

# Entomopathogenic fungus, *Isaria fumosorosea*: a Potential alternative Biocontrol Agent for Management of invasive whiteflies in Coconut

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Fig.1. Different formulations of *Isaria fumosorosea*

Coconut *Cocos nucifera* L. (Arecaceae) is an important plantation crop and millions of people depend directly or indirectly on this crop for their livelihood. India is one of the leaders in coconut cultivation and stands third in coconut production in the world. The coconut palm is attacked by several insect and pests all around the year from seedling stage to aged palm. Among, Eriophid mite, *Aceria guerreronis*, rhinoceros beetle, *Oryctes rhinoceros*, red palm weevil, *Rhynchophorus ferrugineus*, black headed caterpillar, *Opisina arenosella* and white-grub, *Leucopholis coneophora* are considered as the major pests of coconut. While the two whiteflies viz., areca nut whitefly, *Aleurocanthus arecae* and invasive spiralling whitefly, *Aleurodicus dispersus* recorded on coconut in India are considered as minor pests.

Since 2016, coconut ecosystem is facing continuous succession of several invasive whiteflies and poses great threat to its production and productivity. Between 2015-2019, the following four exotic, highly polyphagous whiteflies viz., rugose spiralling whitefly, *Aleurodicus rugioperculatus*; Bondar's nesting whitefly, *Paraleyrodes bondari*;

nesting whitefly, *Paraleyrodes minei* and palm infesting whitefly, *Aleurotrachelus atratus* invaded rapid succession on coconut (Sundararaj et al., 2021). The most insidious spread of these species in India is likely mediated by humans through the movement of infested seedlings and planting materials. Extensive surveys have revealed that these species spread rapidly in the large geographical region of India mostly through transportation of infested seedlings. Extensive spread along the coastal regions and gardens near the backwater of India is predicted owing to the favourable weather factors and availability of host plants.

Nymphs and adults of these exotic whiteflies feeds aggressively on leaf sap results in depletion of nutrients and water which leads to premature leaf drop and it produces wax and sticky honeydew on infested areas leading to extreme growth of black sooty mold which results in the reduction of photosynthetic efficiency of affected palms. These invasive species have now become regular pests of coconut and active throughout years, warranting control measures to avoid crop losses. Species of

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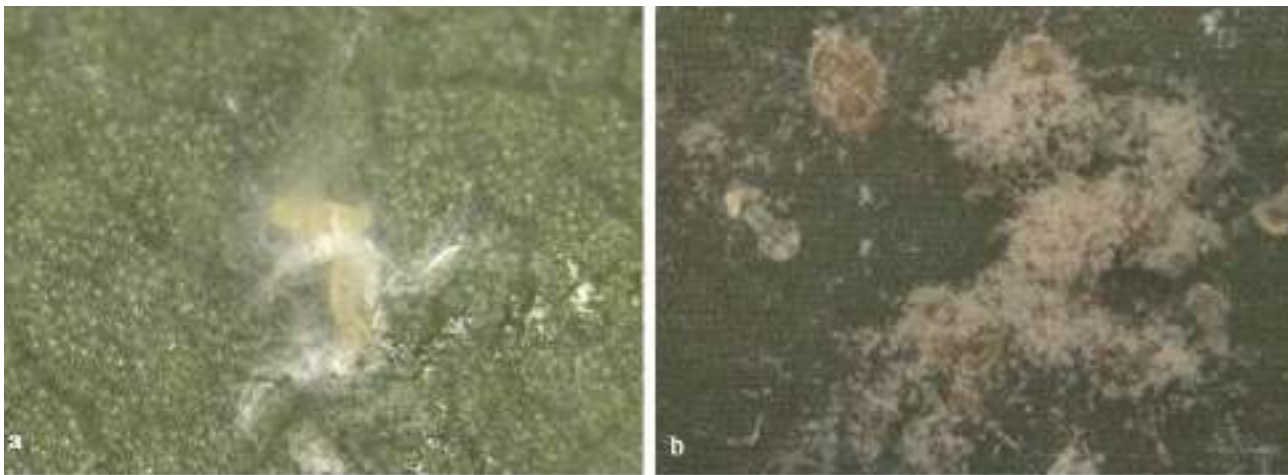


Fig.2. *Isaria fumosorosea* infection on eggs (A) and fourth nymphal instar (B) of RSW

exotic whiteflies with similar habits co-exist in more or less the same niche on coconut and have a similar pattern of growth and development.

