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Ovicidal and insecticidal effects of microbial pathogens against invasive Coconut Rugose spiralling whitefly (RSW), *Aleurodicus rugioperculatus* Martin and impact on their chrysopid predator *Mallada boninensis* (Okamoto)

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Abstract

Background: The Rugose spiraling whitefly (RSW), *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae), has been reported as an invasive pest of coconut in different parts of peninsular India during 2016. It mainly caused damage to coconut palms, bananas, custard apples and other broad-leaved plants as hosts. Presently it is spread all over India and the coconut growing farmers are facing hard ships due to this infestation of RSW in coconut ecosystem. Still now the management of this invasive pest is fully based on the biological control, particularly with predators and parasitoids and the study on the microbial control of RSW was minimum. Hence, an attempt was made to study the ovicidal and insecticidal effects of microbial pathogens, viz. *Isaria fumosorosea* Wize, *Beauveria bassiana* (Balsamo) Vuillemi, *Metarhizium anisopliae* (Metschnikoff), *Lecanicillium lecanii* (Zimmerm.) with a spore load of 1×10^8 cfu against different life stages of RSW and their chrysopid predator, *Mallada boninensis* (Okamoto)(Neuroptera: Chrysopidae) under laboratory as well as field conditions.

Results: The results revealed that under laboratory conditions, *I. fumosorosea* caused 34.54% egg mortality, 37.39% nymphal mortality and 48.30% adult mortality, followed by *L. lecanii* (24.54, 30.76 and 28.01%) and *M. anisopliae* (20.56, 32.51 and 42.92%) for egg, nymphal and adult mortality, respectively. Under field conditions, *I. fumosorosea* caused (29.60%), followed by *M. anisopliae* (24.30%) and *B. bassiana* (21.00%) nymphal mortality, respectively, at 15 days after spray, also, it was found effective in reducing the RSW population on coconut. The impact of fungal entomopathogens against the predator revealed that *I. fumosorosea* treated eggs of *M. boninensis* recorded maximum 90.33% grub emergence than other treatments.

Conclusion: The present study concludes that the RSW can be controlled effectively by *I. fumosorosea* along with the chrysopid predator, *M. boninensis* under field conditions as they showed maximum ovicidal and insecticidal actions

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against different life stages of RSW and safer to the major predator of RSW and fall under harmless category as per the classification of IOBC.

Keywords: Coconut, *Aleurodicus rugioperculatus*, Biological control, Microbials, Predator

Background

Rugose spiralling whitefly (RSW), *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae) is an exotic pest of coconut and first reported by Shanas et al. (2016) from Kerala, India. Further, it has also been reported from peninsular India, viz. Tamil Nadu, Karnataka and Andhra Pradesh (Elango et al. 2019). RSW is a polyphagous insect which is likely to expand its host range and mainly infests the coconut palms and other broad-leaved hosts in its native range (Elango and Nelson 2020). RSW penetrates its stylet in the phloem and consume massive amounts of sap that seems to be high in sugar. It excretes the additional liquids/sugar (Honeydew). Honeydew accumulates on leaves and tender coconut, promoting the development of black sooty mold and the premature fall of leaves. As well, these molds have an effect on the rate of photosynthesis and transpiration, which impedes light penetration, vapor movement and exchange as a result plants reveal yellowish spots on leaves, wilt or die (Taravati and Mannion 2016). This sucking pest is polyphagous, having a high reproductive potential, short life cycle and positively impacted on global food production immensely (Boopathi et al. 2022). As the pest was introduced and reported in India, no effective chemical insecticides and biopesticides exist to deal with this devastating sap sucking insect. Presently it is spread all over India and the coconut growing farmers are facing hard ships due to the infestation of RSW in coconut ecosystem. Up till now, the management of this invasive pest is fully based on the biological control, particularly with indigenous natural enemies and the study on the microbial control of RSW was minimum. Hence, an attempt was made to study the ovicidal and insecticidal effects of microbial fungal pathogens against different life stages of RSW and their chrysopid predator, *M. boninensis* under laboratory and field conditions.

Methods

Insect culture

Different developmental stages of the RSW, *A. rugioperculatus* such as eggs, first (crawlers), second, third, fourth nymphal instars (pseudo pupae) and adults were collected from infested coconut trees (dwarf variety: Chowghat orange) present in orchard maintained under pesticide-free environment in the Kumaraguru Institute of Agriculture, Tamil Nadu Agricultural University, Erode (11.49°N, 77.55°E) and the chrysopid predator, *M. boninensis* egg culture was obtained from biological control laboratory, Tamil Nadu Agricultural University, Coimbatore.

