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Research Article

Effect of antibiotic materials on rugose spiralling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae) oviposition

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Abstract

The rugose spiralling whitefly is an invasive sucking pest on horticultural crops found in India. Elimination of endomicrobial communities harboured in hosts through antibiotic treatments adversely affects the fitness parameters of rugose spiralling whitefly. Keeping this in view, the present study aimed to evaluate the ovicidal action of antibiotics against rugose spiralling whiteflies reared on four host plants. Antibiotics with varied modes of action were erythromycin, ciprofloxacin, carbenicillin and cefotaxime and were applied to coconut, banana, sapota and guava leaves for whitefly oviposition. Antibiotic treatment, carbenicillin 100 µg/mL + ciprofloxazin 5 µg/mL significantly ($p<0.05$) reduced the oviposition and % egg hatchability of whiteflies reared in coconut (13 eggs/spiral and 61.54%), banana (15 eggs/spiral and 60.00%), sapota (15 eggs/spiral and 66.67%) and guava (16 eggs/spiral and 56.25%). The reduction in the number of eggs per spiral and hatchability percentage proved that antibiotic treatments significantly ($P<0.05$) reduced rugose spiralling whitefly fecundity. Antibiotic material affects the fitness parameters of whitefly by disrupting the endomicrobial communities associated with whitefly. Antibacterial materials have a potential plant protection role in the management of whiteflies by reducing population growth.

Keywords: *Aleurodicus rugioperculatus*, Antibiotics, Fecundity, Hatchability, Host plants

INTRODUCTION

In India, Rugose Spiralling Whitefly (RSW) *Aleurodicus rugioperculatus* incidence on coconut plantations was first noted in the Pollachi area (Tamil Nadu) in July 2016, and its damage was also observed in coconut (40-60%) and banana (25-40%) leaves (Selvaraj *et al.*, 2017). RSW produces copious amounts of honeydew, which cover the leaflets and influences sooty mould growth, affecting the photosynthesis of the plant (Stocks and Hodges, 2012). RSW adults were larger in size, lethargic in nature and had a white powdery mass covering the insect's body. Three triangular brown spots were observed on both wings, and long pincer-like structures were found in the caudal abdomen of the male adults. Adults lay whitish-yellow eggs in a spiral manner on the lower surface of the leaves (Stocks and Hodges, 2012).

The rugose spiralling whitefly is a polyphagous, more destructive pest and has a wide host range to infest more than 120 plant species. A severe outburst of RSW on coconut palms, mango and guava, occurred in the Kottayam districts of Kerala (Shanas *et al.*, 2016). In other parts of India, the invasion of RSW is recorded on sugarcane in Andhra Pradesh (Bhavani, 2020), coconut in Chhattisgarh (Patel *et al.*, 2020) and different host plants in Gujarat (Jethva *et al.*, 2020). Among the different host plants, the intensity of RSW infestation was more severe on four host plants, namely, coconut, banana, sapota and guava, which were selected for the present study. Pesticide-based management strategies failed to control RSW due to higher dispersal, fecundity and morphological features.

The functional role of endosymbionts associated with whiteflies has not been well explored, and their role in insects was identified by the elimination of endosymbionts through antibiotic treatments, which significantly affected the fitness parameters of whiteflies (Raina *et al.*, 2015). Antibiotic materials with diverse mechanisms of action alter the endosymbiont communities harboured in whiteflies (Costa *et al.*, 1997). Growth and offspring emergence from adults and enzymes for the synthesis of trehalose present in honeydew secretion were affected using antibiotics for the management of whiteflies (Davidson *et al.*, 1994). Hence, the present study evaluated the effect of antibiotics against oviposition for the sustainable management of rugose spiralling whiteflies.

MATERIALS AND METHODS

Mass culturing of rugose spiralling whitefly

Initially, stock cultures of whiteflies were collected from the Tamil Nadu Agricultural University (TNAU) orchard (11.0123° N, 76.9355° E), Coimbatore (India) and inoculated into potted (41 cm diameter) plants of coconut (Chowghat Orange Dwarf), banana (Ney Poovan), sapota (CO-2), and guava (L-49) maintained in an individual mini nylon net house structure (270 × 150 × 210 cm; mesh size 120 micron) at the Insectary, TNAU at 31 ± 2 °C, 60-75% RH under natural light conditions.

