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

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

***Keywords*— Aleurodicus rugiopercularus, Encarsia guadelopae, Biological control, Coconut whitefly, Parasitoid wasp, Mass rearing**

I. INTRODUCTION

Aleurodicus rugioperculatus (Hemiptera: Sternorrhyncha: Aleyrodidae) commonly known as Coconut whitefly is a serious pest causing economic losses to ornamentals and tropical crops in many countries [2]. Recently, the infestation of coconut whitefly has been observed in various coconut growing regions in Sri Lanka including Colombo, Kaluthara, Gampaha, Kegalle, Batticaloa, Kandy and Jaffna. Whitefly feeding induces stress to the host plants by extracting water and nutrients while also secreting honeydew, which favors the growth of sooty mold on the leaf surface resulting a massive threat to coconut cultivation. Management of coconut Whitefly possess significant challenges because of their wide host range. Biological control of pest becomes an ecologically sound and effective solution for whitefly infestation. Whiteflies have a number of naturally occurring parasites with them *Encarsia guadeluopae* (Hymenoptera: Aphelinidea) has been identified as the most commonly found natural enemy of *A. rugioperculatus* [8]. *E. guadeluopae* is an obligate endoparasitoid against *A. rugioperculatus* nymphs [11]. The adult stage of this parasite damages the nymphal stage of the whitefly. Therefore, *E. guadeluopae* can be utilized as a biocontrol agent for managing coconut whitefly populations. This approach is environmentally friendly and can serve as an alternative to chemical pesticides.

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

II. METHODOLOGY

2.1. Field survey

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

2.2. Identification of whitefly and parasitoids

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

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

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

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

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

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

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

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

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

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

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

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

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

2.4. Field release of parasitoids

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

Two methods were used to field release of parasitoids:

2.4.1. Introduction of adult parasitoids to whitefly infested plants

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

2.4.2 Introduction of parasitoid cards to infested fields

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

This introduction process was repeated five times at two-week intervals in each location. After each round of releasing parasitoids into the field, coconut leaf samples were collected from each location. Number of existing whiteflies puparial cases, both with and without emergence hole were recorded to determine the percentage of parasitism.

