juin 2009 relatif à la gestion des déchets sanitaire, est responsable de la gestion des déchets produits en son sein. Cette gestion est confiée à au Service de Prévention et de Contrôle des Infections (PCI) et au Pôle de gynéco-obstétrique et de la pédiatrie (PGOP).

Les onze (11) personnes travaillant au PCI et au PGOP sont toutes formées, certes à des degrés moindres à l'hygiène et à l'assainissement. 72,72% de ce personnel (A) soit huit (08) formés à la gestion des déchets hospitaliers a connaissance de l'arrêté ministériel portant sur la réglementation des déchets sanitaires. Six (06) personnes soit 54,54% ont une copie à leur disposition et disent l'avoir lu suite à leur affectation au CHU.

Le personnel administratif, trois (03) personnes au total dont deux (02) soit (66,66%) ont connaissance de l'existence de textes à savoir le nouveau code de l'environnement et l'arrêté traitant de la gestion des déchets médicaux.

Le personnel médical (B), ce personnel médical représente 09,86% soit sept (07) de notre échantillon. 85,71% de ce personnel soit six (06) personnes avouent connaître les risques liés à une gestion inadéquate des déchets pour l'homme et de son cadre de vie et de travail. Toutefois, elles affirment détenir cette connaissance de leur cursus scolaire et universitaires, des livres et de l'actualité sur l'environnement dans le monde.

La collecte des déchets est réalisée à 90% et la quantification des déchets est effective.

Les fiches de traçabilité ou de registre au PCI et au PGOP, permettent de faire le suivi de sortie et d'entrée du matériel d'enlèvement des déchets médicaux et faire la demande à temps.

Les contrats entre le CHU et les sociétés d'enlèvement des déchets hospitaliers et d'entretien des locaux sont signés soit au CHU ou au niveau du Ministère de la Santé et de l'Hygiène Publique en présence des Ministères des Marchés Publics et le Ministère en charge du Portefeuille de l'Etat.

Points faibles et à améliorer

Le tri des déchets est encore à améliorer. En effet, dans les services à un risque infectieux, il devrait avoir plus d'une poubelle afin que les déchets médicaux à risques infectieux et les déchets médicaux non infectieux soit séparés. Ce tri pourrait réduire la quantité de déchets à incinérer et partant le coût financier. Nous notons entre autres les points ci-dessous :

- l'observance d'une traîne dans l'enlèvement des déchets ;
- Le non-respect du mode d'introduction des déchets OPCT dans les boîtes de sécurité d'où mauvais usage de celles-ci ;
- L'enlèvement incomplet des déchets médicaux infectieux et autres OPCT et le devenir des 10% de ces déchets non enlevés n'a pu être communiqué à l'occasion de cette recherche ;
- Le personnel (A) 27,28 % soit trois (03) formés à la gestion des déchets hospitaliers n'ont pas connaissance de l'arrêté ministériel portant sur la réglementation des déchets sanitaires. Cinq (05) personnes soit 45,46% n'ont jamais lu ni vu ledit arrêté ministériel ;
- Le personnel médical (B), 14,29% de ce personnel disent ne pas connaître les risques liés à une gestion inadéquate des déchets pour l'homme et de son cadre de vie et de travail.

Les gênes olfactive et visuelle occasionnées par la puanteur et l'état de putréfaction des déchets biodégradables, qui se retrouvent avec des déchets infectieux dans des paniers, des poubelles sans couvercle. Le tri ne peut être ainsi complet car nous avons trouvé dans des salles un seul panier et à la récupération l'agent d'enlèvement rassemble le tout dans un même sachet poubelle pour une même direction.

Existence de politique de deux structures de collecte des déchets avec des agents jeunes dont la fréquence au travail est effective.

Les matériels et équipements de gestion.

L'observance d'une lenteur dans la fourniture au PCI et au PGOP du matériel nécessaire à la précollecte, la collecte et par la suite du tri des déchets sanitaires infectieux.

Le règlement des salaires des travailleurs de SEQUOIA et NETSI n'est pas fait à temps et n'est pas régulier d'où le désintéressement de certains employés de ces deux entreprises.

La méconnaissance des textes réglementaires et législatifs en matière de gestion des déchets sanitaires de l'ensemble du personnel d'enlèvement de collecte et d'entretien des locaux de SEHUOIA et NETSI

Le manque d'imposition d'un niveau d'étude du personnel technique de surface.

Le Plan National de Gestion des Déchets Sanitaires (PNGDS) 2021-2025 n'est pas le document de travail des agents.