Cultivable gut bacterial isolation

Fifty numbers of nymphal stages were collected from all four host plants and starved for 24 h. Then, the nymphs were surface sterilized with 70% ethanol followed by 5% sodium hypochlorite for 1 min and washed with sterile water 3–5 times to remove adhering contaminants. Whitefly nymphs were homogenized with 0.1 M phosphate buffer (pH 7.0) and serially diluted in sterile distilled water and 0.1 mL of was plated on seven different bacterial growth media, *viz.*, nutrient agar, Luria Bertani, MacConkey agar, tryptic soy agar, endo agar, Reasoner's 2A agar, and MRS agar (M/s. HiMedia Laboratories, Mumbai, India). Petri plates containing insect homogenates were incubated for 48 h at 28±2 °C and monitored for the formation of new colonies at an interval of 24 h. The bacterial colonies were selected based on their colony morphology and subjected to genomic DNA isolation using HipurA® Bacterial Genomic DNA Purification Kit (M/s. HiMedia Laboratories, Mumbai, India). Sanger sequencing was performed at the M/s. Barcode Biosciences, Bengaluru, India and then aligned sequences submitted in NCBI database (Saranya *et al*., 2022).

Effect of antibiotics on RSW oviposition

The efficacy of the antibiotic treatments mentioned in Table 1 was assayed on RSW. The antibiotics were sprayed on coconut, banana, sapota and guava, which were placed in an individual net structure. Clip cages with pairs of whiteflies were placed on antibiotic-treated leaves for RSW feeding and oviposition for 48 h. RSW adults were removed after 48 h of oviposition, and the number of eggs per spiral and percent egg hatchability of RSW were observed (Insecticide Resistance Action Committee, 2009- Whiteflies Susceptibility Test Method No. 016).

Statistical analysis

Data were analyzed using IBM SPSS Statistics 22 (SPSS, 2013) for analysis of variance and comparison through a general linear model (GLM) with Tukey's honest significant difference test.

RESULTS AND DISCUSSION

Preliminarily, nineteen antibiotics tested were against cultivable gut bacterial isolates of RSW with their accession numbers are mentioned in Table 2. Among these, erythromycin E15, ciprofloxacin CIP5, carbenicillin CB100 and cefotaxime CTX 30 antibiotics were selected based on their inhibition zone and varied mode of action towards bacterial isolates of RSW. The same were tested for the ovicidal action of RSW.

The antibiotic treatments indicated in Table 1 were tested for oviposition and percent egg hatchability of RSW. Significant ($P<0.05$) reductions in the number of eggs per spiral and percent egg hatchability of RSW were recorded in the antibiotic treatments CB^{100} + CIP^{5} followed by CIP^{5}+ CTX^{30} and CB^{100} when compared to the control in all host plants. CB^{100} + CIP^{5} significantly reduced the RSW oviposition and egg hatchability percentage of RSW reared in coconut (13 eggs/spiral and 61.54%), banana (15 eggs/spiral and 60.00%), sapota (15 eggs/spiral and 66.67%) and guava (16 eggs/spiral and 56.25%).

In the current study, majorly *Bacillus* genera were found in the RSW nymphal stage. Similarly, Davidson *et al*. (2000) reported that *Bacillus* and *Staphylococcus* in the whitefly *Bemisia argentifolii* produce medium-length sugars from derived sucrose and increase the stickiness of honeydew secreted by homopteran insects. *Bacillus* genera associated with RSW may be

Table 1. Effect of antibiotics on rugose spiralling whitefly, *Aleurodicus rugioperculatus* oviposition

