Influence of antibiotics on the fitness parameters of rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae*)*

M. Saranya[1], JS. Kennedy[1], S Jeyarani[2], R. Anandham[3] and N Bharathi[4]
[1,2]Department of Agricultural Entomology, Tamil Nadu Agricultural University,
Coimbatore-641 003, Tamil Nadu - India
[3]Department of Agricultural Microbiology, Tamil Nadu Agricultural University,
Coimbatore-641 003, Tami Nadu – India
[4]Department of Plant Molecular Biology and Bioinformatics, Tamil Nadu Agricultural University,
Coimbatore-641 003, Tamil Nadu – India
E-mail: suganpraveen9@gmail.com (M Saranya)
jskennedy@tnau.ac.in (JS Kennedy**)**
anandhamranga@gmail.com (R Anandham)
jeyarani.s@tnau.ac.in (S Jeyarani)
bharathi.n@tnau.ac.in (N Bharathi)

Corresponding authors

Dr. M. Saranya
Senior Research Fellow
Department of Agricultural Entomology,
Tamil Nadu Agricultural University,
Coimbatore-641 003, India
E-mail id: suganpraveen9@gmail.com

Dr.JS. Kennedy D.Sc.,
Professor (Agricultural Entomology),
Department of Agricultural Entomology,
Tamil Nadu Agricultural University,
Coimbatore-641 003, India
E-mail id: jskennedy@tnau.ac.in

Rugose Spiraling Whitefly (RSW), *Aleurodicus rugioperculatus* is an invasive phloem feeder found in India during July-August 2016. Bacterial communities associated with insects are reported to play a major role in their fitness parameters. To resolve this hypothesis, efforts were taken to disrupt the facultative secondary symbionts harboured in hosts through disparate antibiotic treatments and fitness parameters of the treated whitefly were investigated on the *A. rugioperculatus* was reared on four different host plants *viz*., coconut, banana, sapota, and guava. Antibiotics such as Erythromycin E15, Ciprofloxacin CIP5, Carbenicillin CB100 and Cefotaxime CTX 30 treatment were provided to the whitefly adults by parafilm feeding chamber method and observed for fitness parameters of *A. rugioperculatus* progeny. Antibiotic treatment combinations disrupted the bacterial genera *Bacillus*, *Exiguobacterium*, *Acinetobacter*, *Lysinibacillus*, *Arthrobacter*, and *Pseudomonas* associated with *A. rugioperculatus*. Combinations of Carbenicillin 100 µg ml^{-1} + Ciprofloxacin 5 µg ml^{-1} reduced the egg hatchability (59.44 ± 0.59 %), nymphal survival (31.67 ± 0.40 %), longer developmental time (32.69 ± 0.83 days) and reduced fecundity (82.00 ± 0.09 eggs). Antibiotic treatment reduced the fitness parameters *viz*., egg hatchability, nymphal survival, developmental time and fecundity of *A. rugioperculatus* reared on coconut followed by banana, sapota and guava. Prolonged developmental time would provide sufficient time for the parasitoid attack on *A. rugioperculatus* nymphal stages coupled with decreased offspring emergence through antibiotic treatment could be used for the effective management of whiteflies.

Keywords: Antibiotics, Insect associated bacteria, Fitness parameters, Host plants, Rugose spiraling whitefly

Introduction

India is the third-largest producer of coconut in the world, with the annual coconut production of 20.73 billion nuts from an area of 2.19 million ha with an average productivity of 9430 nuts/ha during 2021[1]. Invasion of, seven alien whitefly species were reported in India over the past six years[2]. Those seven invaders include solanum whitefly, *Aleurothrixus trachoides* Back in 2015[3], rugose spiraling whitefly, *A. rugioperculatus* Martin in 2016[4], legume feeding whitefly, *Tetraleurodes acaciae* Quaintance in 2017[5], bondar's nesting whitefly, *Paraleyrodes bondari* Peracchi in 2018[6], nesting whitefly, *Paraleyrodes minei* Iaccarino in 2018[7], palm infesting whitefly, *Aleurotrachelus atratus* Hempel in 2019[8] and woolly whitefly, *Aleurothrixus floccosus* Maskell in 2019[9].

Increasing insect pests in coconut ecosystem severely affect palm productivity and also negatively affect international trade by restrictions for export of value-added coconut products from India.

Among these severe incidences of rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae) was recorded in coconut gardens of India. Martin[10] originally described the incidence of *A. rugioperculatus* in coconut gardens of Belize and Mexico in 2004. Subsequently, *A. rugioperculatus* was recorded as a pest of gumbo limbo (*Bursera simaruba* L) in Florida from Miami-Dade County in 2009[11]. In India, it was first reported in coconut farms of Pollachi, Tamil Nadu during July 2016[12].

Life cycle of *A. rugioperculatus* contains egg, nymphal, pseudo pupal and adult stages. Eggs are elliptical and yellowish in colour with irregular spiraling deposits of white flocculent wax surrounding each egg. Nymphal stages with white waxy covering and one broad fluff tail-like structure on the posterior side of *A. rugioperculatus*. Taxonomic identification of *A. rugioperculatus* was done by its puparial characters. Presence of reticulated cuticle on dorsal

side, compound pores in abdominal segments VII and VIII with distinct dagger-like process, corrugation on the surface of operculum and acute shape of the apex of lingula were reported as unique features of *A. rugioperculatus*. Adults are in large size and have a pair of irregular light brown bands across the wings and males with pair of claspers at tip of abdomen[13].

Due to its high fecundity and dispersal rate the *A. rugioperculatus* extended its host range to several crops (118) including ornamentals, palms, weeds, and native and invasive plant species[14]. In Florida, 22 per cent of palm species, 16 per cent of gumbo limbo, 10 percent of Calophyllum spp., 9 per cent of avocado, 4 percent of black olive, and 3 percent of mango varieties were infested by *A. rugioperculatus* during 2009- 2012[14]. In India, incidences, and damage of *A. rugioperculatus* were recorded on 12 plant species *viz*., *Psidium guajava*, *Musa* sp., *Myristica fragrans*, *Colacasia* sp., *Garcinia* sp., *Annona muricata*, *Murraya koenigii*, *Spondias mombin*, *Mangifera indica*, *Strelitzia reginae*, *Heliconia stricta* and *Artocarpus heterophyllus*[13].

More occurrence of *A. rugioperculatus* population were observed in the middle and lower leaflets of 4-6 years old coconut trees and also in inflorescence, pedicle and exocarp, in severe cases 60-70% of fronds were infested by *A. rugioperculatus* but does not influence the nut yield[4,13]. Desapping of palms by removal of water and nutrients and produces white waxy material and a copious amount of honeydew deposition on upper surface of leaves leading to sooty mold fungus growth on the plant shoot which disrupts the photosynthesis[13,15]. Chemical management of *A. rugioperculatus* on plantations and crop fields is not only difficult but is also ineffective owing to the white waxy covering on the immature *A. rugioperculatus*. Biocontrol is the best way for the sustainable management of whiteflies.

Insects are harboured with diversified microorganisms in their gut and the interaction between the microorganisms and their hosts varies from symbiosis to pathogenesis[16]. Plant phloem sap is rich

in carbohydrates but deficient in nitrogen and essential amino acids[17]. The gut bacteria of whiteflies provide nutrients and essential amino acids from food[18]. Ecdysteroids are essential for the moulting process in insects. In hemipteran insects, conversion of phytosterol into ecdysteroids can be aided by endosymbionts.

Previous study we reported the cultivable bacterial genera associated with the *A. rugioperculatus* were *Bacillus, Exiguobacterium, Acinetobacter, Lysinibacillus, Arthrobacter,* and *Pseudomonas*[19]. Feeding of adult whiteflies with oxytetracycline caused reduced growth and development of offspring as reported by Costa et al.[20,21]. Treatment of cabbage leaves with five antibiotics (rifampicin, ampicillin, tetracycline, streptomycin sulfate and chloramphenicol) significantly affected the growth and development of larvae and caused larval mortality and malformation of prepupae in *Plutella xylostella*[22]. Incorporation of antibiotics mixture (containing tetracycline, gentamicin, penicillin, and rifampicin) in artificial diet from tea powder reduced the survival, growth, and reproduction of *Ectropis obliqua*[23]. Understanding the effect of antibiotics on the host fitness will provide an idea to develop a management strategy for the sustainable management of whitefly through the elimination of bacterial communities[24]. Being a recent invasive insect, studies on the effect of antibiotics against cultivable gut symbionts vis-à-vis bionomics of *A. rugioperculatus* are lacking and hence, in the present study, was aimed to evaluate the effects of antibiotics to disrupt the insect associated cultivable bacteria against fitness parameters of *A. rugioperculatus* reared on four host plants.