III. RESULT AND DISCUSSION

3.1. Taxonomic identification

3.1.1. Identification of *Aleurodicus rugioperculatus*

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

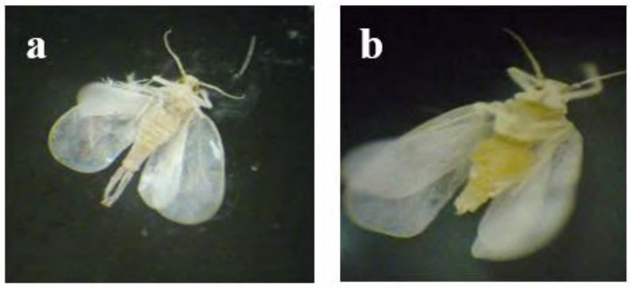

Fig 1: Adult whiteflies. a. Male; b. Female

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

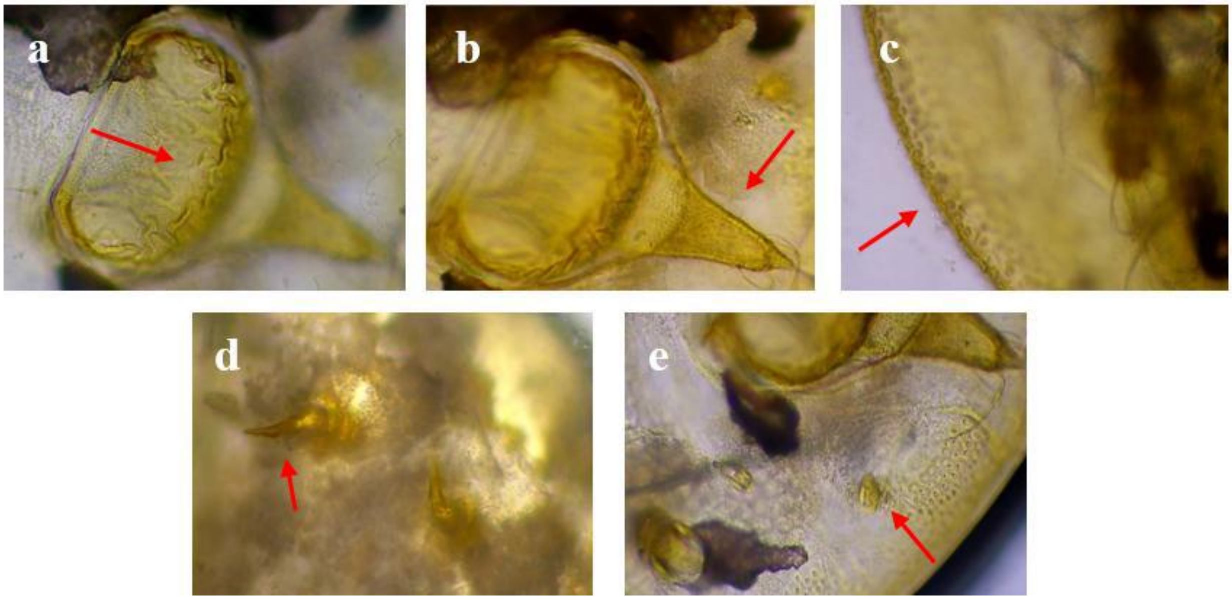

Fig 2: Characters of puparial case. a. rugose operculum; b. triangular lingua; c. reticulated margin on dorsum; d. compound pores with dagger like process; e. presence of smaller compound pores

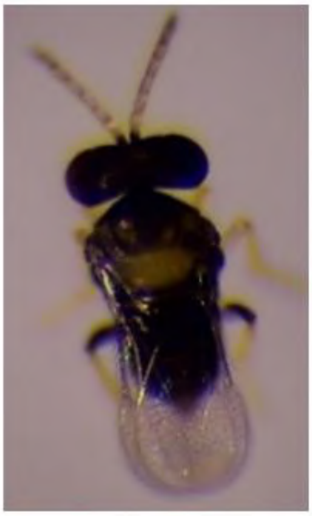

Fig. 3: Adult E. guadeloupae

3.1.2. Identification of *Encarsia guadeloupae*

An average of 98% of emerged parasitoids from the samples showed similar characteristics, and they were identified as *Encarsia guadeloupae* based on the specific features of the species. Adults were dark brown with yellow scutellum. Antenna pale with radical and scape brown. Legs pale except hind coxae and hind femur. Antennal formula 1-1-4-2. Tarsal formula 5-4-5. In the mesosoma 9-11 pairs of setae on the mesocutum and 2 pairs of setae on the scutellum. More than 2 setae on each side of gastral tergites II and III. Fore wings with 3 setae on basal cell, 2 setae on sub marginal vein and 6-7 long setae on the anterior margin of the marginal vein (Fig. 4).