V. CONCLUSION

La gestion des déchets est confiée à des tierces au CHU. L'attribution du marché se fait par appel d'offre. NETSI et SEQUOLIA, sont les deux entreprises chargées de l'entretien des locaux et de l'enlèvement des déchets. Le marché est acquis par appel à l'effet avec la présence du Ministère des Marchés Publics, le Ministère en Charge du portefeuille de l'Etat et du Ministère de la Santé et de l'Hygiène Publique, représenté par le principal intéressé : le CHU. Le marché de gré à gré est le second type, qui se passe entre la Direction du CHU et l'entreprise concernée. Ce dernier cas de figure est instable car appelé à être remis en cause à tout moment s'il y a un changement au niveau de la Direction du CHU. Quant au personnel impliqué dans la gestion des gestions, l'analyse des différents verbatims, nous permet de ressortir l'instabilité à la tête des directions, le manque de civisme de certains des acteurs, le manque d'information et la formation. En effet, L'article 9 de l'arrêté n°131 MSHP/CAB/DGHP/DRHP du 03 Juin 2009 stipule entre autres que : « *toute personne physique ou morale qui produit des déchets médicaux dans le secteur de la santé est tenue de les gérer conformément aux dispositions du présent arrêté…* ». L'article 10 dudit arrêté, indique que cette responsabilité peut être cédée à travers une convention écrite à une personne physique et morale. Quant à l'article 11, nous retenons qu'il faut au préalable une autorisation du Ministre de la Santé et de l'Hygiène Publique pour la gestion des déchets sanitaires.

Cette autorisation peut être octroyée à une personne physique ou morale. Cette observance des mesures institutionnelle est un point fort de la gestion des déchets infectieux du CHU. Toutefois, l'analyse des verbatims ont permis de ressortir des points faibles il s'agit de la gêne visuelle et olfactive des allergies développées par certains professionnels de santé. Ces états psychologiques sont liés, selon bon nombre d'entre eux à la longue cohabitation et la proximité avec les déchets. Quant à la collecte, qui elle-même passe part par la précollecte puis la collecte proprement dite, les professionnels de santé doivent être formés pour le besoin et dans le meilleur des cas sensibilisés pour faciliter la celle.ci.

Le nouveau code de l'environnement, l'Arrêté ministériel portant sur la règlementation des déchets sanitaires constituent l'arsenal réglementaire en matière de gestion des déchets médicaux ou déchets sanitaires.

Le Plan National de Gestion des Déchets Sanitaires (PNGDS) 2021-2025 élaboré avec l'appui technique et financier de l'UCPS-BM[11], trace les chantiers pour une gestion rationnelle des déchets sanitaires en Côte d'Ivoire.

[11] Unité de Coordination Projet Santé – Banque Mondiale

L'objectif général de ce plan qui est d'améliorer la gestion des déchets sanitaires en Côte d'Ivoire, est l'objectif de facto à assigner au CHU. Le CHU pour atteindre cet objectif, doit observer chacun des points faibles énumérés dans l'analyse des résultats de cette recherche.

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Adoption of Technology in Cardamom Cultivation in Taplejung District, Nepal

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Abstract— *A study assessed the farmers' knowledge of technology adoption status in large cardamoms in the Taplejung district of Nepal. For the study, primary data were collected from 60 randomly selected farmers using a semi-structured interview schedule, focal group discussion (FGD), and key informant interview (KII). Secondary information was collected by reviewing different relevant publications. The data were processed and analyzed using descriptive statistics through SPSS and Excel programs. The study shows that farmers are aware of modern technology available for large cardamom cultivation. Most farmers adopt modern irrigation techniques (86%) to irrigate cardamom orchards. Only 2% farmers used chemical fertilizer in cardamom orchards, and 50% of farmers use organic manure. Mainly, cultural practices are used to control diseases, insects and pests. During field preparation, 72% of farmers used traditional tools, i.e., spades. For pruning operations, only a few farmers, 28%, used chainsaws but mainly used secateurs, and for weeding, hand tools were used primarily than brush cutters (only 33% adopted). The study shows that most farmers adopt improved air dryers (68%) rather than traditional dryers for post-harvest technology. The tail trimming method is carried out by 86% of cardamom farmers, and grading operation was not carried out before selling. Farmers mostly received subsidies from PMAMP and a few from the Agriculture Knowledge Center and other organizations. Furthermore, a lack of financial resources, technical issues, a lack of training or capacity-building programs, and difficulties with maintenance and repair were the significant constraints to adopting technology.*

Keywords— ***Cardamom, Adoption, Production, Subsidy, Technology***

I. INTRODUCTION

Large cardamom (*Amomum subulatom Roxb*) is one of the most popular spices crops of the Zingibersceae family under the order Scitaminae. It is also known as Alaichi in Nepal, Badhi Alaichi in Hindi, and renounced as black gold, black cardamom, and queen of spices (Banjade et al., 2023). It is the world's oldest and third most expensive spice, followed by saffron and vanilla (Bohara et al., 2023). It is a high-value spice crop grown commonly in the himalayan region's mid-hill districts. The major producers of this species are Nepal (52%), India (37%) and Bhutan (11%) of total world production per annum (Gautam et al., 2016). It is typically pollinated by bumblebees (Basnet et al., 2021).