Materials and methods

Mass culturing of A. rugioperculatus

A. rugioperculatus infested coconut leaflets were collected at the Tamil Nadu Agricultural University (TNAU) orchard (11.0123° N, 76.9355° E), Coimbatore, Tamil Nadu, India, and

138 released onto mud potted (41 cm diameter) plants of coconut, banana, sapota, and guava, which
139 were kept in a separate mini net house (270 × 150 × 210 cm) with a nylon net mesh size of 120
140 microns). *A. rugioperculatus* rearings were maintained in the Insectary, Department of
141 Agricultural Entomology, TNAU at 31 ± 2 °C, 60 to 75% relative humidity under natural light.
142 *Antibiotic susceptibility test for cultivable bacterial isolates of A. rugioperculatus*
143 *Isolation of cultivable bacteria*
144 Cultivable gut bacteria isolation method was mentioned in the previous study by Saranya et al.
145 (2022). Briefly, second nymphal stages of RSW were collected from coconut, banana and sapota
146 and allowed to starve for 24 h. Surface decontaminated nymphs were homogenized with 0.1 M
147 phosphate buffer (pH =7.0), serially diluted in sterile distilled water and placed on seven different
148 bacterial growth media and incubated for 48 h at 28 ± 2°C. Unique morphotypes were subjected to
149 continuous streaking four-six times to obtain a pure culture. The bacterial isolates were screened
150 based on their chitinolytic activity and subjected to molecular identification of bacterial
151 isolates.16S rRNA gene sequencing results revealed that the cultivable bacterial genera *viz*.,
152 *Bacillus*, *Exiguobacterium*, *Acinetobacter*, *Lysinibacillus*, *Arthrobacter*, and *Pseudomonas* were
153 associated with the *A. rugioperculatus* and same isolated using seven bacterial growth media[19].
154 These bacteria were subjected to sensitivity tests by the Kirby-Bauer disk diffusion method against
155 different antibiotics (Erythromycin E15, Streptomycin S10, Rifampicin RIF5, Polymyxin-B
156 PB300, Vancomycin VA30, Cefotaxime CTX30, Doxycycline DO20, Trimethoprim TR5,
157 Ciprofloxacin CIP5, Colistin CL10, Ampicillin AMP10, Nalidixin NA30, Bacitracin B8,
158 Tetracycline TE30, Carbenicillin CB100, Kanamycin K30, Spectinomycin SPT100,
159 Chloramphenicol C30, and Novobiocin NV30) (M/s. HiMedia Laboratories, Mumbai, India). Gut
160 bacterial isolates of *A. rugioperculatus* were inoculated in nutrient broth and incubated for 24 h at

28 ± 2°C. After incubation bacterial isolates were spread on nutrient agar plates and allowed for 5 minutes and each antibiotic disc (Himedia, India) were placed on the surface of the agar using sterilized forceps and incubated for the 24 h and at 30°C. After 24 h, inhibition zone diameter was measured. The inhibition zone diameter observed for each antibiotic was compared with Clinical Laboratory Standards Institute (CLSI) publication to interpret the sensitivity of the antibiotics. Among the nineteen antibiotics tested, Erythromycin E15, Ciprofloxacin CIP5, Carbenicillin CB100 and Cefotaxime CTX 30 were selected based on the maximum zone of inhibition towards cultivable bacterial isolates of *A. rugioperculatus* and proceeded for further assays[19].

Minimum Inhibition Concentration

Antibiotics that were found to be effective from the above experiments were assayed further for finding out its Minimum Inhibitory Concentration (MIC). Antibiotic strip with different concentrations ranging from 0.002 µg to 256 µg (HiMedia Laboratories Pvt. Ltd, Mumbai) was used to identify the MIC for each selected antibiotics. Based on the MIC assay, antibiotics Carbenicillin (100 µg), Cefotaxime (30 µg), Ciprofloxacin (5 µg), and Erythromycin (15 µg) were found to have broad-spectrum activity at a minimal concentration (0.25µg) and used for the development of aposymbiotic population. As per our result and guidelines of the CLSI, above mentioned concentrations were found to be highly effective and hence, the same concentration was used for further experiment.

Antibiotic treatments through parafilm feeding chambers

A. rugioperculatus adults were fed with the antibiotic added sugar solution using parafilm feeding chamber method adopted by Ruan et al.[24]. Clip cages was placed on potted plants when early nymphal stages turn into pseudopupal stage. Regular monitoring was done to observe the newly emerged whiteflies by pupal cases with T shape exit hole and males have clasper organ at the tip of

abdomen. A newly emerged a pair of *A. rugioperculatus* adult whiteflies were taken into small plastic containers (3.5 cm height, 2.0 cm width). The top portion of the container was stretched with parafilm and the feeding solution was placed above the parafilm layer. Another layer of stretched parafilm is covered with a feeding solution to avoid air bubbles. A minute hole was made on the sides of the container for the aeration of the adults (Fig.1). *A. rugioperculatus* adults were allowed to feed the antibiotic solution for 24 - 48 h and transferred to clip cages. Then, clip cages were placed on four host plants for oviposition and the further progeny development. Antibiotic solutions were fed to the subsequent progeny generations until complete elimination of cultivable gut bacteria which was confirmed through culture-dependent bacterial isolation method. Antibiotic-treated (cultivable gut bacteria eliminated) populations were subjected to fitness parameter *viz*., percentage of egg hatchability, nymphal survival, nymphs developing into adulthood, developmental time from nymphal to adult stage, the fecundity of *A. rugioperculatus* and morphometry was recorded (Fig. 2). Three replications per treatment were maintained for each of the four host plants. Three pairs of *A. rugioperculatus* were used for morphometry studies under stereo zoom microscope (Leica M205C, Germany).

Antibiotic feeding solution (0.2 ml) contains 5 mmol l^{-1} phosphate buffer (pH 7.0), 25% sucrose (w/v) along with selected antibiotics and control feeding solution without antibiotics were used for the above experiment. Antibiotic treatments included were Carbenicillin 100 µg ml^{-1} (CB100), Ciprofloxazin 5 µg ml^{-1} (CIP5), Erythromycin 15 µg ml^{-1} (E15), Cefotaxime 30 µg ml^{-1} (CTX30), Carbenicillin 100 µg ml^{-1} (CB100) + Ciprofloxazin 5 µg ml^{-1}(CIP5), Carbenicillin 100 µg ml^{-1} (CB100) + Erythromycin 15 µg ml^{-1} (E15), Carbenicillin 100 µg ml^{-1} (CB100) + Cefotaxime 30 µg ml^{-1} (CTX30), Ciprofloxazin 5 µg ml^{-1} (CIP5) + Erythromycin 15 µg ml^{-1} (E15), Ciprofloxazin 5

206 μg ml^{-1} (CIP5) + Cefotaxime 30 μg ml^{-1} (CTX30), Erythromycin 15 μg ml^{-1} (E15) + Cefotaxime
207 30 μg ml^{-1} (CTX30) and Control.

208 **Statistical analysis**

209 Data were analyzed using analysis of variance (ANOVA), and means were compared using
210 general linear model (GLM) with Tukey's HSD test. All the analyses were performed by using
211 IBM SPSS Statistics 22[25].

212 **Results**

213 *Effect of Antibiotics on bionomics of A. rugioperculatus reared in different host plants*

214 *A. rugioperculatus* adults reared on four host plants *viz*., coconut, banana, sapota, and guava were
215 treated separately with eleven antibiotic treatments to study the effect on fitness parameters *viz*.,
216 percentage of egg hatchability, nymphal development, developmental time and fecundity of
217 *A. rugioperculatus*. Combined antibiotic treatments (CB100 + CIP5, CB100 + CTX30,
218 CIP5 + CTX30, CB100 + E15, CIP5 + E15, E15 + CTX30) significantly removed the six
219 cultivable bacterial genera *viz*., *Bacillus*, *Exiguobacterium*, *Acinetobacter*, *Lysinibacillus*,
220 *Arthrobacter*, and *Pseudomonas* associated with the *A. rugioperculatus* than individual antibiotic
221 treatments (CB100, CIP5, CTX30, E15). Among the combined antibiotic treatments, CB100 +
222 CIP5 completely eliminated six cultivable bacterial genera associated with the *A. rugioperculatus*
223 and in individual antibiotic treatments, CB100 significantly reduced the population of cultivable
224 bacterial genera associated with the *A. rugioperculatus*.