Fungal isolates and culture maintenance

To evaluate the pathogenicity of EPF against *A. rugioperculatus,* the fungal cultures, viz. *Isaria fumosorosea* Wize, *Beauveria bassiana* (Balsamo) Vuillemi, *Metarhizium anisopliae* (Metschnikoff), *Lecanicillium lecanii* (Zimmerm.) with a spore load of (1×10^8 cfu) was obtained from the National Bureau of Agricultural Insect resources (NBAIR), Bangalore. Isolates were maintained in culture on potato dextrose agar (PDA) slants in universal bottles (30 ml) and stored at 4 °C. Continuous cultures were maintained on slants with subcultures grown for 14 days at 25 °C, following which lids were tightly sealed and cultures stored at 4 °C. Spore suspension was prepared based on the procedure given by Sumalatha et al. (2020)

Ovicidal effect of entomopathogenic fungi on *A. rugioperculatus*

Bioassays were carried out to evaluate the ovicidal effect of EPF, viz. *I. fumosorosea*, *B. bassiana*, *M. anisopliae* and *L. lecanii* with a spore load of 1×10^8 cfu against eggs of RSW under laboratory condition at 25 ± 1 °C, 65 ± 5% RH and 10:14 light: dark regime. EPF were assayed using direct spray bioassay method to evaluate ovicidal effect on RSW eggs as per the method described by Thangavel et al. (2013). Uniform age of *A. rugioperculatus* eggs present in an egg spirals along with 5 cm leaf bit was taken from coconut saplings maintained in the greenhouse culture. The number of eggs presented in each egg spiral was counted with the help of the Leica M205C stereo zoom microscope. The leaf bits were placed on 1.5% agar in a Petri dish (9 cm dia.) to maintain the turgidity of the coconut leaves. EPF treatments were prepared and sprayed with the help of an atomizer over the eggs of *A. rugioperculatus* with five replications in completely randomized design as per the recommended concentration (1×10^8 cfu) (Table 1). All the treated Petri dishes were maintained at 25 ± 1 °C in an incubator and hatchability was recorded every 24-h interval. Later, the eggs were individually examined under a stereo zoom binocular microscope (Carl Zeiss Stemi 2000) at 40 × magnification for verification of fungal infection. Finally, all unhatched eggs were transferred to moist chambers for three days

Table 1 Treatment details

Treatments	Entomopathogens	Concentration	Recommended dose (ml/lit)
T_1	*Isaria fumosorosea*	1×10^8 cfu	5
T_2	*Beauveria bassiana*	1×10^8 cfu	5
T_3	*Metarhizium anisopliae*	1×10^8 cfu	5
T_4	*Lecanicillium lecanii*	1×10^8 cfu	5
T_5	Control (Water)	–	-

to observe fungal outgrowth if any, as an evidence of egg mortality due to fungal infection. Observations were made up to seven days after treatment. The experiments were repeated for three times to confirm the ovicidal action of EPF against RSW eggs. The mortality data were subjected to Abbott's formula (Abbott 1925) for calculating corrected mortality.

Insecticidal effect of entomopathogenic fungi on nymphs and adults of *A. rugioperculatus*

Strains of different EPF were assayed against RSW nymphs and adults by direct spray method in completely randomized design (CRD) (Thangavel et al. 2013). Leaf bits containing 30 nymphs in early and late instars of RSW were placed in a (9 cm diameter) Petri dish containing 1.5% agar and the adults of RSW was collected with the help of an aspirator from the greenhouse culture. EPF was sprayed with the help of an atomizer over the nymphs and adults of *A. rugioperculatus* with five replications. All the treated Petri dishes were maintained at 25 ± 1 °C in an incubator. The nymphs and adults were individually examined under a stereo zoom binocular microscope (Carl Zeiss Stemi 2000) at 40 × magnification for verification of fungal infection. The mortality rates were recorded by counting the dead cadavers, nymphs and adults with fungal spores. Observations on the mortality of RSW by EPF were made at 3, 7 10 and 15 days after treatment (DAT). The mortality data were corrected using Abbott's formula.