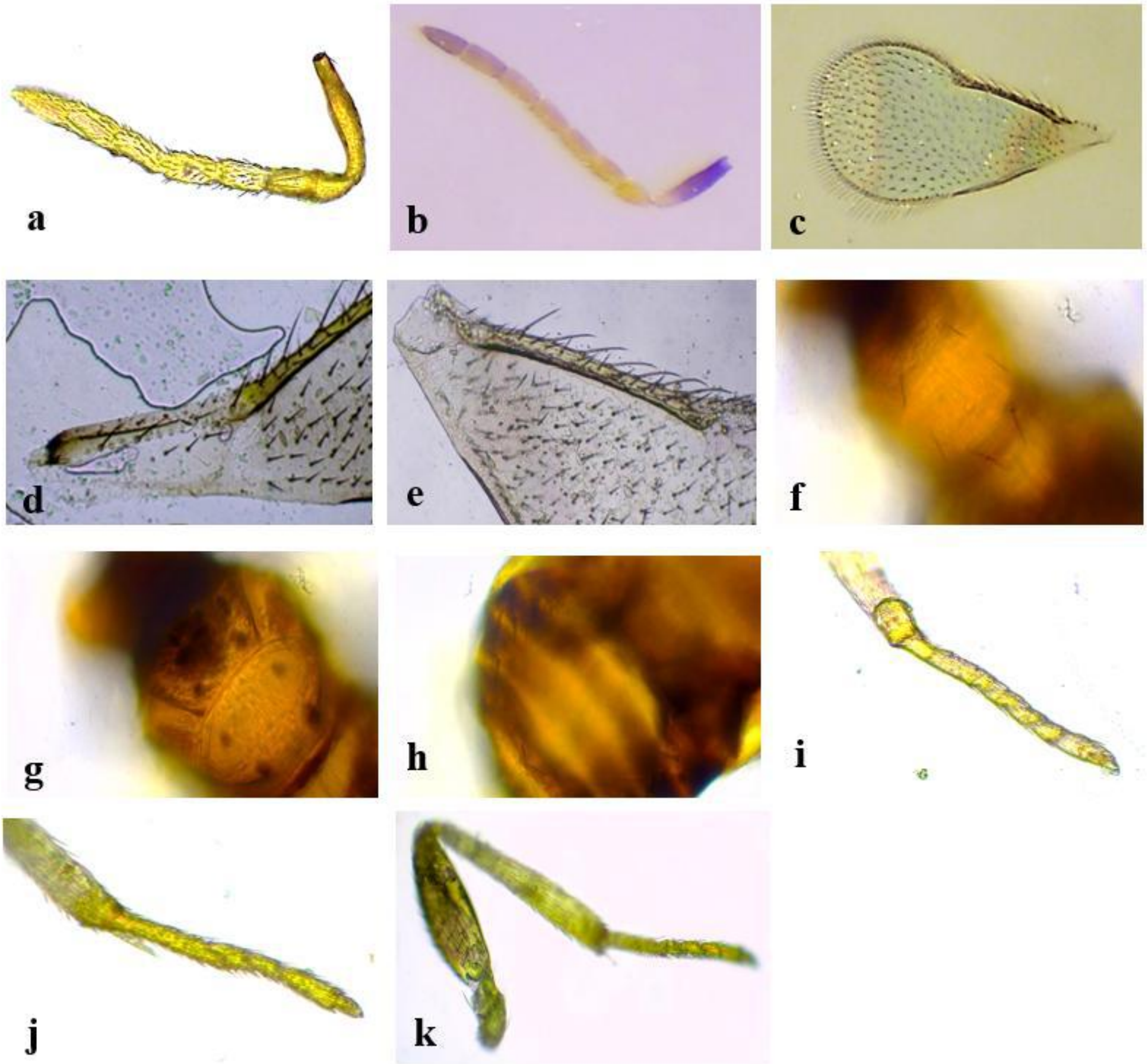

Fig 4: Body parts of E. guadeloupae a-b antenna; c-e fore wing; f-h thorax; i. fore leg; j. mid leg; k. hind leg

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

Six host plants were selected for raring coconut whitefly based on the earlier reports. All the six host plants tested were found to be favorable for the oviposition of whitefly. Highest mean egg spirals were observed in coconut (18), while the lowest were observed in groundnut (2). Oviposition preference of whitefly is influenced by the leaf hairiness. They more prefer rough leaf surfaces. Coconut having rough leaf surface was more favored by whiteflies for their oviposition.

