Record of Potential Exotic Parasitoid Encarsia cubensis on the Neotropical Whitefly Aleurotrachelus atratus Infesting Coconut

Sumalatha, B.V.[1], *B. Hanumanthe Gowda[2], Selvaraj, K[1]., Rameshkumar, A[3] and G. Ragothuman[4]

[1]ICAR- National Bureau of Agricultural Insect Resources, Bengaluru

[2] Coconut Development Board, Kochi

[3]Zoological Survey of India, M-Block, New Alipore, Kolkata

[4]CDB-Centre of Excellence for Coconut, DSP Farm, Udumalpet, Tiruppur

Abstract

The Neotropical palm infesting whitefly, *Aleurotrachelus atratus* Hempel (Hemiptera: Aleyrodidae) is a highly invasive pest that was reported during 2019 on coconut in India. In a short span of time, it spread rapidly across states of Karnataka, Tamil Nadu, Kerala and Andhra Pradesh on coconut, oil palm and many other ornamental palm plants. During the recent survey, the immature stages of *A. atratus* were found parasitized by an aphelinid parasitoid, *Encarsia cubensis* Gahan (Hymenoptera: Aphelinidae). These parasitoids were identified by their morphological characteristics and also characterized by DNA barcoding of adult parasitoids using partial (658 bp) mitochondrial cytochrome oxidase 1 (CO1) gene (GenBank accession number ON881119). This is the first report on occurrence of *Encarsia cubensis* as primary parasitoids on *A. atratus* in India and its natural parasitism ranged from (42-68%) across the host plants and study locations in Karnataka, Andhra Pradesh and Tamil Nadu. It is believed that the parasitoid likely entered India along with A. atratus in India and constitutes a potential biological agent against *A. atratus.* It is expected that the E. cubensis population will become fully established, increase, spread and exert a significant impact on *A. atratus* population in India. Therefore, efforts may be made by growers and other stakeholders to increase the rate of natural parasitism through inundative, conservation and classical biological control approaches to reduce the pest population, crop damage and yield loss.

The invasive palm infesting whitefly, also known as the coconut whitefly, *Aleurotrachelus atratus* Hempel (Hemiptera: Aleyrodidae), entered India during 2019 and was reported to feed on coconut, *Cocos nucifera* L. and areca palm, *Dypsis lutescens* (Selvaraj *et al.*, 2019). Subsequently, it spread rapidly to other coconut growing districts in Karnataka and Tamil Nadu (Selvaraj *et al.*, 2021). Besides, occurrence of pest on coconut was reported from Kerala (Jilu *et al*., 2022) and Andhra Pradesh on coconut and oil palm. The pest also extended its host range to include areca nut, *Areca catechu*, oil palm, *Elaeis guineensis*, foxtail palm, *Wodyetia bifurcata* and lipstick palm, *Cyrtostachys renda*. The whitefly is highly polyphagous and prefers to colonize on palm plants often resulting in moderate to severe infestations (Fig.1). Nymphs and adult colonise on the under surface of leaflets and in severe cases covers more than 90% of the leaflets resulting in loss of vigour and drying of the leaflets (Fig. 1).

Aleurotrachelus atratus is believed to be native to the Neotropical region and is widely distributed in Africa, North and South America, Central America and the Caribbean, Europe and Oceania (Evans, 2008; Delvare *et al*., 2008; Borowiec *et al.*, 2010). It causes direct damage by sucking the sap from the plant cells, causing tremendous stress to the plant by the removal of nutrients and water from affected host plants. In addition, it produces wax and excretes sticky honeydew, which provides a substrate for the development of black sooty mould on an infested plant causing indirect damage by reducing the plant's photosynthetic capacity and decreasing the aesthetic

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Fig.1. Aleurotrachelus atratus: Symptoms of damage and life stages on coconut

quality of the ornamental plants, rendering them unmarketable. Infestations of the whitefly lead to a reduction in the average growth and development, premature leaf drying and dropping and substantial yield and yield parameters. Colonies of *A. atratus* were reported to coexist with other invasive and native whiteflies viz., rugose spiraling whitefly, *Aleurodicus rugioperculatus*; Bondar's nesting whitefly, *Paraleyrodes bondari*; nesting whitefly, *Paraleyrodes minei*; spiraling whitefly, *A. dispersus* and arecanut whitefly, *Aleurocanthus arecae* on coconut and other palm plants (Sundararaj *et al.,* 2021).