Effect of entomopathogens against nymphs of *A. rugioperculatus* under field condition

The effect of EPF against nymphs of RSW was studied in the field of coconut orchard located at Kumaraguru Institute of Agriculture, Erode. Coconut palms (three years old) of the dwarf variety (Chowghat orange) were chosen for the experiment so that observations could be made easily. Five palms per treatment with at least 5–7 infested fronds were selected for each treatment. A battery-operated Aspee Knapsack, a high volume sprayer with a 16-L capacity, was used to spray EPF as per recommended dosage (Table 1) at 15-day intervals. Observations on the RSW nymphal population were taken from 10 leaflets per fronds per palm as pre-treatment count and 3, 7, 10 and 15 days after spray, respectively.

Effect of entomopathogens on the emergence potential of chrysopid predator, *M. boninensis*

Strains of different EPF were assayed on the emergence potential of important predator green lacewing, *M. boninensis* of RSW. The effect of treatments on the eggs of the predator was assessed as per the method described by Krishnamoorthy (1985). The stalked eggs of *M. boninensis* on brown paper stripes were procured from biological control laboratory, Tamil Nadu Agricultural University, Coimbatore. Thirty healthy eggs of *M. boninensis* per replication were selected and kept in the Petri dishes (9 cm dia.). EPF were sprayed with respective treatment solution using a hand atomizer. Each treatment was replicated five times and an untreated check was also maintained by spraying distilled water. The number of grubs hatching from each treatment after spray at 24, 48 and 72 h was recorded and percent hatchability was worked out with below given formula:

$$\text{Per cent hatchability} = \frac{\text{Number of grubs hatched}}{\text{Total number of eggs}} \times 100$$

Statistical analysis

Statistical analysis was done in completely randomized design. The percentages of mortality in eggs, nymphs and adults were recorded and corrected with that in control by using Abbott's formula as follows:

$$P = [C - T/C] \times 100$$

where P = estimated percentage of insects killed by fungus alone, C = percentage of control insects living, and T = percentage of treated insects that are living after the experimentation period.

The data collected under laboratory experiments in completely randomized design were analyzed using analysis of variance (ANOVA) using AGRES 3.01 and AGDATA software. Data in the form of percentages were transformed to arcsine values and those in numbers were transformed to $\sqrt{x} + 0.5$ and analyzed. The mean values of the treatments were compared using DMRT at 5% level of significance**.** The data gathered in the field experiments were analyzed by randomized block design using AGRES 3.01 and AGDATA software. The population of insect pests were subjected to square root transformations (Snedcor and Cochran 1967). Duncan multiple range test (DMRT) was applied for comparing treatment means at 5% level of significance (Duncan 1955).

Results

Ovicidal effect of entomopathogenic fungi on *A. rugioperculatus* eggs

Entomopathogenic fungi were assayed using the direct spray method to evaluate ovicidal effect on *A. rugioperculatus* eggs. The results revealed that RSW egg chorion showed no change for three consecutive days after treatment, but from the fourth day onwards, changes were detected and mortality was observed. Among the EPF, *I. fumosorosea* caused 34.54% egg mortality at 7 Days after treatment (DAT), followed by *L. lecanii* (24.54%) and *M. anisopliae* (20.56%) (Fig. 1). Very minimum ovicidal effect was recorded at 7 DAT in case of *B. bassiana* (16.58%). The egg hatchability was suppressed by all the EPF to some extent. *I. fumosorosea* produced minimum egg hatchability (64.56%), whereas in untreated check 100% RSW egg hatchability was observed at 7 DAT as compared to other fungi.

Insecticidal effect of entomopathogenic fungi against nymphs of *A. rugioperculatus*

Entomopathogenic fungi were assayed using direct spray method to evaluate insecticidal effect on *A. rugioperculatus* nymphs. As a result, nymphal mortality was minimum during the early days but elevated in the subsequent days. Among the treatments, *I. fumosorosea* caused (37.39%) mean nymphal mortality, followed by *M. anisopliae* (32.51%), *L. lecanii* (30.76%) at 15 DAT, respectively. Among the treatments, nymphal mortality was very low in *B. bassiana* (28.66%) (Fig. 2). Due to nymphal mortality, especially in fourth-instar nymphs (pseudo pupae), this caused a significant reduction in adult emergence and continuation of RSW populations. *I. fumosorosea* treated nymphs caused considerable mycosis and distortions in newly emerging adults, which drastically reduced adult emergence and may contribute to reducing the pest's presence in coconut palms.