225 Percent egg hatchability of *A. rugioperculatus* was significantly influenced by antibiotic treatments
226 and host plants (F=12.407, df = 30, P = 0.001). Minimum egg hatchability rates were noted in
227 CB100 + CIP5 (59.44 ± 0.59%) followed by CB100 (67.04 ± 1.20%), CIP5 + CTX30 (76.29 ±

0.04%), CIP5 (80.55 ± 1.01%) while maximum in CB100 + E15 (94.09 ± 0.02%) followed by E15 (89.95 ± 0.14%), CIP5 + E15 (89.48 ± 0.07%) and control (99.00 ± 0.65%) (F=124.911, df = 10, P = 0.001). Among the host plants, higher percentage of egg hatchability was observed on coconut (88.15 ± 0.13%) than other tested host plants (F = 41.64, df = 4, P < 0.001) (Fig.3).

Antibiotic treatments and host plants significantly influenced the development of *A. rugioperculatus* from nymphal stage into adulthood (F=16.42, df = 30, P=0.001) (Fig. 4). Percentage of nymphs developing into adulthood was lower in CB100 + CIP5 (31.67 ± 0.40) followed by CIP5 + CTX30 (37.50 ± 0.31) and CIP5 + E15 (41.75 ± 1.00) while higher in E15 (80.84 ± 1.35) and CTX30 (73.53 ± 1.34) than control (99.48 ± 2.23) (F=1610.79, df =10, P=0.001). In host plants, significantly the lowest per cent of nymphs developing into adulthood were noted in coconut (51.16 ± 0.46) followed by banana (56.65 ± 1.41), sapota (67.28 ± 0.87) and guava (73.99 ± 0.50) (F=886.07, df = 3, P = 0.001).

Time needed for the development from nymphal to the adult stage of *A. rugioperculatus* was significantly influenced by both antibiotic treatments and host plants (F=4.833, df = 30, P=0.05) (Table 1). Developmental time of *A. rugioperculatus* from nymphal to adult stage was significantly longer in antibiotic treatment of CB100 + CIP5 (32.69 ± 0.83 days) followed by CB100 + E15 (29.55 ± 0.66 days), CB100 +CTX30 (27.24 ± 0.13 days), CIP5 + CTX30 (28.58 ± 0.12 days), CB100 (26.76 ± 0.16 days), CTX30 (27.35 ± 0.41 days) than control (22.93 ± 0.02 days) (F =24.94, df = 10, P = 0.05) population. Significantly, longer developmental time (nymph-adult) needed for the nymphal stage reared in coconut (27.66 ± 0.63 days) while shorter in sapota (25.62 ± 0.46 days) (F=5.738, df = 3, P = 0.001).

Antibiotic treatments and host plants had significant effect on *A. rugioperculatus* oviposition (F=57.571, df = 30, P = 0.001) (Table 2). Number of eggs laid by female adult was negatively affected by the

CB100 + CIP5 (82.00 ± 0.09 eggs) followed by CIP5 + CTX30 (95.25 ± 1.19 eggs), CB100 (102.50 ± 1.24 eggs), CB100 + CTX30 (103.00 ± 1.18 eggs), CIP5 (108.75 ± 0.11 eggs) antibiotic treatments than control (237.25 ± 2.10 eggs) (F=1711.601, df = 10, P = 0.001). The lowest number of eggs laid by *A. rugioperculatus* female was recorded in banana (115.54 ± 0.96 eggs) followed by coconut (120.27 ± 1.55 eggs), guava (124.90 ± 2.01eggs) and sapota (132.90 ± 0.41 eggs) (F=247.621, df = 3, P = 0.001).

Morphometric analysis

The progenies of antibiotic-treated *A. rugioperculatus* adults showed elevated variation in morphometry of egg and nymphal stages (Table 3). Morphometric analysis indicated that there is an increased length and decreased width in eggs and nymphal stages in treatment with CB100 + CIP5 antibiotics than in the control population of *A. rugioperculatus*. Significant changes in mean body length of *A. rugioperculatus* life stages were observed in both control and antibiotic treated population (F= 9.960, df = 3, P= 0.001). Both mean body length and width of *A. rugioperculatus* life stages were influenced by the host plants. Increased body length was observed for various life stages of *A. rugioperculatus* reared in the test crops coconut and banana (0.48 mm) (F= 18.227, df = 12, P= 0.001) and decreased with guava (0.56 mm) followed by sapota (0.57 mm) (F= 3.143, df = 12, P= 0.001).

Egg length ranged from 0.30-0.32 mm while width was 0.11-0.14 mm and 0.10-0.12 mm in control and antibiotic treated population, respectively. Length of nymphal stages ranged from 0.30-0.34 mm (first nymphal), 0.50-0.57 mm (second nymphal), 0.46- 0.54 mm (third nymphal) and 0.62-0.74 mm (fourth nymphal) in control population and in antibiotic treated population, it was 0.30-0.36 mm (first nymphal), 0.51-0.54 mm (second nymphal), 0.52- 0.55 mm (third nymphal) and 0.63-0.65 mm (fourth nymphal). Width of nymphal stage was 0.17-0.20 mm (first nymphal), 0.76-0.80 mm (second nymphal), 0.78-0.81 mm (third nymphal) and 1.00-1.06

mm (fourth nymphal) for control population and in antibiotic treated population it was 0.15-0.18 mm (first nymphal), 0.75-0.79 mm (second nymphal), 0.73-0.79 mm (third nymphal) and 1.00-1.04 mm (fourth nymphal).

Discussion

Eliminating or reducing the endobacterial population in insect through antibiotic materials have negative effect on the growth and development of host insects[26-28]. Endosymbiont based plant protection measures to control insect pest was achieved with antibiotics[29]. In previous study, totally 81 morphologically unique bacteria were selected of which 58 isolates showed positive for chitinase activity subjected to 16S rRNA gene sequencing and duplicates were removed. 16S rRNA gene sequencing revealed the presence of cultivable bacterial genera *viz*., *Bacillus*, *Exiguobacterium*, *Acinetobacter*, *Lysinibacillus*, *Arthrobacter*, and *Pseudomonas* associated with the *A. rugioperculatus* were isolated subjected to antibiotic sensitivity test. Similarly, 11 bacterial genera were isolated from sweet potato whitefly, *Bemisia tabaci*, which included *Pseudomonas*, *Deinococcus*, *Sphingomonas*, *Acinetobacter*, *Staphylococcus*, *Modestobacter*, *Micrococcus*, *Bacillus*, *Kocuria*, *Microbacterium*, *Erwinia*, *Brevibacterium*, *Exiguobacterium*, and *Moraxella* [30-32].

In a previous study, we isolated RSW-associated bacteria and subjected to antibiotic susceptibility tests. Screened antibiotics were used for the current study with antibiotics administration of RSW adults and fitness parameter studies. Progenies from antibiotic-treated whiteflies were subjected to culture-dependent bacterial isolation, it did not yield any bacterial colonies which confirmed the antibiotic disruption. Four antibiotics namely Erythromycin E15, Ciprofloxacin CIP5, Carbenicillin CB100 and Cefotaxime CTX30 were selected based on susceptibility test and their mode of action[19].

Carbenicillin is the first semisynthetic penicillin that showed a broad range of action on both gram-positive and negative bacteria[33-35]. Carbenicillin negatively disrupts the components of bacterial cell-wall peptidoglycan by inactivating the transpeptidase enzyme[36]. Ciprofloxacin is a broad-spectrum fluoroquinolone group of antibiotics. Ciprofloxacin, impaired the secretion of DNA gyrase which is responsible for DNA synthesis[37]. Erythromycin belongs to the macrolide class which inhibits protein synthesis by binding to the 50S subunit of prokaryotic ribosomes[38].

Cefotaxime is the third-generation cephalosporin group of antibiotics and its mode of action is similar to carbenicillin which inhibits bacterial cell wall synthesis[39].

Functional role of bacterial endosymbionts associated with host insects could be identified through the elimination of these endosymbionts using antibiotics. Complete elimination of endosymbionts from *A. rugioperculatus* may not be achieved by antibiotic treatments. Because if one microbe disappears, insects particularly de-sapping *A. rugioperculatus* have the ability to activate some other microbes to compensate for it. Temporary setback is possible but subsequent revitalization is always occurred. Antibiotics with a different mode of action alter the endosymbiont population of the whiteflies[20,40]. Growth, offspring emergence from the adult and enzyme for the synthesis of trehalose present in the honeydew secretion affected by the antibiotics were used for the management of whiteflies. *A. rugioperculatus* produces copious amount of honeydew which covers the leaflets and influence the sooty mould growth affecting the photosynthesis of the plant[11]. Antibiotic treatment eliminates the endosymbionts responsible for honeydew production in whiteflies.