Alarmed by the invasion of a pest unknown to them, farmers resorted to spraying of chemical pesticides to control RSW. But the efforts were in vain as the chemicals turned out to be a temporary fix and moreover, other ill effects like environmental pollution, killing of natural enemies and health risks to the people involved in spraying operations were also reported. Therefore, biological control is the most sustainable solution to tackle these invasive whiteflies. Explorations were carried out so that biological control of the pest could be accomplished through insect predators and parasitoids which are economically feasible, ecologically compatible and environmentally benign. Among natural enemies encountered, two aphelinid parasitoids, *Encarsia guadeloupae* and *E. dispersa* were found to have colonized the rugose spiralling whitefly and naturally suppressing the pest. *E. guadeloupae* emerged to be the dominant one among which recorded maximum natural parasitism of 56-82% while it was only 5-10% for *E. dispersa* (Selvaraj *et al.*, 2017). Apart from these, predators viz., *Dichochrysa astur*, *Jauravia pallidula*, *Cheilomenes sexmaculata* and *Cybocephalus sp.* were also observed to be feeding on rugose spiralling whitefly. In India, *E. guadeloupae* was well established by augmentation, re-distribution and various conservation strategies, no natural parasitism was observed on other invasive whitefly species except few generalist predators. Moreover, using a single biocontrol agent to suppress the whitefly population may be difficult under severe outbreak conditions especially in the case of invasive pests.

Keeping this in view, ICAR-NBAIR initiated the preliminary studies on evaluation of available entomopathogenic fungus available at microbial repository. The studies showered two strains of *Isaria fumosorosea* (ICAR-NBAIR Pfu-1) and (ICAR-NBAIR Pfu-5) high virulence against eggs, nymphs and adults of *A. rugioperculatus* life stages (Sumalatha *et al.*, 2019). This entomopathogenic fungus, *Isaria fumosorosea* is used worldwide as potential biocontrol agent against *A. rugioperculatus* and *Paraleyrodes bondari* on coconut (Ali *et al.*, 2015; Kumar *et al.*, 2016) in Florida. Further, Pfu-5, Pfu-6, Pfu-8 and Pfu-9 strains tested and evaluated against rugose spiraling whitefly, nesting whiteflies and palm infesting whitefly.

Considering the importance of these invasive pest complex in coconut, the project on "Exploration of entomopathogenic fungus *Isaria fumosorosea* for the management of emerging invasive whiteflies in coconut" was sanctioned by Coconut Development Board, Kochi with following objectives viz., to standardize liquid state fermentation technology for the mass production of *Isaria fumosorosea* to develop suitable formulations with longer shelf-life, persistence and higher bio-efficacy against the invasive whiteflies on coconut; to evaluate the bio-efficacy of *I. fumosorosea* against invasive whiteflies in coconut and to assess the impact of *I. fumosorosea* on non-target organisms (compatibility studies with predators, parasitoids and pollinators) and to conduct areawide demonstrations in collaboration with farmer producing organizations (FPOs) and Demonstration-cum-Seed Production (DSP) farms in Karnataka and Tamil Nadu.



Fig. 3. *Isaria fumosorosea* infection and growth on adults of RSW



### 1. Standardization of liquid state fermentation technology for the mass production of *Isaria fumosorosea*

The entomopathogenic fungus, *Isaria fumosorosea* strains viz., ICAR-NBAIR Pfu-5, Pfu-6, Pfu-8 and Pfu-9 were identified as a promising microbial bio control agent against rugose spiralling whitefly, palm infesting whitefly, nesting whiteflies of coconut. Evaluation of different liquid fermentation culture technology for mass production of *I. fumosorosea* with potato dextrose broth (PDB), molasses yeast extract broth (MYB), jaggery yeast extract broth (JYB) and Sabouraud dextrose yeast extract broth (SDYB) were carried out for growth and sporulation of *I. fumosorosea* (ICAR-NBAIR Pfu-5, Pfu-6, Pfu-8 and Pfu-9). Among these SDYB was found to be the best suitable media for mass production of *I. fumosorosea*.

### 2. Development of oil, rice and talc based formulations of *Isaria fumosorosea* for longer shelf-life, persistence and higher bioefficacy

- Based on the liquid fermentation technology, talc, rice grain and oil based formulations were standardized for *I. fumosorosea* (strains ICAR-NBAIR Pfu-5, Pfu-6, Pfu-8 and Pfu-9) with longer persistence under field conditions (Fig.1), shelf life and bio-efficacy. Shelf-life for about 90, 180, 210 days, talc, oil and rice grain based formulations, respectively.