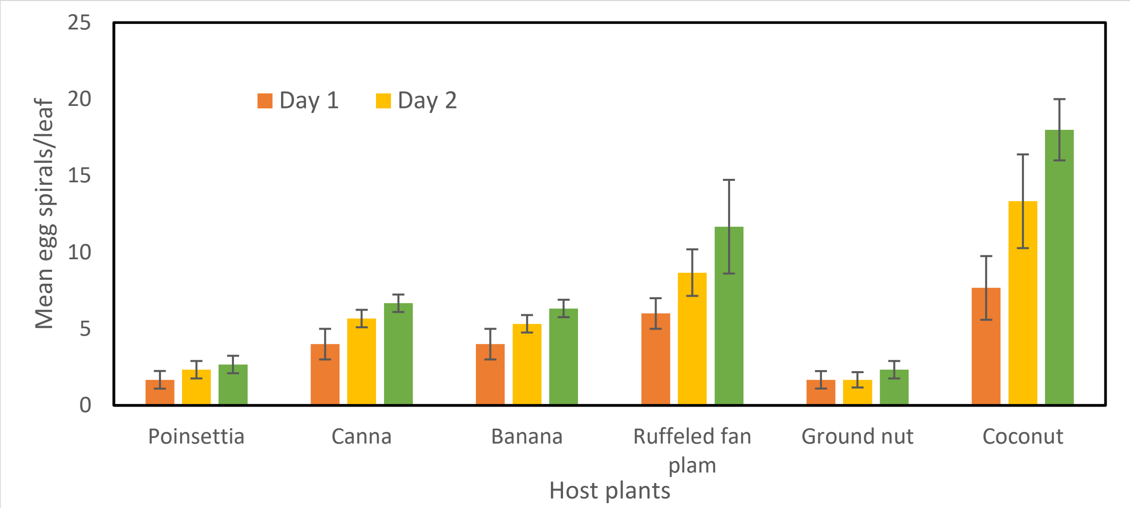

Fig 5: Mean egg spirals per leaf in tested host plants

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

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

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

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

3.3. Field survey

Field survey revealed that all the locations were severely infested with coconut whitefly. Natural parasitism level was significantly lower in each location. This may be due to the usage of synthetic insecticides and the changes of the climatic conditions.

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

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

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

The Pearson correlation coefficient of 0.225 and a p value of 0.089 indicates a positive correlation between the percentage of whitefly and the percentage of parasitism, but the correlation is not statistically significant (Fig. 6). The positive correlation suggests as the percentage of whitefly increases; there is a tendency for the percentage of natural parasitism to also increase. The behavioral response of *E. guadeloupae* involves more attraction to the whitefly infesting host plants [7]. Therefore, natural parasitism level of *E. guadeloupae* increase as the whitefly infestation increase.

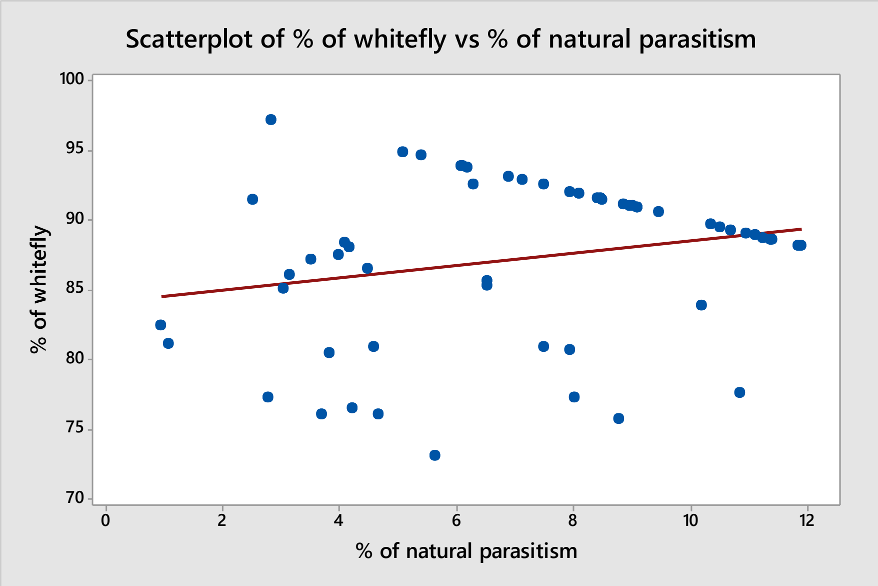

Fig 6: Relationship between Whitefly (A. rugioperculatus) and Parasitoid (E. guadeloupae) populations in Coconut Cultivations in selected locations at the initial stage

3.4. Field release of parasitoids

There is a significant difference between the percentage of parasitism before field release and the after the field release of parasitoids. Parasitoid population in coconut cultivations were gradually increase after the field release of parasitoids. It indicates that, *E. guadeloupae* has successfully established in whitefly infested coconut plantations with the time.