In the present study, both antibiotic treatments and host plants were influenced the fitness parameters of *A. rugioperculatus*. Among the ten antibiotics tested CB100 + CIP5 significantly reduced the percentage of egg hatchability, nymphs developing into adulthood, fecundity and prolonged the developmental time of *A. rugioperculatus*. A decline in the percentage of egg hatchability was observed in antibiotic-treated *A. rugioperculatus* on the host plants coconut (61.54 ± 1.92%), banana (60.00 ± 0.59%), sapota (66.67 ± 0.56%) and guava (56.25 ± 0.95%). Percentage of nymphs developing into adulthood was decreased in antibiotic-treated *A. rugioperculatus* on coconut (16.67 ± 0.43%), banana (26.67 ± 0.56%), sapota (36.67 ± 1.21%) and guava host (46.67 ± 0.21%) compared to untreated population. Similarly, Zhang et al.[41] recorded

the effects of ciprofloxacin feeding caused prolonged developmental time and reduced the larval development, fecundity, and egg hatchability of the oriental fruit moth *Grapholita molesta*.

Prolonged developmental time needed from nymphal to adult stage of antibiotic-treated *A. rugioperculatus* reared on coconut (34.10 ± 0.57 days), banana (33.52 ± 0.79 days), sapota (32.04 ± 0.79 days) and guava (31.11 ± 0.79 days) than control population. Fecundity of *A. rugioperculatus* was significantly lower in antibiotic-treated *A. rugioperculatus* reared coconut (62.00 ± 0.26 eggs) followed by banana (70.00 ± 1.10 eggs), sapota (97.00 ± 0.33 eggs) and guava (99.00 ± 2.32 eggs) than control coconut (266.00 ± 3.74 eggs) followed by guava (246.00 ± 0.64 eggs), banana (235.00 ± 1.83 eggs), sapota (202.00 ± 3.99 eggs) and on *A. rugioperculatus* population. Antibiotic concentration prolonged the development period of *Pieris canidia*[42]. Duan et al.[43] reported that antibiotic treatment impaired nutrient metabolism, development process, and the immune system of honeybee larvae.

A single pair of *A. rugioperculatus* (control) produced 19 egg (colonies) spirals (13-17 eggs/spiral). White waxy fluff covering the egg spiral was gently blown off using a straw to facilitate the visualization of eggs and the eggs were counted using hand lens. Higher fecundity of *A. rugioperculatus* (control) due to adult whiteflies spent less energy for dispersal (flight) in clip cages and most of energy might be utilized for oviposition to increase the progenies.

Influence of antibiotics on the endosymbionts of host insects depends on species of the endosymbiont, antibiotic dose and treatment duration[40]. Costa et al.[21] reported that carbenicillin disrupts bacterial cell wall and caused a negative effect on developmental time and offspring emergence in whiteflies which corroborates with our findings. Tetracycline (0.01%) and rifampicin (0.005%) treatment disrupt the bacterial protein synthesis and negatively affect the growth and development of whitefly which correlates with the findings of Ruan et al.[24] who

reported that the rifampicin adversely affects the development of offspring in *B. tabaci.* Antibiotics would disrupt the intestinal symbiotic balance by the removal of gut bacteria and also larval gut cells and have a deleterious impact on the normal growth and metabolism of the larvae[22].

Tetracycline treatment (50 µg ml^{-1}) reduced the *Arsenophonus* and *Wolbachia* population which influenced the growth, development and reproduction pattern of *B. tabaci* and affected the further progeny development [40,44,45]. Prolonged developmental times and higher mortality in antibiotic-treated *Eurygaster integriceps* was also reported[46]. Elimination *Buchnera* population by rifampicin treatment reduced the body size, body mass, soluble sugar, protein and glycogen content which affected the nutrient efficiency and reproductive ability of pea aphid[47].

Karimi et al.[48] also reported that the elimination of *Arsenophonus* prolonged the developmental time showing reduced nymphal survival and adult longevity of date palm hopper, *Ommatissus lybicus*. Antibiotic treatments disrupt whitefly mitochondria which directly influence the fitness of whiteflies and endosymbiont densities[49].

Reduction or elimination of primary and secondary endosymbionts using antibiotic possessing substances as biorational insecticides may be deployed for integrated management of whitefly. Curing of secondary symbionts through antibiotics on *B. tabaci* caused negative effects on host insect[24,50,51]. Rifampicin and oxytetracycline treatment on *B. tabaci* negatively affected the growth and development of the offsprings[20,21,24,52].

Elimination of the bacterial genera *Buchnera* with rifampicin, negatively influenced the body size, body mass, length and width, fertility, and nutritional requirement of *A. pisum*[53]. Morphometric analysis of antibiotic-treated whitefly stages showed longer length and shorter width of egg and nymphal stages, respectively compared to the control. Current study gains support from the findings of Ruan et al.[24] who reported the body length of offspring of *B. tabaci* was longer in

rifampicin and tetracycline treated than control. In conclusion, CB100 + CIP5 antibiotic treatment significantly influence the fitness parameters by the disruption of facultative endosymbionts associated with *A. rugioperculatus*. Prolonged developmental time would provide sufficient time for the parasitoid attack on *A. rugioperculatus* nymphal stages coupled with decreased offspring emergence through antibiotic treatment could be used for the effective management of whiteflies.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors

Consent for publication

All authors gave their consent for the publication of the manuscript.

Conflict of Interest

The authors reported no conflict of interest.

Authors' contributions

M. Saranya: conducted all laboratory experiments and drafted the manuscript

Dr. J.S. Kennedy: Conceived the hypothesis, designed the experiments, and reviewed the manuscript

Dr S. Jeyarani and Dr. R. Anandham: Assisted in laboratory experiments and drafted the manuscript

Dr. N. Bharathi: Reviewed the manuscript

Funding declaration

This work was supported by Department of Science and Technology, Government of India-New Delhi, under grant GOI- DST (SERB) /EMR/2016/00581

References

1. ICC (2021) Coconut Statistical Year Book-2021. International Coconut Community, Jakarta, Indonesia (Accessed from Coconut Development Board website on 05 August 2021)
2. Sundararaj, R., Selvaraj, K., Vimala, D. and Venkatesan, T. Whiteflies (Hemiptera: Aleyrodidae) of India. In. Indian Insects: Diversity and Science, (Eds. S. Ramani, P. Mohanraj, and H.M. Yeshwanth) CRC press, 2020, Tayler & Francis group, UK. pp. 103-120
3. Dubey, A.K. and Sundararaj, R., A new combination and first record of the genus Aleurothrixus Quaintance and Baker (Hemiptera: Aleyrodidae) from India. *Biosystematica*, 2015, **9**(1/2), pp.23-28.
4. Sundararaj, R. and Selvaraj, K., 2017 Invasion of rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae): a potential threat to coconut in India. *Phytoparasitica.* **45** (1):71-74.
5. Sundararaj, R. and Vimala, D., New record of the legume feeding whitefly Tetraleurodes acaciae (Quaintance) (Hemiptera: Aleyrodidae) from India. *Indian. J. Entomol.*, 2018, **80**(3), 1168-1169.
6. Josephrajkumar, A., Mohan, C., Babu, M., Krishna, A., Krishnakumar, V., Hegde, V. and Chowdappa, P., First record of the invasive Bondar's nesting whitefly, *Paraleyrodes bondari* Peracchi on coconut from India. *Phytoparasitica*, 2019, **47**, 333-339. https://doi.org/10.1007/s12600-019-00741-2
7. Mohan, C., Josephrajkumar, A., Babu, M., Krishna, A., Prathibha, P.S., Krishnakumar, V. and Hegde, V., Non-native neotropical nesting whitefly, *Paraleyrodes minei* Iaccarino on coconut palms in India and its co-existence with Bondar's nesting whitefly, *Paraleyrodes bondari* Peracchi. *Curr. Sci.* **117**, 2019, 515-519.

8. Selvaraj, K., Sundararaj, R. and Sumalatha, B.V., Invasion of the palm infesting whitefly, *Aleurotrachelus atratus* Hempel (Hemiptera: Aleyrodidae) in the Oriental region. *Phytoparasitica*, **47**, 2019, 327-332. https://doi.org/10.1007/s12600-019-00742-1

9. Sundararaj, R., Selvaraj, K., Swamy, C.M., Ranjith, M. and Sumalatha, B.V., First record of the invasive woolly whitefly *Aleurothrixus floccosus* (Maskell) from India. *Indian. J. Entomol.*, 2020, **82**, 88-91.