The Ramsai, Golsai, Dambersai, Jirmale, and Bharlyange are five registered varieties under Nepal government (Basnet et al., 2021). It is a tall, evergreen, perennial, herbaceous monocot plant. The height of this plant ranges from 1.5 to 3.0 meters (Bisht et al., 2011). It has several tillers consisting of pseudo stems with leaves on the upper part. The inflorescence (spike) appears on the rhizome from where the pseudo stems shoot up (Sharma et al., 2000). The ripened fruit is trilocular, reddish brown, and

contains dark pink seeded capsules (Hussain et al., 2009). Capsules of large cardamom are held together inside the spike with viscous sugary pulp and are 20–25 millimeters (mm) long and oval to globular in shape (Thomas et al., 2009). It is a climate-dependent crop; the best production is between a temperature of 4-20ºC, two annual rainfall of 2000-2500 mm, and more than 90 % humidity (Rijal, 2013; Banjade et al., 2023). This research can guide us in providing the support and tools to improve cardamom farming methods, making it easier and more profitable for farmers. The findings could help to know different technologies adopted at different stages of cardamom production, from field preparation to harvesting, about subsidies provided by government bodies at various scales and constraints to adopt technology in cardamom production.

II. METHODS

2.1 Selection of research site

The study was conducted in the Taplejung district of Koshi province from January to June 2024. The district is located in the Himalayas in eastern Nepal. The district covers an area of 3,646 square kilometers and has a total population of 120,590 (2022 Nepal census). Tibet surrounds the district in the north, Sankhuwasabha district in the west, Tehrathum district and Panchthar district in the south, and Sikkim (India) in the east. Geographically, the district is located at a latitude of 27º 06' to 27º 55'N and a longitude of 87º57' to 87º40' E. The research was conducted in the Prime Minister Agriculture Modernization Project (PMAMP) command area.

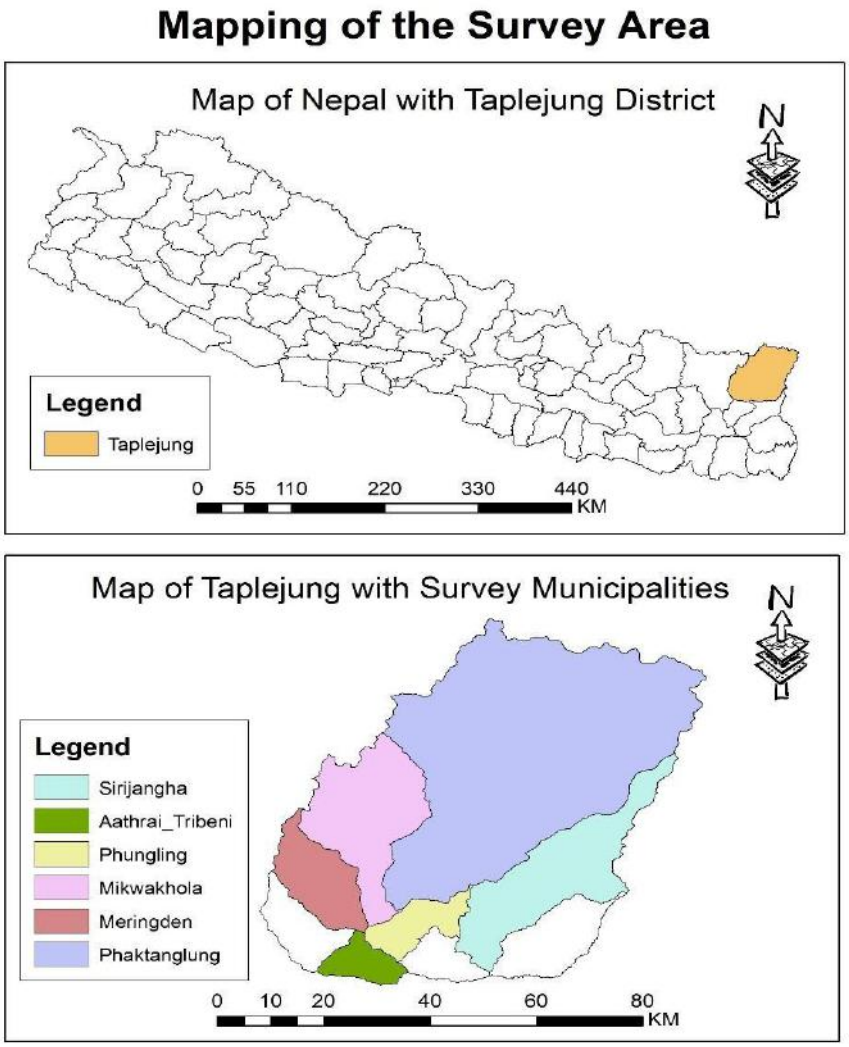

Fig.1. Map of Nepal showing study area (ArcGIS, Esri, 2024)