S. No	Antibiotics	No of eggs/Spiral (Mean ± SE)				Egg hatchability (%) (Mean ± SE)			
		Coconut	Banana	Sapota	Guava	Coconut	Banana	Sapota	Guava
1	CB^{100}	16.00 ± 0.04^{bc}	18.00 ± 0.12^{b}	19.00 ± 0.19^{bc}	17.00 ± 0.13^{ab}	81.25 ± 0.90^{b}	61.11 ± 0.56^{a}	68.42 ± 0.37^{ab}	64.71 ± 0.78^{b}
2	CIP^{5}	18.00 ± 0.09^{de}	20.00 ± 0.45^{c}	21.00 ± 0.48^{de}	18.00 ± 0.07^{b}	88.89 ± 0.51^{c}	75.00 ± 1.80^{b}	66.67 ± 0.87^{a}	83.33 ± 0.18^{d}
3	E^{15}	20.00 ± 0.17^{fg}	23.00 ± 0.43^{d}	22.00 ± 0.30^{ef}	22.00 ± 0.22^{d}	95.00 ± 2.60^{cd}	86.96 ± 2.08^{cd}	90.91 ± 1.89^{def}	90.91 ± 1.30^{f}
4	CTX^{30}	20.00 ± 0.17^{fg}	22.00 ± 0.10^{d}	23.00 ± 0.07^{fg}	20.00 ± 0.39^{c}	90.00 ± 2.70^{c}	77.27 ± 0.24^{b}	82.61 ± 0.33^{c}	85.00 ± 0.66^{de}
5	CB^{100} + CIP^{5}	13.00 ± 0.04^{a}	15.00 ± 0.38^{a}	15.00 ± 0.25^{a}	16.00 ± 0.12^{a}	61.54 ± 1.92^{a}	60.00 ± 0.69^{a}	66.67 ± 0.56^{a}	56.25 ± 0.95^{a}
6	CB^{100} + E^{15}	16.00 ± 0.13^{bc}	18.00 ± 0.25^{b}	18.00 ± 0.22^{b}	16.00 ± 0.12^{a}	93.75 ± 2.69^{cd}	94.44 ± 2.36^{de}	72.22 ± 0.41^{b}	93.75 ± 1.28^{fg}
7	CB^{100} + CTX^{30}	17.00 ± 0.07^{cd}	20.00 ± 0.07^{c}	20.00 ± 0.43^{cd}	18.00 ± 0.23^{b}	94.12 ± 2.82^{cd}	75.00 ± 1.76^{b}	90.00 ± 0.42^{de}	83.33 ± 0.40^{d}
8	CIP^{5}+ E^{15}	19.00 ± 0.47^{ef}	22.00 ± 0.06^{d}	21.00 ± 0.01^{de}	21.00 ± 0.39^{cd}	94.74 ± 2.96^{cd}	86.36 ± 2.16^{c}	95.24 ± 1.19^{f}	90.48 ± 1.07^{cef}
9	CIP^{5}+ CTX^{30}	15.00 ± 0.26^{b}	17.00 ± 0.33^{b}	19.00 ± 0.18^{bc}	18.00 ± 0.19^{b}	80.00 ± 1.38^{b}	76.47 ± 1.95^{b}	94.74 ± 0.59^{f}	72.22 ± 0.32^{c}
10	E^{15}+ CTX^{30}	21.00 ± 0.40^{g}	23.00 ± 0.17^{d}	24.00 ± 0.12^{g}	21.00 ± 0.22^{cd}	90.48 ± 2.13^{cd}	91.30 ± 1.05^{cde}	87.50 ± 1.05^{d}	80.95 ± 0.18^{d}
11	Control	29.00 ± 0.27^{h}	27.00 ± 0.11^{e}	31.00 ± 0.34^{h}	26.00 ± 0.53^{e}	100.00 ± 0.29^{d}	100.00 ± 0.76^{e}	93.55 ± 0.83^{ef}	100.00 ± 0.65^{g}