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

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

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

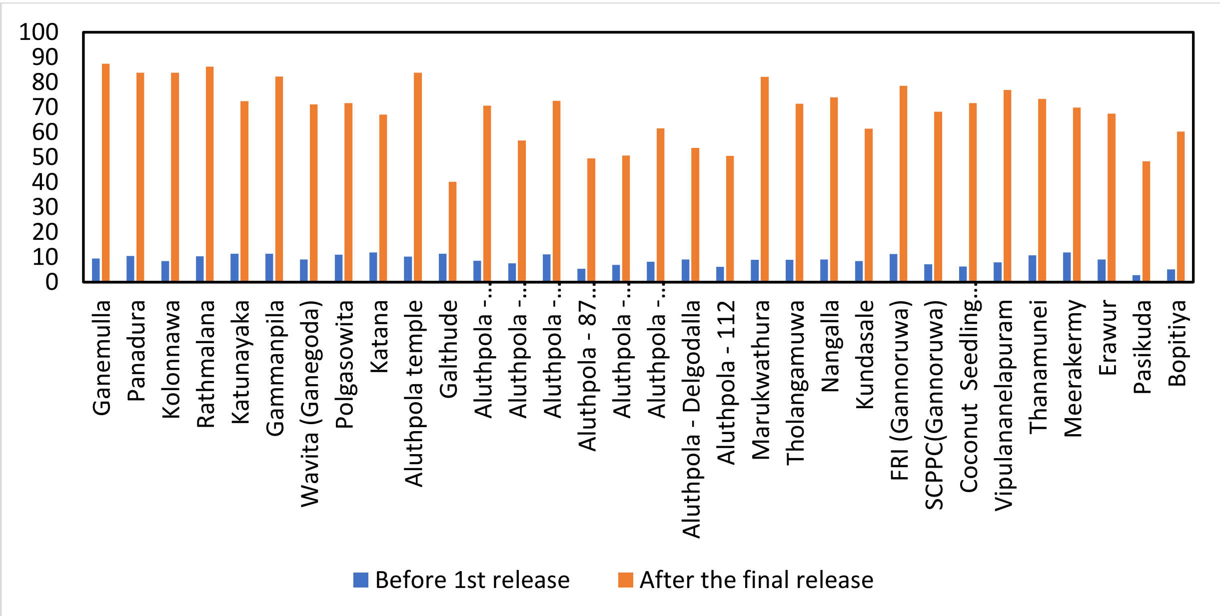

Fig 7: Percentage of parasitoid (E. guadeloupae) population in coconut cultivations at selected locations before and after field release of parasitoids

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

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

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

Observed a perfect negative correlation (-1.000) between the percentage of parasitism and the percentage of whitefly after the field release of parasitoids (Fig. 8). The correlation is highly significant. It revealed that when there is an increase in parasitism, there is a corresponding tendency for a decrease in the percentage of whitefly. It is due to the fact that, the adult stage of *E. guadeloupae* damages the larval stage of the whitefly. Here, the parasite sucks the essence of the whitefly, which is mainly in the second larval stage and lays its eggs on it. As a result, the parasite population grows, and the whitefly population decreases over time.

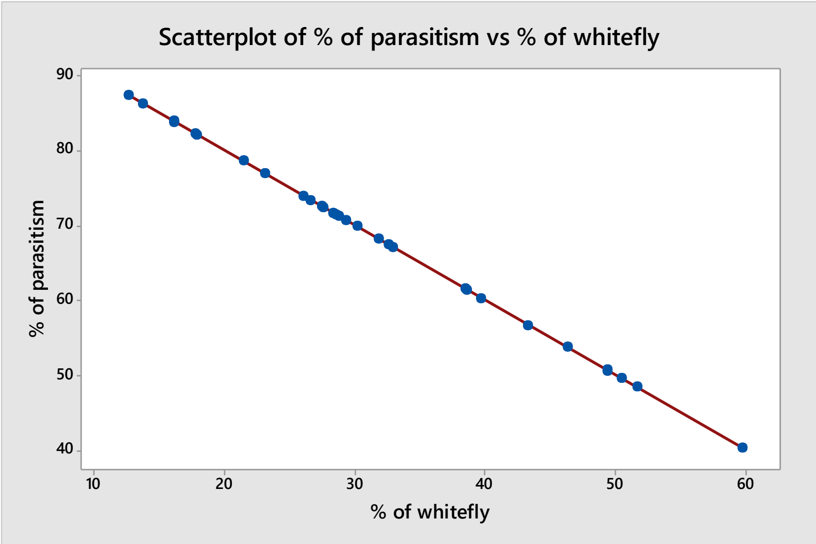

Fig 8: Relationship between whitefly (A. rugioperculatus) and parasitoid (E. guadeloupae) populations in coconut cultivations after field release of parasitoids