2.2 Sampling size and procedures

The farmers of the local areas, i.e., Taplejung municipality, Sirijangha rural municipality, Phaktanglung rural municipality, Meringden rural municipality, Mikwakhola

rural municipality, and Aathrai Tribeni rural municipality, were selected. Sixty cardamom farming households were selected based on a purposive random sampling method.

2.3 Survey design and field survey

The pre-survey field visit was conducted to gather preliminary information regarding the site's socioeconomic, demographic, and topographical settings. An interview schedule design was prepared to collect the farmers' primary information. Pre-testing of the interview schedule was done before the field survey. The interview schedule was designed for the nearby respondents in the study area with the help of the site supervisor of PMAMP, Taplejung. Focus Group Discussion was done to obtain detailed information about individual farmers' feelings, perceptions, and opinions. It was conducted before and after the final survey to build up ideas for interview schedule preparation. The participants of the focus group discussion were cardamom farmers for this survey. The primary key informants were farmers, the government agriculture officer, and the Agriculture Knowledge Center. The socio-demographic and farm characteristics were used for descriptive analysis of the study site and population. Different variables like land size, education status, family size, ethnicity, etc., were analyzed using simple descriptive.

2.4 Data analysis

The collected data were edited, and local units of measurement were standardized into scientific units. Data were entered and analyzed using computer software like Microsoft Excel and SPSS (Statistical Package for Social Science). The information was analyzed using descriptive statistical tools like mean, standard deviation, frequency, etc.

III. RESULTS AND DISCUSSIONS

3.1 Socio demographic characteristic

The total number of cardamom-growing households sampled was 60. Among those 60 respondents, 26 were female, and the remaining 34 were male. The age of the respondent in the study area ranged from 27 to 71 years. 58% of the surveyed households were Jana Jati, mainly Limbu and Gurung, 30% were Kshettri, 10% were Brahmin, and 2% of the respondents were found to be Dalit. The majority of the respondents were found to be Hindu. And, 42% of the survey households were Hindu, 30% were Kirat, 26% were Buddhist, and the remaining 2% were Christian. Household size determines the supply of labor force to the farm operations. The maximum family size was found to be 10, and the minimum to 3. The study revealed that 38% of respondents' main occupation was cardamom farming, 42% were engaged in other agricultural work, mainly cultivating oranges, kiwi, and livestock, 2% were involved in agri-tech professional, 2% were students, and the remaining 16% respondents engaged in other types of occupation.

3.2 Status of Cardamon farming

Only medium- and large-scale cardamom production farmers were identified during the study. The respondents of the study area cultivated an average of 40.88 ropani of land, ranging from a minimum of 12 ropani to a maximum of 200 ropani of land. The respondents of the study area had an average of 33.34 of self-owned land and remaining on an average of 7.54 ropani of contracted/rented land. Cardamom farming in the study area has been done for many years. Most of the respondents have done cardamom farming for more than ten years. It revealed that cardamom farming in the study area was a primary farming practice, and cultivation practices are transferred from generation to generation. The main cardamom cultivated in the study area is the Ramsai variety. Other varieties grown in the study area include Golsai, Chibasai, and Jirmale varieties. The maximum area is covered by the Ramsai variety, followed by Golsai and Chibasai. Topography and climatic conditions determine the varieties of cardamom to be cultivated, and climatic conditions and topography of this study area favored the cultivation of the Ramsai variety.

Table 1: Varieties of cultivated cardamom

Variety	Percentage
Ramsai	54%
Ramsai,Bharlang	2%
Ramsai,Chibasai	8%
Ramsai,Chibasai,Sawne	2%
Ramsai,Dambarsai	4%
Ramsai, Golsai	16%
Ramsai,Golsai,Dambarsai,Jirmale	2%
Ramsai,Golsai,Jirmala,Chibasai, Dambarsai	2%
Ramsai,Golsai,Jirmale	2%
Ramsai,Jirmala	2%
Sawne	6%
Total	100%

On average, the respondent's farmers have 18.24 years of experience in cardamom cultivation, with a minimum of 5 years and a maximum of 40 years of experience in cardamom farming.