Values with the same letters in the last column do not differ significantly according to Tukey's HSD test at the 5% level of significance. Values in each column are the mean of 3 replications ± standard error (SE); CB^{100}- Carbenicillin 100 mcg CIP^{5}- Ciprofloxacin 5 mcg CTX^{30}- Cefotaxime 30 mcg E^{15}- Erythromycin 15 mcg

responsible to produce copious amounts of honeydew which favours sooty mould growth. Among tested, a combination of antibiotics CB^{100} + CIP^{5} with a different mode of action than single antibiotics effectively disrupts the endomicrobial communities of whitefly, which cause a nutritional deficiency in the host and direct influence whitefly oviposition. And also affect symbionts responsible for the honeydew secretion and enzyme synthesis. The present study gain supports from the studies of bio efficacy of antibiotics based on the type of antibiotics, endosymbionts and whitefly biotypes. Antimicrobial materials with a different mechanism of action influence the endomicrobial communities harboured in whiteflies (Costa *et al.*, 1997; Ahmed *et al.*, 2010). Carbenicillin and cefotaxime negatively disrupt the components of bacterial cell wall peptidoglycan by deactivating the transpeptidase enzyme (Butler *et al.*, 1970; Murray and Moellering Jr, 1981). Ciprofloxacin inhibits DNA synthesis by inactivating the secretion of the DNA gyrase enzyme (Zweerink and Edison, 1986). Erythromycin disrupts protein synthesis (Brock and Brock, 1959).

And also per cent egg hatchability of RSW was reduced in guava followed by banana, coconut and sapota. This might be due to the food quality, food quantity, temperature, host plants and biotype influence the developmental rate and reproduction of the host insect (Samih *et al.*, 2014). Host plant characteristics include physical (waxy covering, fibrous lamina, trichomes on foliage) and biochemical substances that influence the growth period of the rugosa whitefly (Pradhan *et al.*, 2020). In the present study, combinations of CB^{100} + CIP^{5} treatment eliminated secondary endosymbionts associated with rugose spiralling whitefly, which negatively affected the rugose spiralling whitefly oviposition. Similar reports from Pais *et al*. (2008) found that rifampicin and oxytetracycline removed endosymbionts and decreased the fecundity of tsetse flies. Shan *et al*., (2016) observed that curing of secondary symbionts through antibiotics on *B. tabaci* may cause negative effects on insects. Xue *et al*. (2012) and Zhao *et al*. (2020) reported that rifampicin and oxytetracycline treatments reduced the development of immature *B. tabaci* .

Conclusion

In conclusion, among the tested combinations of various antibiotics, CB^{100} + CIP^{5} antibiotic treatment significantly ($P<0.05$) influenced RSW oviposition by indirectly eliminating endosymbionts associated with *A. rugioperculatus*. Antibiotic-based materials negatively influence the RSW fecundity. Botanicals which possess antimicrobial properties can be used as biopesticides in the integrated pest control aspect for the sustainable management of whiteflies.

Table 2. Cultivable bacterial isolates of rugose spiralling whitefly, *Aleurodicus rugioperculatus*

S.No.	Bacterial Isolate	NCBI Accession No
1.	*Bacillus licheniformis* SCRSW1	MN782273
2.	*Exiguobacterium mexicanum* SCRSW2	MN782274
3.	*Acinetobacter refrigeratoris* SCRSW5	MN907646
4.	*Bacillus manliponensis* SCRSW7	MN782275
5.	*Bacillus velezensis* SCRSW8	MN782276
6.	*Bacillus zanthoxyli* SCRSW10	MN782277
7.	*Bacillus albus* SCRSW11	MN782278
8.	*Bacillus zanthoxyli* SCRSW13	MN782279
9.	*Bacillus altitudinis* SCRSW14	MN782280
10.	*Bacillus aryabhattai* SCRSW16	MN907647
11.	*Lysinibacillus xylanilyticus* SBRSW13	MN907653
12.	*Arthrobacter nitrophenolicus* SBRSW21	MN907660
13.	*Bacillus subtilis* subsp. *stercoris* SBRSW19	MN907658
14.	*Pseudomonas stutzeri* SBRSW22	MT027239
15.	*Bacillus tequilensis* SBRSW24	MN907664
16.	*Bacillus siamensis* SBRSW28	MN907667

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Conflict of interest

The authors declare that they have no conflict of interest.

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