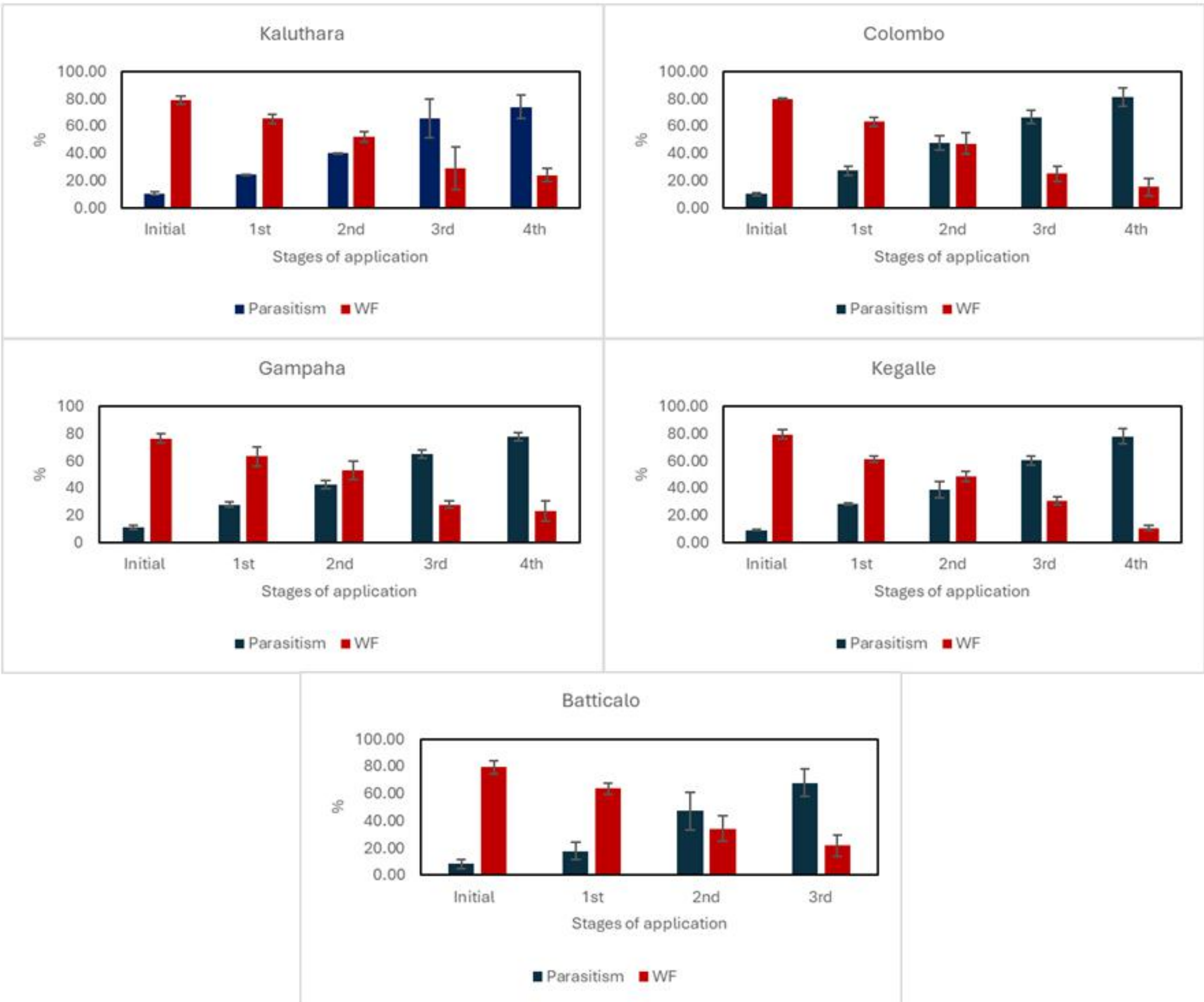

Fig 9: Average percentage of whitefly (A. rugioperculatus) and parasitoid (E. guadeloupae) population in Coconut cultivations in selected districts at different stages of release of parasitoids

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

In Kaluthara district, the initial whitefly and parasitoid populations were recorded at 79% and 10%, respectively. After the introduction of parasitoids, whitefly population exhibited a decline to 24% by the end of the 4th release stage, while the parasitoid population increased to 74%. This trend of whitefly population reduction with an increase in parasitoid population can be observed in all other districts. Ultimately, whitefly populations decreased to 24%, 15%, 23%, 11%, 22% in Kaluthara, Colombo, Gampaha, Kegalle, and Batticaloa districts, respectively.