3.3 Technology awareness and access

The study revealed that all the respondents were aware of modern technology available for large cardamom cultivation. Out of 60 respondents, 100% respondent were aware. Out of 60 respondents, 86% respondent had access to modern technology, and only 14% hadn't access due to certain reasons. The results showed that most of the farmers become aware through extension services from agriculture authorities (36%) on technology, followed by training programs/workshops (28%), peer recommendations (18%), internet/online research (10%), and 10% through agriculture fairs/exhibitions. The result also showed that the respondents with bachelor and above education status were more aware through the internet and online research.

3.4 Adoption of Intercultural Operation

Cardamom farmers of Taplejung district mostly used modern irrigation methods (88%) and 12% of farmers used traditional irrigation methods. There were no fully rain-fed depended orchards. Most of the respondents adopted modern irrigation techniques. Out of 88% of modern irrigation, 86% adopted sprinkler irrigation, 2% adopted pipe irrigation, and no cardamom farmers have adopted drip irrigation. In cardamom orchards, only half of the respondents use manure and fertilizer, and half don't use any kind of fertilizer in the orchards. FYM, compost, and vermicompost are applied around the plant, and urine is applied by spraying in the cardamom plant. The results showed that most farmers (98%) in the study area don't use chemical fertilizers. Only 2% of the farmers use chemical fertilizers, such as urea and DAP.

Table 2: Status of adoption of organic manure

Organic manure	Percentage
FYM	52%
Compost	20%
Vermicompost	4%
Urine	24%
Total	100%

They determine the quantity of manure/fertilizer to apply in large cardamom orchards mainly by experience-based estimation followed by advice from agriculture extension services and visual inspection of the plant. No one farmer has done soil testing to measure soil nutrients. The result showed that half of the respondents who apply fertilizer mainly apply it once a year, followed on an as-needed basis.

The result showed that 46% of the respondents adopted the pruning technique due to the motivation of disease prevention and management, 22% due to enhanced light penetration to lower parts of the plant, 14% due to improved air circulation around the plant, 12% due to improved flowering and fruiting and 6% due to enhance plant health. Out of 98% of farmers who adopted weeding operations, 78% conducted them on an as-needed basis, 18% conducted them once a month, and 6% conducted them twice a month. The study revealed that 90 percent of respondents used cultural practices to control diseases, and 10 percent used chemicals to prevent diseases. In the cultural method, farmers mostly used sanitation processes and diseased-free plants; in the chemical method, most farmers used Allcop.

Table 3. Frequency of manure application

Application	Percentage
Once a year	48%
Twice a year	12%
Thrice a year	12%
As needed basis	28%
Total	100%

Respondents whose orchards are more than 20 years old have plowed their orchards. Farmers on terraced land have used mini tillers or tractors for field preparation. The result showed that most of the farmers, about 72%, used spades for field preparation, mainly to dig pits.

3.5 Post-harvest technology

The result showed that cardamom farmers adopt modern technology in the drying process. Among them, 62% of the respondents used improved air dryers, and only 38% used traditional dryers. Most respondents (86%) carried out the tail-trimming process, and only 14% didn't. It showed that no equipment is used for the tail trimming process, which is done by the rubbing process. The result showed that no respondent carried out grading operations before selling. The farmer sold the cardamom to a wholesaler without grading operation. All respondents have used jute bags as a storage method for dried cardamom. It also showed that no respondent used air-tight containers and vacuum-sealed containers.

3.6 Subsidy regarding technology adoption

The result showed that 84% of the respondents applied for and received a subsidy, and 16% of the respondents didn't apply for a subsidy. Similarly, the result showed that most respondents received an air dryer (Bhatti) and irrigation, chainsaw brush cutter, sprayer, and tractor mini tiller as a subsidy. Air dryers are obtained mainly from PMAMP and a few from the Agriculture Knowledge Center. Most respondents want air dryers with a 100% subsidy from the government and then irrigation, chainsaws, brush cutters, and grading machines, respectively.

3.7 Constrains to adopting technology

The result showed that almost half of the respondents (50%) moderately used modern technology. 30% of respondents did not easily access modern technology, 16% of respondents had high accessibility, and 4% of respondents did not have access at all. The main factor that prevented the adoption of modern technology was that more than half of the respondents did not know how to use modern technology. Financial assistance, training programs, and access to information support are equally required to encourage cardamom farmers to adopt modern technology. All respondents had encountered challenges related to the maintenance or repair of technology used in cardamom cultivation. The result showed that 92% of the respondents participated in training sessions.

IV. CONCLUSION

The study revealed that farmers in the area were engaged in various agricultural activities, with cardamom cultivation being a primary focus. Most farmers grew the Ramsai variety of cardamom, adopting modern irrigation techniques like sprinkler systems and primarily using organic fertilizers. While traditional tools were commonly used for field preparation, some farmers had started adopting improved technologies like air dryers, which were often subsidized. However, challenges such as insufficient knowledge of modern technology and difficulty in accessing qualified technicians hindered broader adoption. Financial support and training are identified as key to encouraging the use of modern technology in cardamom farming.