- Viable spores of *Isaria fumosorosea* were persistent in the phyllosphere as well as in the field conditions up to three weeks after spray at Bengaluru conditions and re-isolated from spray leaves surface and soil rhizosphere. This study also indicates that the fungus grows optimally between 25-28°C in Karnataka region but tolerates up to 30°C.

- Under laboratory conditions, *Isaria fumosorosea* showed high virulence and effective against all the life stages of rugose spiralling whitefly including eggs and adults. Mortality was ranged from 35.2 to 99.6%, 48.8 to 91.8%, 36.9 to 86.1% and 28.6 to 80.6% on the eggs, first, third and fourth instars of RSW, respectively with  $1 \times 10^4$  -  $1 \times 10^8$  spores/ml at 5 days of treatment (Fig.2 &3).

- Isaria fumosorosea* strains (Pfu-6, Pfu-8 and Pfu-9) showed significantly less eggs mortality as compared to Pfu-5 and less virulence against nymphal stages as well. Moreover, these *I. fumosorosea* strains took slightly longer time to grow on RSW and kill them.

- Isaria fumosorosea* strain (Pfu-5) was found pathogenic to eggs and nymphs of Bondar's nesting whitefly, *Paraleyrodes bondari* (Fig.4). The nymphal stages were found to be more susceptible as compared to egg stage and 70-80% mortality was observed on 5<sup>th</sup> day and it was enhanced up to 95% on 7<sup>th</sup> days after treatment. The results revealed that Pfu-6, Pfu-8 and Pfu-9 were showed significantly lesser virulence as compared to Pfu-5 under laboratory conditions.

- Isaria fumosorosea* strains (Pfu-5) was found pathogenic to eggs and nymphs of nesting whitefly, *Paraleyrodes minei* (Fig.5). The nymphal stages were more susceptible to Pfu-5 as compared to egg. Nymphal mortality was about 70% mortality on 5<sup>th</sup> day and about 95% on 7<sup>th</sup> days after treatment under laboratory condition. The results revealed that Pfu-6, Pfu-8 and Pfu-9 were showed significantly lesser virulence as compared to Pfu-5.

- Similarly, *I. fumosorosea* strain (Pfu-5) was found pathogenic to eggs and nymphs of *Aleurotrachelus atratus* (Fig.6). The nymphal stages were found to

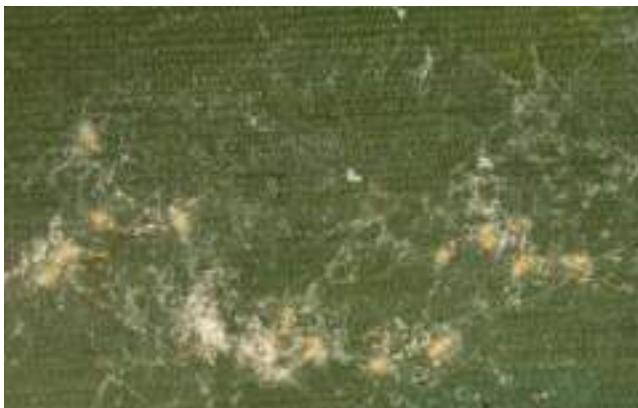


Fig. 4. *Isaria fumosorosea* infection on different stages of *Paraleyrodes bondari*



Fig. 5. *Isaria fumosorosea* infection on different stages of *Paraleyrodes minei*



be more susceptible as compared to egg stage and overall mortality 72-82% was observed on 7<sup>th</sup> days after treatment. Adult whitefly mortality was not observed during the study. The results revealed that Pfu-6, Pfu-8 and Pfu-9 showed significantly lesser virulence as compared to Pfu-5.

- Among the formulation, talc formulation was found to be less effective against different life stages of invasive whiteflies as compared to oil and rice formulation of *I. fumosorosea*. The effectiveness of rice and oil formulation was on par with respect to mortality among the formulations against all the invasive whiteflies and persists for longer time under field conditions.

### 3. Assessment of the impact of *Isaria fumosorosea* on non-target organisms (compatibility studies with predators, parasitoids and pollinators)

- Studies on non-target effects of *I. fumosorosea* on parasitoids, *Encarsia guadeloupae*, *Goniozus nephantidis*, predator, *Pseudomallada astur* and silk worm, *Bombyx mori* (Fig.6) revealed that no

infectivity was observed on different stages of *P. astur*, *G. nephantidis*, *B. mori* and insignificant effect (5-12% inhibition in parasitoid adult emergence). Therefore, these biocontrol agents can be integrated simultaneously for the management of invasive whiteflies in coconut.