10. Martin, J.H., Whiteflies of Belize (Hemiptera: Aleyrodidae). Part 1-introduction and account of the subfamily Aleurodicinae Quaintance & Baker. Moscas blancas de Belice (Hemiptera: Aleyrodidae). Parte 1-introducción y descripción de la subfamilia Aleurodicinae Quaintance & Baker. *Zootaxa*, 2004, **681**,1-119.

11. Stocks, I.C., and Hodges, G., The rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin, a new exotic whitefly in South Florida (Hemiptera: Aleyrodidae). Florida Department of Agriculture and Consumer Services, Division of Plant Industry, *Aleurodicus rugioperculatus*, 2012, pest-alert. pdf (Accessed 7 March, 2017).

12. Selvaraj, K., Gupta, A., Venkatesan, T., Jalali, S.K., Ballal, C.R. and Sundararaj, R., First record of invasive rugose spiralling whitefly *Aleurodicus*. *rugioperculatus* Martin (Hemiptera: Aleyrodidae) along with parasitoids in Karnataka. *J. Biol. Control*., 2017, **31**,74-78. https://doi.org/10.18311/jbc%2F2017%2F16015.

13. Chandrika Mohan, Josephrajkumar, A., Merin Babu, Prathibha, P.S., Krishnakumar, V., Hegde, V. and Chowdappa, P., Invasive Rugose Spiralling Whitefly on Coconut, Technical Bulletin No. 117, (Centenary Series 60), 2017, ICAR-CPCRI, Regional Station, Kayamkulam, 16 p.

14. Francis, A.W., Stocks, I.C., Smith, T.R., Boughton, A.J., Mannion, C.M. and Osborne, L.S., Host plants and natural enemies of rugose spiralling whitefly (Hemiptera: Aleyrodidae) in Florida. *Fla. Entomol.*, 2016, **99**, 150-153. https://doi.org/10.1653/024.099.0134.

15. Kumar, V., McKenzie, C.L., Mannion, C., Stocks, I., Smith, T. and Osborne, LS., Rugose spiralling whitefly *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae). EENY578. University of Florida, IFAS Extension, 2013.

16. Mrazek, J., Strosova, L., Fliegerova, K., Kott, T. and Kopecny, J., Diversity of insect intestinal microflora. *Folia. Microbiologica.,* 2008, **53** (3), 229-233. https://doi.org/10.1007/s12223-008-0032-z.

17. Pandey, N., Singh, A., Rana, V.S. and Rajagopal, R., Molecular characterization and analysis of bacterial diversity in *Aleurocanthus woglumi* (Hemiptera: Aleyrodidae). Environ. Entomol., 2013, **42**(6):1257– 1264. https:// doi. org/ 10. 1603/ EN131 10

18. Douglas, A.E., Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. Annu Rev Entomol., 1998, **43**(1):17–37. https:// doi. org/ 10. 1146/ annur ev. ento. 43.1.17

19. Saranya, M., Kennedy, J.S. and Anandham, R., Functional characterization of cultivable gut bacterial communities associated with rugose spiralling whitefly, *Aleurodicus rugioperculatus* Martin. 3 Biotech., 2022, **12**, 1-14. https://doi.org/10.1007/s13205-021-03081-3.

20. Costa, H.S., Johnson, M.W., Ullmann, D.E. and Tabashnik, B.E., The antibiotic, oxytetracycline interferes with *Bemisia tabaci* oviposition, development, and ability to induce squash silverleaf. *Ann. Entomol. Soc. Am.,* 1993, **86**,740-748. https://doi.org/10.1093/aesa/86.6.740.

21. Costa, H.S., Enneberry, T.J. and Toscano, N.C., Effects of antibacterial materials on *Bemisia argentifolii* (Homoptera: Aleyrodidae) oviposition, growth, survival, and sex ratio. *J. Econ. Entomol.*, 1997, **90**, 333-339. https://doi.org/10.1093/jee/90.2.333.m

22. Lin, X.L., Kang, Z.W., Pan, Q.J. and Liu, T.X., Evaluation of five antibiotics on larval gut bacterial diversity of *Plutella xylostella* (Lepidoptera: Plutellidae). *Insect Sci.*, 2015, **22**, 619-628. https://doi.org/10.1111/1744-7917.12168

23. Gao, T., Zhou, X., Jiang, R., Zhang, C., Liu, S., Zhao, T., Li, X., Long, Y. and Yang, Y., Evaluation of different concentrations antibiotics on gut bacteria and growth of *Ectropis obliqua* Prout (Lepidoptera: Geometridae). *bioRxiv*, 2021, 2021-04. https://doi.org/10.1101/2021.04.14.439780

24. Ruan, Y.M., Xu, J. and Liu, S.S., Effects of antibiotics on fitness of the B biotype and a non-B biotype of the whitefly *Bemisia tabaci*. *Entomol. Exp. Appl.*, 2006, **121**,159-166. https://doi.org/10.1111/j.1570-8703.2006.00466.x.

25. Spss., IBM SPSS statistics 22. IBM Corp New York,2013.

26. Griffiths, G.W. and Beck, S.D., Effects of antibiotics on intracellular symbiotes in the pea aphid, *Acyrthosiphon pisum*. *Cell. Tissue. Res.*, 1974, **148**, 287-300. https://doi.org/10.1007/BF00224257.

27. Mittler, T.E., Some effects on the aphid *Myzus persicae* of ingesting antibiotics incorporated into artificial diets. *J. Insect. Physiol.*, 1971, **17**,1333-1347. https://doi.org/10.1016/0022-1910(71)90198-3.

28. Nogge, G. and Gerresheim, A., Experiments on the elimination of symbionts from the tsetse fly, *Glossina morsitans morsitans* (Diptera: Glossinidae), by antibiotics and lysozyme. *J. Invertebr. Pathol.*, 1982, **40**, 166-179. https://doi.org/10.1016/0022-2011(82)90112-4.

29. Jaynes, J.M., Burton, C.A., Barr, S.B, Jeffers, G.W., Julian, G.R., White, K.L., Enright F.M., Klei, T.R. and Laine, R.A., In vitro cytocidal effect of novel lytic peptides on

Plasmodium falciparum and *Trypanosoma cruzi* 1. *FASEB. J.*, 1988, **2**, 2878-2883. https://doi.org/10.1096/fasebj.2.13.3049204.

30. Ateyyat, M.A., Shatnawi, M, and Al-Mazra'awi, M., Isolation and identification of culturable forms of bacteria from the sweet potato whitefly *Bemesia tabaci* Genn. (Homoptera: Aleyrodidae) in Jordan. *Turk. J. Agric.*, 2010, **34**,225–234. https:// doi. org/ 10. 3906/tar- 0902- 35
31. Indiragandhi, P., Yoon, C., YangJO, C.S., Sa, T.M. and Kim, G.H., Microbial communities in the developmental stages of B and Q biotypes of sweetpotato whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). *J. Appl. Biol. Chem.,* 2010, **53**, 605–617. https:// doi. org/ 10. 3839/ jksabc.2010. 093
32. Visotto, L.E., Oliveira, M.G.A., Ribon, A.O.B., Mares-Guia, T.R. and Guedes, R.N.C., Characterization and identification of proteolytic bacteria from the gut of the velvetbean caterpillar (Lepidoptera: Noctuidae). *Environ. Entomol.*, 2009, **38**,1078–1085. https:// doi. org/ 10. 1603/022. 038. 0415
33. Acred, P., Brown, D.M., Knudsen, E.T., Rolinson, G.N. and Sutherland, R., New semi-synthetic penicillin active against *Pseudomonas pyocyanea*. *Nature*, 1967, **215**, 25-30.
34. Knudsen, E.T., Rolinson, G.N. and Sutherland, R., Carbenicillin: a new semisynthetic penicillin active against *Pseudomonas pyocyanea*. *Br. Med. J.*, 1967, **3**,75. https://doi.org/10.1136/bmj.3.5557.75.
35. Smith, C.B. and Finland, M., Carbenicillin: activity in vitro and absorption and excretion in normal young men. *Appl. Microbiol.*, 1968, **16**,1753-1760. https://doi.org/10.1128/am.16.11.1753-1760.1968.