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Impact of adding powdered Azolla (Azolla pinnata) leaves to the diet on the carcass and economic characteristics of turkey poults

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Abstract— *The current study was planned to examine the "Impact of adding powdered Azolla (Azolla pinnata) leaves to the diet on the carcass and economic characteristics of turkey poults". One hundred and twenty-eight-day-old turkey poults were randomly assigned to four treatment groups, each consisting of eight poults and four replicates. Azolla meal was added to the basal diet at 2.5% (T2), 5% (T3), and 7.5% (T4) levels to create three experimental diets. The control group (T1) was fed a basal diet without azolla supplementation. The dietary treatments were arranged in complete randomized design and analysed for the carcass traits and economics of rearing turkey poults. The findings ascribed to improved live weight, eviscerated weight, dressed weight and B: C ratio. The results indicate that up to 5% of Azolla leaf powder can be added to turkey feed as an unusual feed item without causing any negative side effects.*

Keywords— ***Azolla, poults, live weight, organ and economics***

I. INTRODUCTION

Turkey farming is still relatively new in India. However, the Central Poultry Development Organization (Southern Region), located in Hessarghatta, Bangalore, is working hard to increase turkey farming. Tamil Nadu and Kerala are the two states that generate the most turkeys. Turkey farming is quickly becoming more and more popular in the South. Red meat consumption is declining while white meat consumption—which includes chicken and turkey—is rapidly increasing. In India, this pattern has also been noted. Turkey meat has less fat and cholesterol than red meat and other fowl meats. The remarkable growth in chicken production has led to competition with traditional human food supplies, resulting in a shortage of conventional feed ingredients and an increase in their cost (CAST, 2013). According to **Johari and Hussain** (1996), 65–75% of the total broiler output is spent on feed alone, which eventually drives up the price of poultry meat. The ability of chicken to obtain protein from non-protein sources is extremely limited. The average broiler feed has between 22 and 24 percent protein. Protein synthesis in chickens is required at a very rapid rate for compensating the broken tissues of the adult body (Banerjee, 2000). The amount of study on the usage of green feed and forages has increased dramatically in recent years. Compared to other poultry, turkeys are known to consume more green feed, or vegetables. Therefore, the feed component is crucial since turkeys raised in intensive settings do not directly interact with plant feeds, especially green feeds. Additionally, the exponential growth in chicken production has led to a shortage and an increase in the cost of conventional feed ingredients due to competition with traditional human food supplies. Changing from conventional to unconventional feedstuffs will reduce the cost of turkey feed and increase the profit margin because feed costs nearly 75% of the total cost of producing a turkey.

II. METHODOLOGY

Preparation of Azolla meal: The Poultry Farm, Department of Animal Production, Rajasthan College of Agriculture, MPUAT, Udaipur provided the Azolla culture. After maturing, a fresh Azolla culture was harvested and collected, covering the tank's water. The harvested azolla was washed and dried using a brine solution. The dried Azolla was pulverized in a grinder to a uniform size prior to being added to the feed.

Experimental bird details: The day-old turkey poults were purchased for Rs 90 each from the hatchery section of the poultry farm inside the department of animal production of the Rajasthan College of Agriculture, MPUAT, Udaipur. A basic meal and different dosages of Azolla leaf powder were given to them, and they were split up into different dietary treatment groups. The cost of feeding was determined for each treatment group based on the materials' composition and the current market pricing of each feed ingredient.

Statistical analysis

The experiment was carried out using a completely randomized design (CRD), and Snedecor and Cochran's (1994) analysis of variance were used to examine the data pertaining to various parameters that were gathered during the current study.

III. RESULTS AND DISCUSSION

Carcass characteristics: The information on the carcass characteristics of the young turkeys in the various treatment groups is presented in Table 1. Significantly highest live weight was observed in T3 (1225.77±26.93 g) and T4 (1185.23±8.89 g), followed by T2 (1024.44±9.56 g) and significantly lowest in T1 (916.86±9.33 g). The difference between T3 and T4 were found statistically non-significant. The mean dressed weight was 711.94±4.22, 801.62±0.00, 968.97±1.83 and 933.36±4.76 g respectively in T1, T2, T3 and T4. Significantly highest dressed weight was observed in T3 (968.97±1.83 g) followed by T4 (933.36±4.76 g) followed by T2 (801.62±0.00 g) and significantly lowest in T1 (711.94±4.22 g). The eviscerated weight was significantly highest in T3 (856.04±8.12 g) followed by T4 (820.29±19.64 g), T2 (715.28±19.49 g) and significantly lowest eviscerated weight was observed in T1 (633.90±2.34 g). The differences between the various treatments were determined to be statistically insignificant. The differences in the weights of the liver, heart, and gizzard as a percentage of live weight between the various treatments were minimal and were deemed to be statistically insignificant.

