DOI : 10.58993/ijh/2025.82.2.11

Indian J. Hortic. 82(2), June 2025: 195-199

Invasive whiteflies (Hemiptera) infesting sword bean (*Canavalia gladiata*) in India

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ABSTRACT

Sword beans has been recommended as a sustainable way to address protein malnutrition and provide nutritional security. Sword bean is a good supplement to a cereal-based diet due to its protein content being higher than that in any other legumes and crops, and it is known to be resistant to attack of insect pests and diseases. However, the sword bean was found to be severely infested by a complex of invasive whitefly species in India. Three species of whiteflies were identified for the infestations based on morphological features and molecular characterization by sequencing of mtCOI. The species were confirmed as *Tetraleurodes acaciae* (Quaintance) (legume whitefly), *Paraleyrodes bondari* Peracchi, (Bondar's nesting whitefly) and *Aleurodicus dispersus* Russell (spiralling whitefly). Although mixed infection of these species was observed on the same leaves, populations of *P. bondari* were more on upper and lower surfaces of matured and older leaves, while populations of *T. acaciae* were observed on the lower surface of younger leaves. The population of *A. dispersus* was negligible when compared to the other two identified species. This is the first report of *T. acaciae* and *P. bondari* on sword bean. Severe incidences of *P. bondari* infestation on this legume could be devastating and a cause of concern. Regular monitoring may be needed to check its spread to other legume crops which are very important for food and nutritional security.

Key words: Mixed infestation, mt COI, *Tetraleurodes acaciae*, *Paraleyrodes bondari*, *Aleurodicus dispersus*.

INTRODUCTION

Sword bean *Canavalia gladiata* (Jacq.) de Candolle (Fabaceae) originated in Asia and spread across Africa, South America, Australia and Africa. India has a wide genetic diversity of sword bean genotypes in North-Eastern hill region (Debbarma *et al*., 3). Seeds of sword bean are mostly consumed by some tribal groups of North-Eastern India. Seeds are also used in Chinese herbal medicine and as raw-material in phytochemical and pharmaceutical products (Nanda *et al*., 12; Eknayake *et al*., 7; Arun *et al*., 1). Young green pods are utilized as a vegetable in countries like India, Sri Lanka, and Indonesia (Rajaram and Janardhanan, 13; Siddhuraju and Becker, 15). Regular consumption of sword bean is reported to reduces the risk of cardiovascular diseases, prevent cancer, type II diabetes, obesity and osteoporosis (Troszynska *et al*., 19). Cultivation of sword is profitable due to its characters like high biomass, resistance to drought stress, resistance to pest and disease, produces high seed yield of 800-1000 kg/ hectare (Siddhuraju and Becker, 15). Sword bean is normally cultivated in India as border crop, intercrop or shade crop. Wide genetic diversity of sword bean found in India particularly in North-Eastern hill region (Debbarma *et al*., 3). Several efforts to promote sword bean cultivation were made through development of better yielding in short duration, and photo-insensitivity verities like, SBS1 by Tamil Nadu Agricultural University, Coimbatore (Annonymous). Although sword been is not known to suffer from server attack of pest and disease, few pests like leaf miner, *Liriomyza* sp, pod borer *Maruca* sp., striped mosaic disease and wilting have been reported on the sword bean crop (Uge *et al*., 20). Sword bean plants grown as edge plants at the research farm of ICAR-Indian Agricultural Research, Institute, Regional Station, Pune, Maharashtra, India, displayed severe infestation by the mixed whitefly species, *Tetraleurodes acaciae* (Quaintance), (legume whitefly) Bondar's nesting whitefly, *Paraleyrodes bondari* (Peracchi,) *(*Bondar's nesting whitefly), and spiralling whitefly, *Aleurodicus dispersus (*Russell) (spiralling whitefly). To our knowledge, the present communication is the first report on the occurrence of the *T. acaciae* and *P. bondari* on the sword bean.