Since its invasion to India, intensive and continuous surveys were conducted to document the potential natural enemies of this pest in the country. The use of natural enemies is considered an important and effective strategy in pest management, particularly of exotic and invasive pests which have been used effectively through classical, augmentation or conservation biological control to manage the pest population. Still now, four species of predator's viz., *Apertochrysa astur, Jauravia pallidula, Chilocorus nigrita, Cybochephalus indicus* were found in association with *A. atratus* colonies in India (Selvaraj *et al*., 2019). No natural parasitism was observed India although as many parasitoids viz., *Cales noacki, Encarsia cubensis , Encarsia brasiliensis, Encarsia lanceolata, Encarsia nigricephala, Eretmocerus cocois and Eretmocerus desantisi, Encarsia longifasciata, E. hispida (Hymenoptera: Aphelinidae)* on *A. atratus* were reported elsewhere (Evans and Polaszek, 1998; Delvare *et al.,* 2008; Borowiec *et al.,* 2010; Noyes, 2012). Among these parasitoids, *Eretmocerus cocois* is the only one that has been recorded exclusively on *A. atratus* (Delvare *et al.*, 2008; Borowiec *et al.,* 2010). Similarly, Kityo *et al.* (2017) reported four parasitoid species viz., *Encarsia basicincta, Eretmocerus cocois, Encarsia sp.* (Hymenoptera: Aphelinidae) are suppressing the *A. atratus* population with overall parasitism was 10.74+2.03% varying significantly among districts in Inhambane province of Mozambique.

Recently study revealed the occurrence of an exotic parasitoid, *Encarsia cubensis* Gahan (Hymenoptera: Aphelinidae) as primary parasitoids of palm infesting whitefly, *Aleurotrachelus atratus* in Karnataka and Tamil Nadu. Further, the morphological as well as molecular characteristics of parasitoid, the extent of parasitism and its utilization in biocontrol programme of palm plants were discussed.

Materials and Methods

Survey: Extensive systematic surveys were conducted in three states viz., Karnataka, Tamil Nadu and Andhra Pradesh during February 2019 to June 2024 to investigate the occurrence of natural enemies of palm infesting whitefly, *Aleurotrachelus atratus* in India. Infested plant leaves were collected during survey with immature stages and puparium with collection details. The samples were placed in a rearing jar (21×10 cm) and daily observed for the emergence of parasitoids. The emerged parasitoids were collected using an aspirator and preserved in vials containing 90% ethanol for further identification. Identification of parasitoid was confirmed by morphological and molecular characterization. Assessment of parasitism (%) was determined based on the number puparium parasitized versus un-parasitized pupae on the host leaves. Identification of host insect species, A. atratus was confirmed after preparing permanent slide mounts of the puparia.

Morphological and molecular identification

The aphelinid parasitoids were processed either mounted on cards or on microscope slides as per the standard protocol (Noyes, 1982). The parasitoids were identified by original description (Gahan, 1931) and associated literature (Schauff & Evans, 1996; Evans & Polaszek, 1999). Specimens of parasitoid adults were used in molecular characterization after confirmation by morphological characteristics.

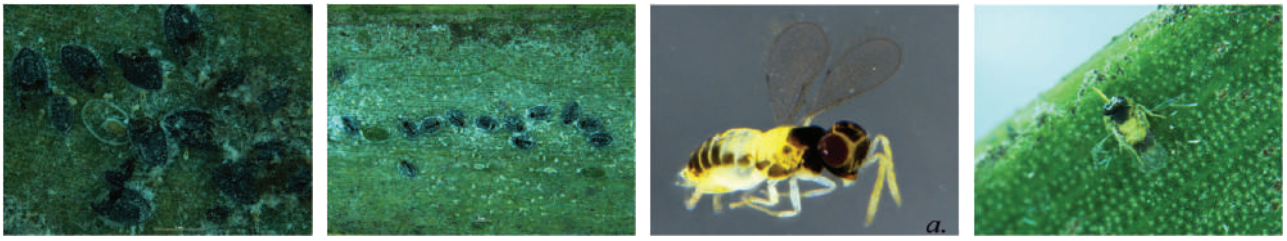

Fig.2. Aleurotrachelus atratus adult emerged (a), E. cubensis emerged puparium (b), E. cubensis adults (c-d)

Molecular characterization of the partial (658 bp) mitochondrial cytochrome c oxidase I (COI) gene was done using adult parasitoids. Genomic DNA extraction from individual adult parasitoids using the DNAase Qiagen kit method based on the manufacturer's protocol. The extracts were subjected to polymerase chain reaction amplification of the 5' terminus of the COI gene following the standard protocol. The amplified products were sequenced by M/s Chromous Biotech Pvt Ltd, Bengaluru and the same were uploaded to GenBank after annotation.