The results obtained in current study fall in line with the findings of Naghshi *et al*. (2014) observed that supplementation of 5% Azolla powder significantly (p<0.05) increased the carcass efficiency and thigh relative percentage. Tawasoli *et al.* (2020) reported positive and beneficial effects of herbals like Azolla meal feeding on dressing percentage up to 6% inclusion of Azolla in poultry diets which is in close agreement with the present study. Shinde *et al.* (2017) revealed that supplementation of Azolla at the rate of 5% showed significant increase (p<0.05) in dressing percentage which is closely in agreement with the results of present study. Kashyap *et al.* (2018) and Bhattacharya *et al*. (2016) reported there was no significant difference among the treatment groups on the carcass traits.

Table 1: Effect of feeding Azolla leaf powder on carcass traits of turkey poults

Parameters / Treatments	**T1**	**T2**	**T3**	**T4**	**SEm±**	**CD at 5%**
Live weight (g)	916.86±9.33^{c}	1024.44±9.56^{b}	1225.77±26.93^{a}	1185.23±8.89^{a}	14.61	45.02
Dressed weight (g)	711.94±4.22^{d}	801.62±0.00^{c}	968.97±1.83^{a}	933.36±4.76^{b}	3.07	9.47
Eviscerated weight (g)	633.90±2.34^{d}	715.28±19.49^{c}	856.04±8.12^{a}	820.29±19.64^{b}	10.40	32.06
Dressing weight (%)	77.65±0.84	78.25±0.72	79.05±1.81	78.75±0.82	1.07	NS
Organ weight as percent of live weight						
Liver weight (%)	2.14±0.04	2.13±0.01	2.12±0.01	2.17±0.02	0.02	NS
Heart weight (%)	1.16±0.01	1.17±0.01	1.12±0.02	1.13±0.02	0.01	NS
Gizzard weight (%)	3.01±0.03	3.06±0.03	3.09±0.03	2.99±0.02	0.03	NS

Means with the same superscripts in a particular row do not differ significantly (p<0.05) from each other.

Economics

The data pertaining to economic parameters of turkey poults in different treatment groups are tabulated in Table-2. Feed efficiency during entire period of study was significantly higher in T_4 group which were fed with 7.5% ALP as compared to rest of the treatment groups. The total

feed cost per poults was significantly higher in T_4 and significantly lowest feed cost was observed in T1. B:C ratio was significantly higher in T_3 (5% Azolla) group. The results of present investigation revealed that inclusion of 5% Azolla had positive effect on economic performance of turkey. However, beyond this level (5% Azolla) slightly decrease in production parameter in terms of gross income and net income was reported in the present study. Data revealed that the benefit cost ratio was significantly highest in T_3 as compared to rest of the treatment groups. However, the difference in benefit cost ratio among T_1, T_2 and T_4 was found to be non- significant. The present results are in agreement with those reported by Borkar *et al*. (2021) who observed that feeding of Azolla meal up to 7.5% in Kadakanath poultry have positive impact in terms of profit as compared to control. Kamel and Hamed (2021) reported significantly highest total return and net return on inclusion of 12% dried azolla in the ration of broilers. Shinde *et al.* (2017) and Ara *et al.* (2015) reported that net profit per bird was maximum in 5% Azolla fed treatment group and beyond this level there was decrease in term of profit in poultry farming. Rathod *et al*. (2013) observed that the use of 7.5% Azolla meal is profitable as compare to other feeding groups. Dhumal *et al.* (2009) and Basak *et al.* (2002) reported that Azolla could be included up to 5% for better profit which is similar to findings of present study.

Table 2: Economics of rearing of turkey poults on feeding Azolla leaf powder

Parameters / Treatments	T1	T2	T3	T4	**SEm±**	**CD at 5%**
Poult cost (Rs/bird)	90	90	90	90		
Feed intake (kg/bird)	2.23±0.01^{d}	2.45±0.01^{c}	2.53±0.01^{b}	2.62±0.01^{a}	0.01	0.02
Total feed cost (Rs/bird)	65.82±0.16^{d}	79.55±0.25^{c}	89.71±0.36^{b}	100.90±0.09^{a}	0.21	0.65
Miscellaneous cost (Rs/bird)	15	15	15	15		
Total cost (Rs/bird)	170.82±0.16^{d}	184.55±0.25^{c}	194.71±0.36^{b}	205.90±0.09^{a}	0.21	0.65
Body weight (g)	907.92±3.08^{d}	1014.50±15.67^{c}	1215.76±19.85^{a}	1116.48±6.20^{b}	9.99	30.79
Gross income (Rs/bird)	226.98±4.62^{d}	253.62±9.17^{c}	303.94±3.95^{a}	279.12±1.93^{b}	4.54	13.99
Net return (Rs/bird)	56.16±4.62^{c}	69.07±9.00^{bc}	109.23±4.08^{a}	73.22±1.97^{b}	4.55	14.01
B:C ratio	1.33±0.03^{b}	1.37±0.05^{b}	1.56±0.02^{a}	1.36±0.01^{b}	0.02	0.08

Means with the same superscripts in a particular row do not differ significantly (p<0.05) from each other.