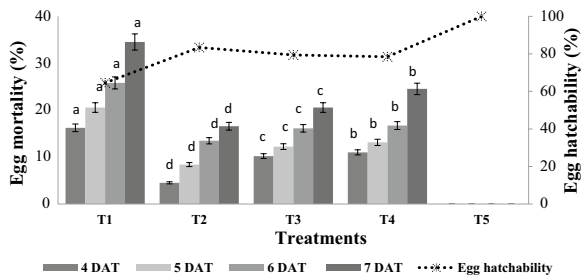

Fig. 1 Ovicidal effect of entomopathogenic fungi on *Aleurodicus rugioperculatus* eggs

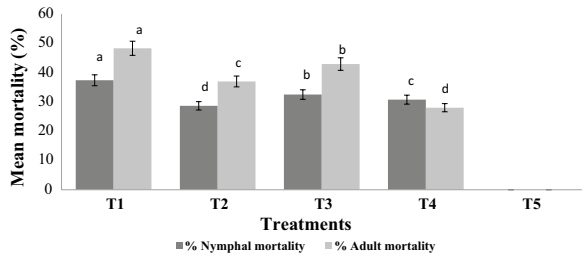

Fig. 2 Pathogenicity of entomopathogenic fungi against nymphs and adults of *Aleurodicus rugioperculatus* under laboratory condition

Pathogenic effect of entomopathogenic fungi against adults of *A. rugioperculatus*

All EPF caused high rates of pathogenicity among *A. rugioperculatus* population. *I. fumosorosea* caused (48.30%) adult mortality rate compared to other EPF isolates and the next higher adult mean mortality was by *M. anisopliae* (42.92%) and *B. bassiana* (36.98%), respectively. Minimum pathogenic effect was observed in case of *L. lecanii* (28.01%) at 15 days after treatment (Fig. 2).

Entomopathogenic fungi against *A. rugioperculatus* under field condition

The effect of EPF was tested against *A. rugioperculatus* under field conditions. In overall statistics, significant differences in the pathogenicity of EPF were observed among the treatments. The results revealed that among the treatments, *I. fumosorosea* caused (29.60%) nymphal, followed by *M. anisopliae* (24.30%) and *B. bassiana* (21.00%) (Fig. 3). Minimum fungal pathogenic effect was observed in case of *L. lecanii* (19.50%) at 15 days after spray under field conditions.

Effect of entomopathogens on the emergence potential of *M. boninensis*

The recommended concentrations of EPF were diluted in the water to prepare different treatments solution. Freshly laid eggs of *M. boninensis* were kept in Petriplate and the treatments were sprayed as topical application

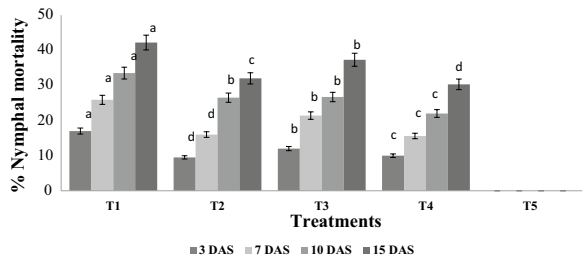

Fig. 3 Entomopathogenic fungi against nymphs of *Aleurodicus rugioperculatus* under field condition

with help of an atomizer and treated eggs were kept for grub emergence. Every 24 h the emergence was observed and 3 days after treatment the tiny grubs (First instar) of *M. Boninensis* was emerged in control (Untreated) pedicellate eggs as well as in treated eggs. The emergence percentage was varying in different treatments. Among the treatments, maximum emergence percentage was observed in untreated eggs (97.45%), followed by *I. fumosorosea* (90.33%) and recorded minimum (9.67%) ovicidal effect on *M. boninensis* eggs at 72 HAT (Fig. 4). The other EPF also recorded the hatchability percentage from 78.88 to 89.65%, while the mortality range was minimum (10.35–21.12%). Hence, the EPF are safer to major natural enemy of RSW and fall under harmless category as per International Organization for Biological Control (IOBC) classification (Harmless < 50%, slightly harmful (50–79%), moderately harmful (80–99%) and harmful > 99%).