36. Butler, K., English, A.R., Ray, V.A. and Timreck, A.E., Carbenicillin: chemistry and mode of action. *J. Infect. Dis.*, 1970, **122**, S1-S8. https://www.jstor.org/stable/30108303.

37. Zweerink, M.M. and Edison, A., Inhibition of *Micrococcus luteus* DNA gyrase by norfloxacin and 10 other quinolone carboxylic acids. *Antimicrob. Agents. Chemother.*, 1986, **29**, 598-601. https://doi.org/10.1128/AAC.29.4.598.

38. Brock, T.D. and Brock, M.L., Similarity in mode of action of chloramphenicol and erythromycin. *Biochim. Biophys. Acta.*, 1959, **33** (1), 274-275. https://doi.org/10.1016/0006-3002(59)90535-9.

39. Murray, B.E. and Moellering Jr, RC., Cephalosporins. *Ann. Rev. Med.*, 1981, **32**, 559-581. https://doi.org/10.1146/annurev.me.32.020181.003015.

40. Ahmed, M.Z., Ren, S-x., Xue, X., Li, X-X., Jin, G-h. and Qiu, B-L., Prevalence of endosymbionts in *Bemisia tabaci* populations and their in vivo sensitivity to antibiotics. *Curr. Microbiol.*, 2010, **61**, 322-328. https://doi.org/10.1007/s00284-010-9614-5.

41. Zhang, X., Wang, X., Guo, Z., Liu, X., Wang, P., Yuan, X. and Li, Y., Antibiotic treatment reduced the gut microbiota diversity, prolonged the larval development period and lessened adult fecundity of *Grapholita molesta* (Lepidoptera: Tortricidae). *Insects*, 2022, **13**, 838. https://doi.org/10.3390/insects13090838

42. Wang, Y., Zhu, J., Fang, J., Shen, L., Ma, S., Zhao, Z., Yu, W. and Jiang, W., Diversity, composition and functional inference of gut microbiota in Indian cabbage white *Pieris canidia* (Lepidoptera: Pieridae). *Life*, 2020, **10**, 254. https://doi.org/10.3390/life10110254

43. Duan, X., Zhao, B.A., Jin, X., Cheng, X., Huang, S. and Li, J., Antibiotic treatment decrease the fitness of honeybee (*Apis mellifera*) larvae. *Insects*, 2021, **12,** 301. https://doi.org/10.3390/insects12040301

44. Raina, H.S., Rawal, V., Singh, S., Daimei, G., Shakarad, M. and Rajagopal, R., Elimination of *Arsenophonus* and decrease in the bacterial symbionts diversity by antibiotic treatment leads to increase in fitness of whitefly, *Bemisia tabaci*. *Infect. Genet. Evol.,* 2015, **32**, 224-230. https://doi.org/10.1016/j.meegid.2015.03.022.

45. Zhong, Y. and Li, Z-X., Influences of tetracycline on the reproduction of the B biotype of *Bemisia tabaci* (Homoptera: Aleyrodidae). *Applied. Entomol. Zool.,* 2013, **48**, 241-246. https://doi.org/10.1007/s13355-013-0180-8.

46. Kafil, M., Bandani, A.R., Kaltenpoth, M., Goldansaz, S.H., Alavi, S.M. and Miller, T., Role of symbiotic bacteria in the growth and development of the Sunn pest, *Eurygaster integriceps*. *J. Insect. Sci.,* 2013, **13**, 99. https://doi.org/10.1673/031.013.9901.

47. Lv, Z-H., Wei, X-Y., Tao, Y-L. and Chu, D., Differential susceptibility of whitefly-associated bacteria to antibiotic as revealed by metagenomics analysis. *Infect. Genet. Evol.,* 2018b**, 63**, 24-29. https://doi.org/10.1016/j.meegid.2018.04.024.

48. Karimi, S., Askari Seyahooei, M., Izadi, H., Bagheri, A. and Khodaygan, P., Effect of *Arsenophonus* endosymbiont elimination on fitness of the date palm hopper, *Ommatissus lybicus* (Hemiptera: Tropiduchidae). *Environ. Entomol.,* 2019, **48**, 614-622. https://doi.org/10.1093/ee/nvz047.

49. Milenovic, M., Gouttepifre, A., Eickermann, M., Junk, J. and Rapisarda, C., Plant-mediated rifampicin treatment of Bemisia tabaci disrupts but does not eliminate endosymbionts. *Sci. Rep.*, **12,** 2022, 20766.

50. Ridley, E.V., Wong, A.C.N. and Douglas, A.E., Microbe-dependent and nonspecific effects of procedures to eliminate the resident microbiota from *Drosophila melanogaster*. *Appl. Environ. Microbiol.,* 2013, **79**, 3209-3214. https://doi.org/10.1128/AEM.00206-13.

51. Shan, H.W., Zhang, C.R., Yan, T.T., Tang, H.Q., Wang, X.W., Liu S.S. and Liu, Y.Q., Temporal changes of symbiont density and host fitness after rifampicin treatment in a whitefly of the *Bemisia tabaci* species complex. *Insect. Sci.*, 2016, **23**, 200-214. https://doi.org/10.1111/1744-7917.12276.

52. Xue, X., Li, S-J., Ahmed, M.Z., De Barro, P.J., Ren, S-X. and Qiu, B-L., Inactivation of *Wolbachia* reveals its biological roles in whitefly host. *PloS One.*, 2012, **7**, e48148. https://doi.org/10.1371/journal.pone.0048148

53. Lv, N., Wang, L., Sang, W., Liu, C-Z. and Qiu, B-L., Effects of endosymbiont disruption on the nutritional dynamics of the pea aphid *Acyrthosiphon pisum*. *Insects.*, 2018a, **9**, 161. https://doi.org/10.3390/insects9040161

573 **Table 1. Effect of antibiotics on developmental time of *A. rugioperculatus* reared in different host plants**

S.No	AT	Developmental time (Nymph-adult) (Days)				
		Host plants				AT
		Coconut	Banana	Sapota	Guava	(Mean)
1	CB100	29.77 ± 0.69^{bc}	26.27 ± 0.14^{cde}	25.05 ± 0.14^{de}	25.98 ± 0.14^{def}	26.76 ± 0.16^{BCD}
2	CIP5	27.05 ± 0.17^{de}	25.30 ± 0.18^{def}	24.16 ± 0.18^{ef}	25.06 ± 0.18^{efg}	25.39 ± 0.22^{ABC}
3	E15	24.08 ± 0.11^{gh}	24.03 ± 0.54^{fgh}	23.02 ± 0.54^{f}	23.16 ± 0.54^{gh}	23.57 ± 0.60^{A}
4	CTX30	28.08 ± 0.47^{cd}	27.07 ± 0.66^{bcd}	26.11 ± 0.66^{cd}	28.17 ± 0.66^{bcd}	27.35 ± 0.41^{CD}
5	CB100 + CIP5	34.10 ± 0.57^{a}	33.52 ± 0.79^{a}	32.04 ± 0.79^{a}	31.11 ± 0.79^{a}	32.69 ± 0.83^{E}
6	CB100 + E15	31.05 ± 0.37^{b}	29.13 ± 0.29^{b}	28.01 ± 0.29^{bc}	30.02 ± 0.29^{ab}	29.55 ± 0.66^{D}
7	CB100 +CTX30	25.76 ± 0.21^{efg}	27.16 ± 0.37^{bcd}	29.02 ± 0.37^{b}	27.06 ± 0.37^{cde}	27.24 ± 0.13^{CD}
8	CIP5 + E15	26.07 ± 0.54^{ef}	24.27 ± 0.35^{efg}	23.15 ± 0.35^{ef}	24.08 ± 0.35^{fgh}	24.39 ± 0.11^{AB}
9	CIP5 + CTX30	30.07 ± 0.01^{bc}	28.10 ± 0.45^{bc}	27.06 ± 0.45^{bc}	29.09 ± 0.45^{abc}	28.58 ± 0.12^{D}
10	E15 + CTX30	24.77 ± 0.22^{fgh}	23.20 ± 0.31^{gh}	23.20 ± 0.31^{ef}	22.16 ± 0.31^{h}	23.31 ± 0.25^{A}
11	Control	23.45 ± 0.28^{h}	22.18 ± 0.32^{h}	21.08 ± 0.32^{g}	25.03 ± 0.32^{efg}	22.93 ± 0.02^{A}
	HP (Mean)	$27.66 \pm 0.63^{\$}$	$26.38 \pm 0.28^{\#\$}$	$25.62 \pm 0.46^{\#}$	$26.44 \pm 0.35^{\#\$}$	
	HP	$F = 5.738$, df=3, $P < 0.001$				
	AT	$F = 24.94$, df=10, $P < 0.05$				
	AT x HP	$F = 4.833$, df=30, $P < 0.05$				

574

575 Values with the lower-case letters, capital letters and symbols do not differ significantly among them according to Tukey's HSD test
576 at < 0.001 level of significance. Letters a–g for interaction (AT * HP), A–J for antibiotic treatments (AT), and #$ for host plants (HP).
577 Values in each column are means of three replications ± standard error (SE).