### 4. Field evaluation of *Isaria fumosorosea* for management invasive whiteflies on coconut in Karnataka and Tamil Nadu

- Field evaluation of *I. fumosorosea* was carried in Karnataka (Fig.7) and Tamil Nadu (Fig.8) for management invasive whiteflies on coconut to determine the effectiveness under different locations. The results showed that *I. fumosorosea* reduced the overall population of RSW range from 78.57-80.10% as compared to untreated control (11.69-13.24%) after 28 days treatment across different location in Karnataka and it was 71.05-76.84%) in the treated palms as compared to untreated palms (11.67-13.81%) across other location in Tamil Nadu.

- In case of nesting whiteflies, maximum overall



Fig. 6. *Isaria fumosorosea* infection on different stages of *Aleurotrachelus atratus*



Fig. 6. Evaluation of *Isaria fumosorosea* on silkworm larvae under laboratory condition

reduction (67.57%) was significantly greater as compared to the untreated palms (5.50%) at 28 days after treatment. Similarly, the mortality of palm infesting whitefly was significantly higher (64.24%) in the treated palms as compared to untreated palms (5.52%) at 28 days after treatment on coconut in Karnataka.

- Effect of *Isaria fumosorosea* on natural enemies in coconut ecosystem under field conditions revealed that no significant variation in activity of predators, *P. astur* and parasitoids, *E. guadeloupae* and *G. nephantidis* in the *I. fumosorosea* (ICAR-NBAIR Pfu-5) treated palms and on par with untreated coconut palms. Further, there was no significant variation in visiting activity and behavior of honey bees observed in *I. fumosorosea* treated palms and *I. fumosorosea* untreated palms.

- Extensive extension activities such as 28 area wide demonstrations, farmers meet and awareness programme/ field day were conducted in collaboration with farmer producing organizations

(FPOs) and Demonstration-cum-Seed Production (DSP) Farms, ICAR- Krishi Vigyan Kendra, Department of Horticulture (Fig.9), Department of Agriculture, All India Coordinated project on Biological control centres and Coconut Development Board in Karnataka & Tamil Nadu, and few in Andhra Pradesh and Lakshadweep islands for the management of invasive whiteflies in coconut using *I. fumosorosea*.

- Around 10 training programme on large scale mass production of *I. fumosorosea* was conducted and training was given to 60 progressive farmers from Andhra Pradesh and Maharashtra, 15 officials working in different biocentres, parasite laboratory of department of Horticulture, Government of Karnataka, five officials of Dr Reddy foundation officials, Srikakulam, two technical persons from Godraj Agrovvet, Vijayawada and DSP Farm, Mandya.

- About 400 kg or liters of *Isaria fumosorosea* formulations were distributed to farmers, Coconut Development Board, Department of Horticulture, KVKs, FPOs, Department of Agriculture, and AICRP



Fig.7. Field demonstration on *Isaria fumosorosea* at KRS, Mandya



Fig.8. Field demonstration on *Isaria fumosorosea* at Pollachi



Fig.9 Shree R. Shanker, Minister of Horticulture and Sericulture, Government of Karnataka, briefing about *Isaria fumosorosea* to farmers and stakeholders

on BC centres for the field evaluation against invasive whiteflies in coconut. Besides, Nucleus culture of *I. fumosorosea* also distributed to FPOs, KVKs, Farmers, Agricultural universities and private organization for their mass production and distribution.

- Foliar application of *I. fumosorosea*, two sprays at 15 days intervals with 5 g of talc formulation or 5 ml of oil formulation in one litres of water during evening hours result in Rs 9500/ha reduction in crop protection and 900 ml of pesticides/ha. Besides bio-suppression of invasive whiteflies, this also conserves the natural enemies in coconut ecosystem and saving environment.

- Besides core studies on *I. fumosorosea*, it was also documented the geographical and host range expansion and their associated natural enemies of these invasive whiteflies in India through regular survey and monitoring programme. Natural infection of *I. fumosorosea* on RSW was also documented in Andhra Pradesh.

- The project's salient achievements were published as eight research articles in national and international reputed publications in one book and book chapters, three extension folders (Kannada, English), ten abstracts were presented in national and international conference and symposium. Thirteen newspaper articles were published in different regional languages and one radio talk (Kodai

FM) was broadcast on biological control of invasive whiteflies infesting coconut.

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