Results and Discussion

Morphological identification: The aphelinid parasitoids reared from *Aleurotrachelus atratus* were identified as *Encarsia cubensis* Gahan (Hymenoptera: Aphelinidae), an invasive species reported for the first time in India. *Encarsia cubensis* is minute, solitary and develops as primary endoparasitoid on the puparium (4th nymphal instar). Therefore, it is believed that the parasitoid likely entered India either along with *A. atratus* or other recently invaded whiteflies viz., *A. floccosus*, *A. trachoides* during 2015 and 2019, respectively. This parasitized is well distributed in Brazil, Costa Rica, Cuba, Dominican Republic, Guadeloupe, Haiti, Mexico, Puerto Rico, USA (Schauff & Evans 1996) and India (new record).

Description: *Encarsia cubensis* was described by Gahan (1931) from Cuba based on a female reared from the woolly whitefly, *Aleurothrixus howardi*, a junior synonym of *Aleurothrixus flocossus*. Dozier (1937) reported the species in very abundant numbers from *A. floccosus* on *Spondias mombin* in Haiti and transferred the species to the genus *Trichaporus*. Later, Evans and Polaszek (1998) reviewed and provided a key and illustrations of species in the *Encarsia cubensis* species group.

Diagnosis: *Encarsia cubensis* is minute, solitary and develops as primary endoparasitoid on the puparium (2th nymphal instar) and emerge during fourth instar nymphs of *A. atratus*. Body of the adult female of *E. cubensis* mostly dark coloured except for the scutellum, the lateral margins of the mid-lobe of the mesoscutum, and the central portion of metasomal tergites I and II which are yellow and the 2nd funicle segment of the antenna longer than the 3rd funicle segment (Fig 2). The species is placed in the *Encarsia cubensis* group which is characterized by having the tarsus of the middle leg 4 segmented and the fore wing broad with a large asetose area under the stigma vein.

Molecular characterization: The partial (658 bp) mitochondrial cy¬tochrome c oxidase I (COI) gene of *Encarsia cubensis* was amplified and sequenced. Further, the COI sequence of species was submitted to the National Center for Biotechnology Information database and obtained GenBank under accession number ON881119. This represents the first DNA sequence of this species, which was based on specimens reared from, the palm infesting whitefly, *Aleurotrachelus atratus* on coconut in India. The COI sequences of *E. cubensis* showed a 90.46% match with *Encarsia formosa* (GenBank accession number MG813797) reported elsewhere and submitted in NCBI database. The mtCOI sequence of *E. cubensis* provided sufficient genetic variation to characterize and distinguish them from other closed related Encarsia species.

Natural parasitism: Hymenopterous parasitoids are only known to parasitize the immature stages of their hosts which kill their host in the process of development and emerge as free-living adults. The present study revealed the parasitism rate of *E. cubensis* on the puparia of *A. atratus* varied from 42-68% on coconut across the study locations in Karnataka, Andhra Pradesh and Tamil Nadu. The parasitized nymphs having circular exit hole on the thoracic region whereas whitefly adults emerge by splitting the “T” shape structure formed by longitudinal and transverse moulting sutures on the cephalothorax of the whitefly puparium (Fig.2). Borowiec *et al.* (2010) reported five species

of aphelinid parasitoids viz., *Eretmocerus cocois* Delvare, *Cales noacki* Howard, *Encarsia longifasciata, Encarsia brasiliensis* De Santis, *E. cubensis* Gahan on *A. atratus* in La Reunion. *Encarsia cubensis* is also reported from the Neotropical solanum whitefly, *Aleurotrachelus trachoides* (Back) (Evans & Polaszek, 1998), the woolly whitefly, *Aleurothrixus floccosus* (Gahan, 1931) and *Bemisia tuberculata Bondar* (Myartseva & Evans, 2007) and was reared from *A. atratus* in Guadeloupe. This species was evidently unintentionally introduced into La Reunion Island to control the citrus woolly whitefly *A. floccosus* (Etienne, 1978). It was previously recorded on *A. atratus* in the Canary Islands (Hernandez-Suarez et al., 2003).

The infestation of *Aleurotrachelus atratus* is very high on coconut and oil palm and spreading rapidly in other coconut and oil palm growing areas in India. The parasitoid species recovered in the present study is one of the most promising biological control agents of *A. atratus* and has been reported, reared and/or released in other African countries including Comoro Islands, La Rèunion, Seychelles, Canary Islands (PRPV, 2016) where *A. atratus* populations are now under control due to the activities of *E. cubensis.* Although the parasitoid population is known to reduce pest density, it is only possible when the parasitoid is well established and the rate of parasitism is sufficiently high enough to exert a significant impact on the *A. atratus* population in India.

Therefore, efforts should be made to increase the rate of parasitism through conservation of native natural enemies and inundative releases of classical biological control agents. Therefore, further extensive surveys need to be conducted on the occurrence of *E. cubensis* on these invasive species for the implementation of effective biological control strategies. ■

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