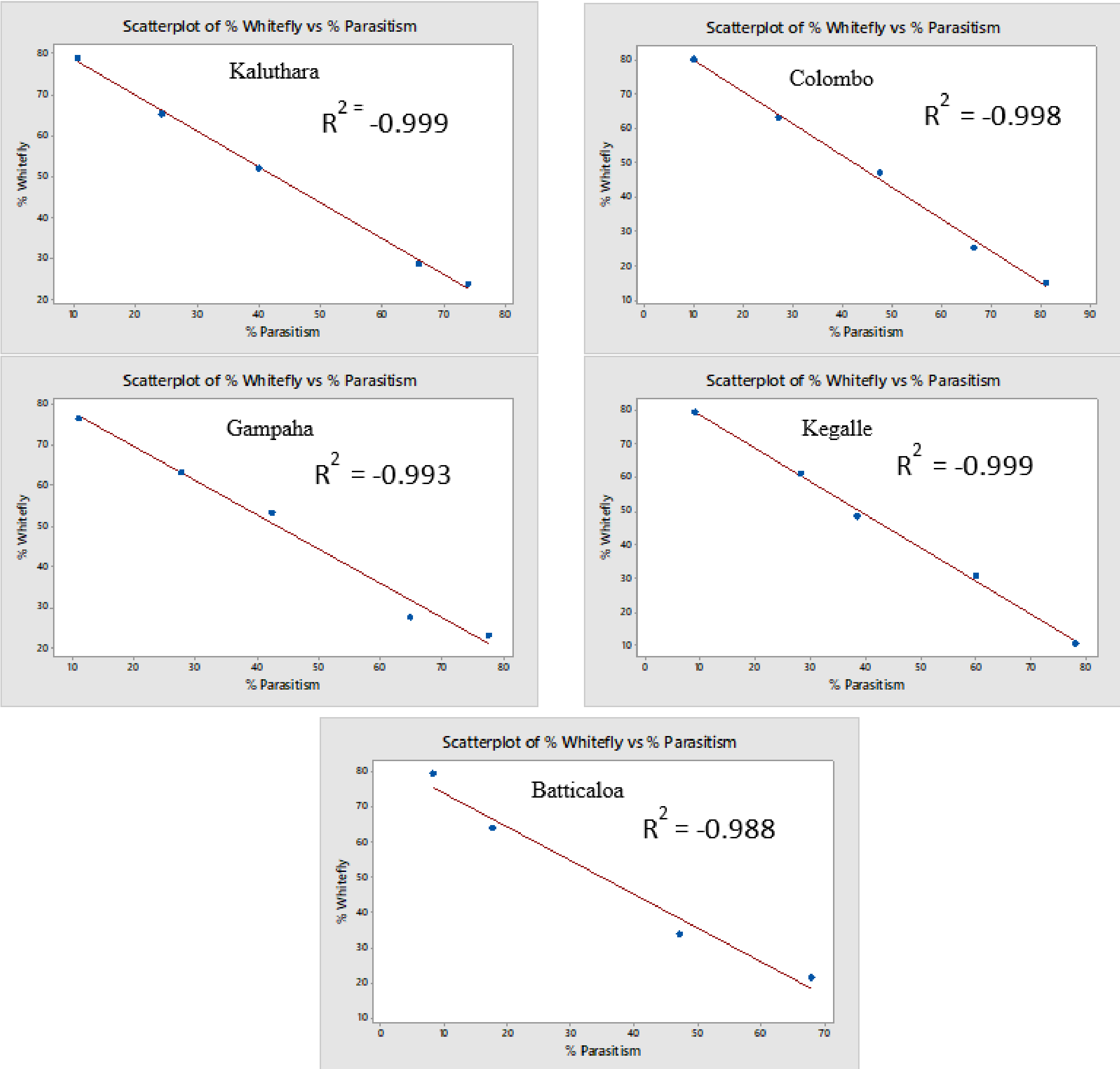

Fig 10: Correlation between population of whitefly and parasitoids in selected locations at different stages of release of parasitoids

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

IV. CONCLUSION

The parasitic wasp, *E. guadeloupae*, identified from the natural environment, can be successfully mass reared in the laboratory and release into invaded areas to effectively control coconut whitefly infestations. Both adult parasitoids of *E. guadeloupae* and parasitized pupae-cards can be used for field release to control the whitefly populations. This control strategy involves releasing the parasitoids five times at two-week intervals. Therefore, this parasitoid has proven to be effective in controlling existing whitefly damage in coconut cultivations in Sri Lanka.

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