Discussion

Entomopathogenic fungi (EPF) are identified as promising biocontrol agents for regulation of invasive insect pests' population without harming the natural enemies. In the present findings, under laboratory condition, *I. fumosorosea* caused (34.54%) egg mortality rate, (37.39%) nymphal mortality rate, (48.30%) egg nymphal adult mortality rate. The next maximum means of mortality rates were recorded as: *L. lecanii* (24.54, 30.76 and 28.01%) and *M. anisopliae* (20.56, 32.51 and 42.92%) egg, nymphal and adult mortality rates, respectively. Under field conditions, *I. fumosorosea* caused (29.60%) followed by *M. anisopliae* (24.30%) and *B. bassiana* (21.00%) nymphal mortality rate, at 15 days after spray in reducing the RSW population on coconut. The present findings agreement with Sumalatha et al. (2020) who studied the effect in different strains of EPF, *I. fumosorosea* against RSW, among the strains *I. fumosorosea* (Pfu-5) showed virulence and maximum mean mortality rates of (44.03, 44.80, 36.42 and 28.82%) on the eggs, first, third and fourth nymphal instars, respectively. Similarly, Sandeep et al. (2022) stated that RSW can be controlled effectively by *I. fumosorosea* alone and in combination with novel insecticides at a reduced rate, which showed better toxicity, ovicidal action and preserve natural enemies and reduced environmental load of chemical pesticides. *I. fumosorosea* is one of the most promising and extensively studied EPF attacking nymphs and adults of whiteflies such as *Bemisia* spp. and *Aleurodicus disperses* (Hemiptera: Aleyrodidae) and *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) (Lacey et al. 2008); *A. rugioperculatus* and *Paraleyrodes bondari* Peracchi (Hemiptera: Aleyrodidae) on coconut (Ali et al. 2015). Effectiveness of EPF against various species of whiteflies has been documented in several previous reports. Boopathi et al. (2013) reported that (37.3 and 22.6%) of egg mortality with *M. anisopliae* (M2 Strain) and *P. fumosoroseus* Wize (Hypocreales: Cordycipitaceae), (P1 strain), respectively at 8 DAT. The egg mortality is one of the important attributes of EPF by which pest is suppressed at initial stage. So that subsequent pest population build up and crop damage is reduced. Mallappanavar (2000) reported that the most effective pathogens against *A. dispersus* as *L. lecanii* at 1.33×10^7 conidia/ml. Boopathi et al. (2015) reported that *B. bassiana* had a comparatively higher efficacy against *A. dispersus* under laboratory conditions than *M. anisopliae*. The results of present study suggested the EPF as potential biopesticide against *A. rugioperculatus* with very slight negative effects on beneficial predator, *M. boninensis*. The compatibility of two different types of biological control agent is very important for sustained and successful pest management. Sumalatha et al. (2020) also reported that *I. fumosorosea* is safer to the natural enemies of RSW. Mingotti Dias et al. (2020) tested the EPF, viz. *B. bassiana*, *M. anisopliae* and *M. rileyi* on the larvae of *Chrysoperla externa* (Neuroptera: Chrysopidae) and reported that these three EPF may be used in association with *C. externa* for sustainable sucking pests' management.

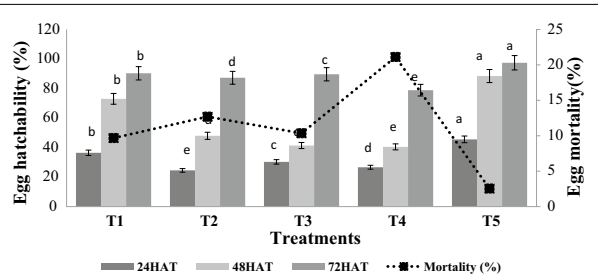

Fig. 4 Effect of entomopathogens on the emergence potential of *Mallada boninensis* Okamoto

Conclusion

The present findings conclude that among the EPF tested against RSW, *I. fumosorosea* showed the maximum egg, nymphal and adult mortality potential under both laboratory and field conditions with a very slight effect on the beneficial predator, *M. boninensis*. Hence, EPF may be used as a tool for control of RSW along with natural enemies. The present research clearly demonstrates the existence of beneficial interactions between the fungal entomopathogens *I. fumosorosea* and the chrysopid predator, *M. boninensis*, these interactions may be used to successful management of RSW in the coconut eco system.

Abbreviations
RSW: Rugose spiraling whitefly; cfu: Colony forming units; IOBC: International Organization for Biological Control; °C: Degree centigrade; RH: Relative humidity; L:D: Light and dark period; NBAIR: National Bureau of Agricultural Insect resources; PDA: Potato dextrose agar; CRD: Completely randomized design; DAT: Days after treatment; DAS: Days after spray; ANOVA: Analysis of variance; PCA: Principal component analysis; %: Per cent; cm: Centimetre.

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Author contributions
KE and AK performed the idea of this article and wrote the manuscript. KE and VD performed the data analysis. All authors read and approved the final manuscript.

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Availability of data and materials
All presented data are original.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
I agree to publish this paper in the EJBC.

Competing interests
The authors declare that they have no competing interests.

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