578

579

Table 2. Effect of antibiotics on fecundity of *A. rugioperculatus* reared in different host plants

S.No	AT	Fecundity (Eggs/female) (Mean ± SE)				
		Host plants				AT (Mean)
		Coconut	Banana	Sapota	Guava	
1	CB100	103.00 ±0.12^{f}	99.00 ± 0.77^{de}	108.00 ± 1.08^{e}	100.00 ± 1.70^{ef}	102.50 ± 1.24^{G}
2	CIP5	110.00 ± 1.03^{def}	103.00 ± 0.16^{cd}	117.00 ± 2.8^{e}	105.00 ± 1.58^{de}	108.75 ± 0.11^{F}
3	E15	131.00 ± 2.52^{b}	165.00 ± 2.66^{b}	141.00 ± 2.27^{bc}	169.00 ± 1.93^{b}	151.50 ± 2.68^{B}
4	CTX30	125.00 ± 0.85^{bc}	116.00 ± 1.45^{c}	139.00 ± 0.87^{bcd}	120.00 ± 0.31^{c}	125.00 ± 2.47^{C}
5	CB100 + CIP5	62.00 ± 0.26^{h}	70.00 ± 1.10^{g}	97.00 ± 0.33^{f}	99.00 ± 2.32^{ef}	82.00 ± 0.09^{I}
6	CB100 + E15	112.00 ± 0.80^{de}	101.00 ± 2.42^{de}	137.00 ± 3.07^{bcd}	109.00 ± 1.65^{d}	114.75 ± 0.78^{E}
7	CB100 +CTX30	94.00 ± 0.78^{g}	90.00 ± 1.73^{ef}	134.00 ± 1.12^{cd}	94.00 ± 0.39^{f}	103.00 ± 1.18^{G}
8	CIP5 + E15	117.00 ± 0.77^{cd}	109.00 ± 1.42^{c}	130.00 ± 2.84^{d}	111.00 ± 0.12^{d}	116.75 ±0.49^{DE}
9	CIP5 + CTX30	87.00 ± 1.86^{g}	82.00 ± 1.75^{f}	112.00 ± 0.06^{e}	100.00 ± 2.55^{ef}	95.25 ± 1.19^{H}
10	E15 + CTX30	108.00 ± 2.19^{ef}	109.00 ± 1.13^{c}	145.00 ± 2.04^{b}	121.00 ± 1.39^{c}	120.75 ± 1.32^{D}
11	Control	266.00 ± 3.74^{a}	235.00 ± 1.83^{a}	202.00 ± 3.99^{a}	246.00 ± 0.64^{a}	237.25 ± 2.10^{A}
	HP (Mean)	120.27 ± 1.55$^{@}$	115.54 ± 0.96$^{\#}$	132.90 ± 0.41$^{\$}$	124.90 ± 2.01*	
	HP	F =247.621, df=3, P < 0.001				
	AT	F = 1711.601, df= 10, P < 0.001				
	AT x HP	F = 57.571, df= 30, P < 0.001				

AT- Antibiotic treatments, HP- Host plants, CB100- Carbenicillin 100 µg ml^{-1}, CIP5- Ciprofloxacin 5 100 µg ml^{-1}, CTX30- Cefotaxime 30 µg ml^{-1}, E15- Erythromycin 15 µg ml^{-1}. Values with the same lower-case letters, capital letters and symbols do not differ significantly according to Tukey's HSD test at < 0.001 level of significance, marked by small a–g for interaction (AT * HP), A–J for antibiotic treatments (AT), and @#$* for host plants (HP). Values in each column are means of three replications ± standard error (SE).

586 **Table 3. Morphometric analysis of symbiotic and antibiotic treated *A.rugioperculatus* from four different host plants**

Biotype	Life stages	Length (mm) (Mean ± SE)				Breadth (mm) (Mean ± SE)			
		Coconut	Banana	Sapota	Guava	Coconut	Banana	Sapota	Guava
Symbiotic	Egg	0.31 ± 0.01	0.32 ± 0.01	0.30 ± 0.01	0.30 ± 0.02	0.14 ± 0.01	0.13 ± 0.02	0.12 ± 0.01	0.11 ± 0.03
	N1	0.34 ± 0.01	0.33 ± 0.02	0.31 ± 0.01	0.30 ± 0.03	0.20 ± 0.02	0.19 ± 0.01	0.17 ± 0.02	0.18 ± 0.03
	N2	0.53 ± 0.01	0.57 ± 0.03	0.50 ± 0.02	0.55 ± 0.01	0.80 ± 0.02	0.79 ± 0.01	0.76 ± 0.03	0.77 ± 0.04
	N3	0.54 ± 0.02	0.48 ± 0.01	0.51 ± 0.03	0.46 ± 0.03	0.81 ± 0.02	0.80 ± 0.03	0.79 ± 0.02	0.78 ± 0.01
	N4	0.63 ± 0.01	0.74 ± 0.03	0.62 ± 0.02	0.72 ± 0.01	1.02 ± 0.00	1.06 ± 0.01	1.01 ± 0.03	1.00 ± 0.04
Antibiotic treated	Egg	0.32 ± 0.00	0.31 ± 0.01	0.31 ± 0.01	0.30 ± 0.01	0.11 ± 0.02	0.12 ± 0.01	0.10 ± 0.04	0.10 ± 0.03
	N1	0.36 ± 0.01	0.35 ± 0.02	0.30 ± 0.01	0.33 ± 0.03	0.18 ± 0.01	0.17 ± 0.02	0.16 ± 0.03	0.15 ± 0.04
	N2	0.54 ± 0.01	0.53 ± 0.02	0.51 ± 0.03	0.52 ± 0.04	0.79 ± 0.02	0.78 ± 0.03	0.75 ± 0.01	0.75 ± 0.01
	N3	0.55 ± 0.02	0.54 ± 0.01	0.52 ± 0.02	0.53 ± 0.01	0.79 ± 0.01	0.75 ± 0.02	0.77 ± 0.02	0.73 ± 0.02
	N4	0.65 ± 0.02	0.65 ± 0.03	0.63 ± 0.01	0.64 ± 0.04	1.00 ± 0.01	1.04 ± 0.03	1.03 ± 0.02	1.04 ± 0.01
	Mean	0.48 ± 0.01^{C}	0.48 ± 0.02^{C}	0.45 ± 0.03^{A}	0.47 ± 0.02^{B}	0.58 ± 0.02^{Q}	0.58 ± 0.03^{Q}	0.57 ± 0.02^{P}	0.56 ± 0.03^{P}
Life stages x Biotype		F= 41.036, df=4, P= 0.001				F= 7.100, df=4, P= 0.001			
Life stages x host plants		F= 18.227, df=12, P= 0.001				F= 3.143, df=12, P= 0.001			
Biotype x host plants		F= 9.960, df=3, P= 0.001				F= 0.690, df=3, P= 0.561			
Life stages x Biotype x host plants		F= 14.744, df=12, P= 0.001				F= 1.425, df=12, P= 0.172			

587 Values with the same capital letters do not differ significantly according to Tukey's HSD test at < 0.001 level of significance, marked
588 by A-C for mean body length of *A. rugioperculatus* life stages and P–Q mean body width of *A. rugioperculatus* life stages reared in
589 different host plants. Values in each column are means of three replications ± standard error (SE).

Figure Legends

Figure 1 Antibiotic feeding of *A. rugioperculatus* adults through parafilm feeding chamber

Figure 2 Antibiotic fed *A. rugioperculatus* adults in clip cage placed on different host plants to study the fitness parameters a. Coconut b. Banana c. Sapota d. Guava

Figure 3 Effect of antibiotics on egg hatchability of *A. rugioperculatus* reared in different host plants

CB100- Carbenicillin 100 µg ml^{-1}, CIP5- Ciprofloxacin 5 100 µg ml^{-1}, CTX30- Cefotaxime 30 µg ml^{-1}, E15- Erythromycin 15 µg ml^{-1}. Values with the same lower-case letters do not differ significantly according to Tukey's HSD test at < 0.001 level of significance, marked by small a–h for antibiotic treatments, and p–q for host plants. Values in each column are means of three replications ± standard error (SE).