IV. CONCLUSION

The experiment's findings showed that adding 5% of Azolla leaf powder to the diet increased live weight, dressed weight, and eviscerated weight, all of which were comparable to those of a typical basal diet. Azolla leaf powder added at a quantity of 5% demonstrated the greatest economic advantage.

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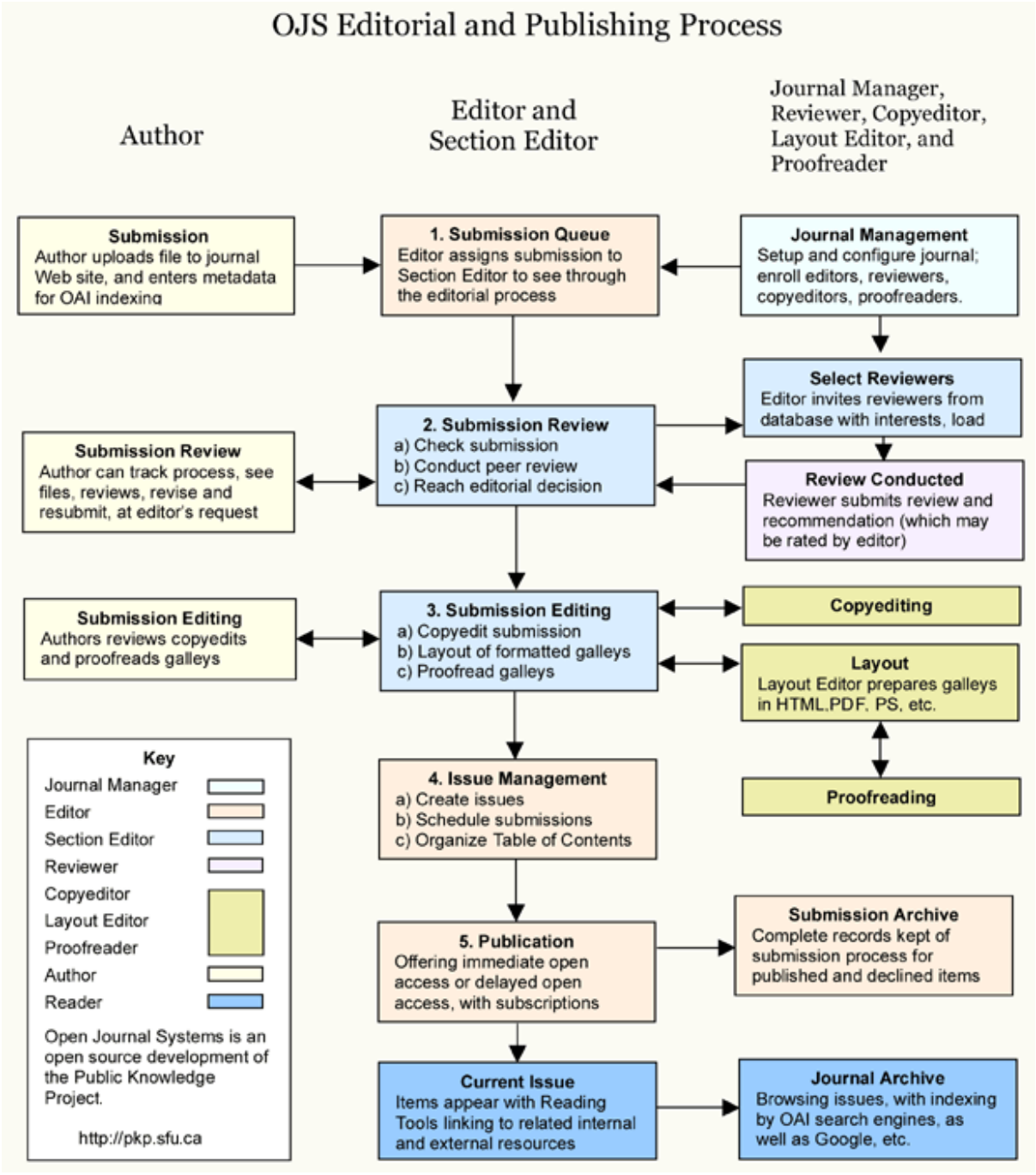

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