Figure 4 Effect of antibiotics on nymphal development of *A. rugioperculatus* reared in different host plants

CB100- Carbenicillin 100 µg ml^{-1}, CIP5- Ciprofloxacin5 100 µg ml^{-1}, CTX30- Cefotaxime 30 µg ml^{-1}, E15- Erythromycin 15 µg ml^{-1}. Values with the same lower case letters do not differ significantly according to Tukey's HSD test at < 0.001 level of significance, marked by small a–k for antibiotic treatments, and p–s for host plants. Values in each column are means of three replications ± standard error (SE)

Figure 1

Coconut

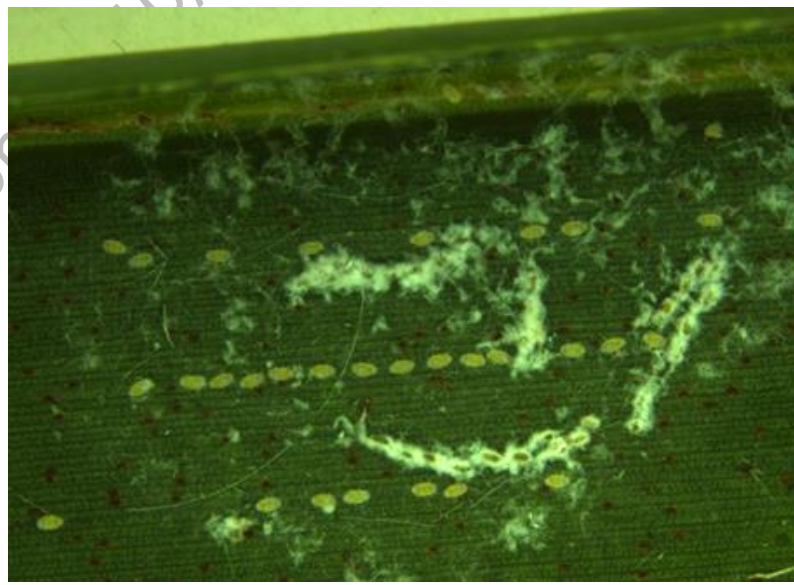
Banana

Sapota

Guava

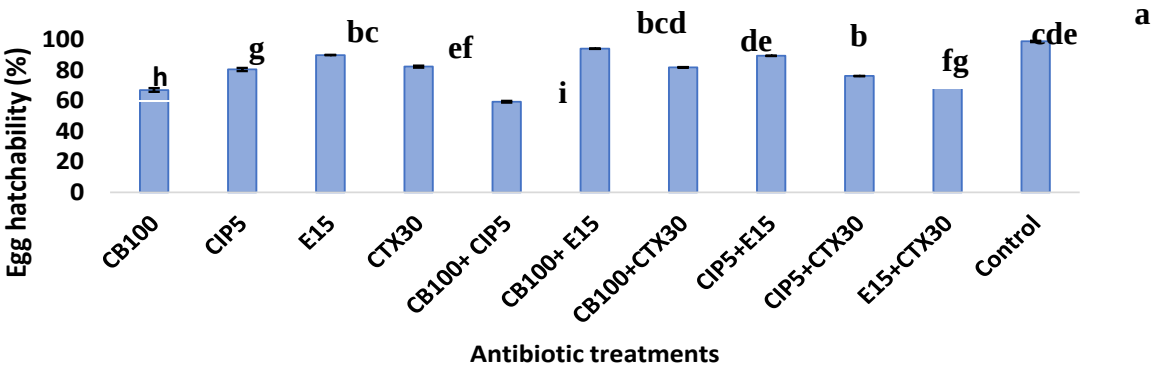
Figure 2a

Egg colonies with crawler stages of antibiotic treated population

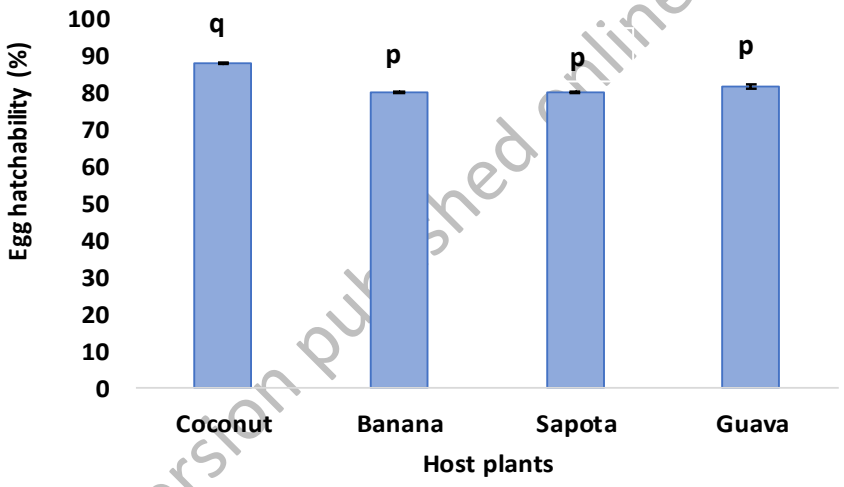


Egg colonies with crawler stages of control population

Figure 2b

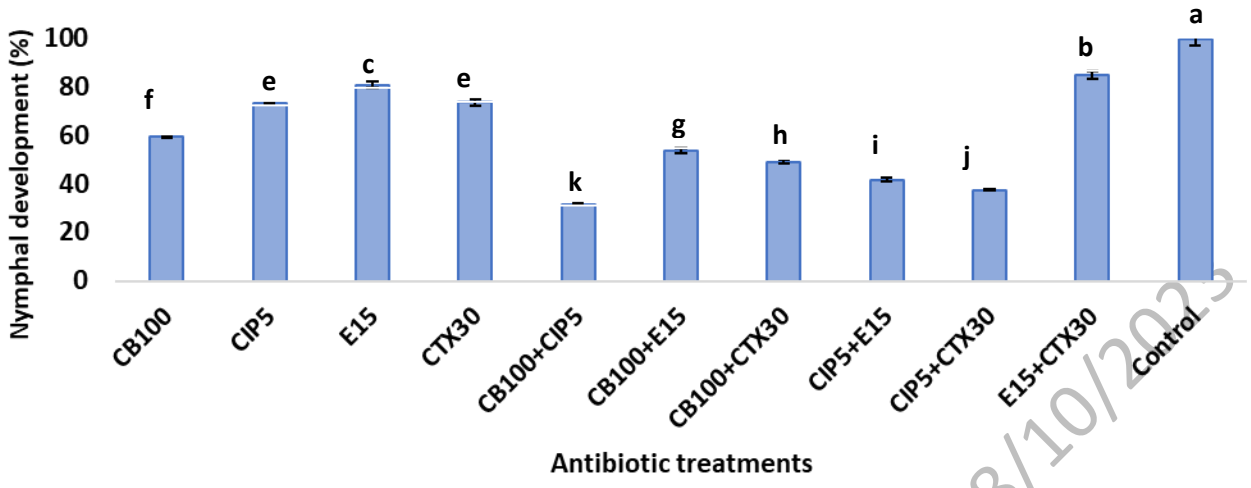


(a)

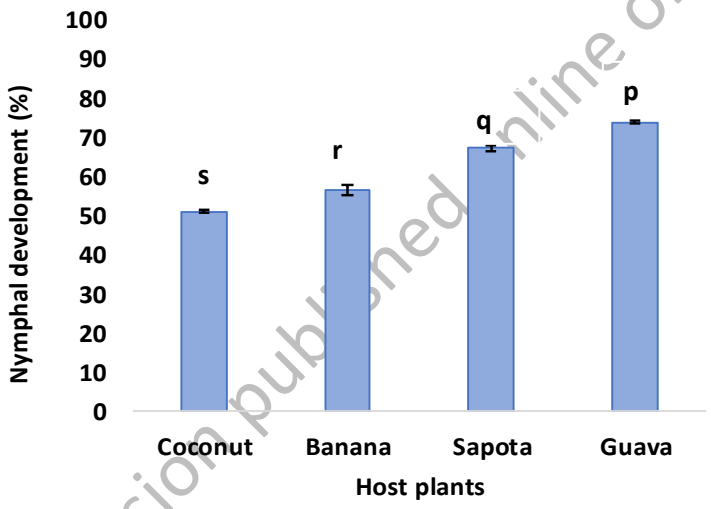


(b)

Figure 3



(a)



(b)

Figure 4