India, have been invaded by more than 110 exotic insect species among these whiteflies and mealybugs constituted a major part (Mandal, 10). The immatures of whiteflies are small sized and often overlooked on leaf surfaces, and their accidental introduction along with associated host plants is likely in newer areas by anthropogenic activities (Dubey, 4). So far, eighth invasive whitefly species are reported in India along

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with their host plants (Sundararaj *et al*., 18). Spiralling whitefly *A. dispersus* was the first invasive whitefly to be reported in India during 1995 (David and Regu, 2) followed by solanum whitefly, *Aleurothrixus trachoides* in 2015 (Dubey and Sundararaj, 6), rugose spiralling whitefly, *Aleurodicus rugioperculatus* during 2016 (Sundararaj and Selvaraj, 16), *T. acaciae* during 2017 (Sundararaj and Vimala, 17) and during year 2018, *P. bondari* (Josephrajkumar *et al*., 9) and, *P. minei* (Mohan *et al*., 11) were reported. *P. bondari* was reported on 34 species of host plants and *T. acaciae* (Quaintance) on 5 species of host plants (Sundararaj *et al*., 18). The whitefly, *P*. *bondari* is smaller in size compared to other species of the subfamily Aleurodicinae invasive from the Neotropical region. Adults and immatures of these species secrete copious amount of wax that leads to sooty mold development on leaf surfaces and yellowing of leaves (Dubey, 4). The present study aimed to identify invasive whiteflies infesting sword bean.

MATERIALS AND METHODS

Adults and puparia of whiteflies infesting *C. gladiata* leaves were collected from the plants grown at the research farm of ICAR-Indian Agricultural Research Institute, Regional Station, Pune, Maharashtra, India. Adults of whitefly species were collected individually directly in 1.5 ml Eppendorf tubes, preserved in ethanol and used for DNA extraction using DNeasy® Blood and Tissue Kit (Qiagen, Amph, Germany). DNA of individual whiteflies were used for amplification of the mitochondrial cytochrome oxidase 1 (*mtCOI-I*) using universal primers LCOI490-F and HCO2198-R (Folmer *et al*., 8) by PCR (volume 20 µl, F and R primers 1µl each, annealing 55 °C for 1 min, 35 cycle) which amplified approx. 658bp DNA.

All the PCR products were sequenced in both forward and reverse direction. Consensus sequences derived from forward and reverse complement used for BLAST search with default settings in the NCBI databases (http://www.ncbi.nlm.nih.gov/). Altogether eight *mtCOI* sequences of three whiteflies were submitted to NCBI and GenBank Accession number obtained (Table 1).

The puparia and a few adults collected from sword bean were slides mounted at the Hemiptera Section, Zoological Survey of India (ZSI), Kolkata, India by following the method of Dubey and David (5) and the identity of whitefy species *T. acaciae*, *P. bondari*, and *A. dispersus* were confirmed. The identification and imaging of whiteflies were done using a compound microscope BX 41 at the ZSI, Kolkata.

RESULTS AND DISCUSSION

Indian tribal legumes sword bean is an underutilized legumes having advantages like adaptability to adverse environmental conditions, can thrive under extreme stress conditions and resists biotic and abiotic stresses in India (Siddhuraju and Becker,15). There are also several efforts for nutritional, chemical and genetic profiling of the crop (Eknayeke *et al*., 7; Arun *et al*., 1; Rajaram and Janardhanan, 13). Although sword bean has been known for its resistance to incidence of insect-pests and diseases, we observed the crop was severely infested by intermingled whiteflies species (Fig. 1). Morphological and molecular characterization (mtCOI-I) confirmed incidence of three whitefly species, *T. acaciae*, *P. bondari and A. disperses* on sword bean. In the case of *T*. *acaciae* two phenotypes, white and pale colored (early emerged) adults were observed (Fig. 1 E & F). However, sequences of both types matched with *T. acaciae*.

In vivo, the puparia of *P*. *bondari* are pale and *T*. *acaciae* are black (Fig. 2); adults of *P*. *bondari* have two pairs of bands on the forewings (absent in *A*. *dispersus* and *T*. *acaciae*), *A*. *dispersus* adults are larger than the other two, and *T*. *acaciae* adults are small, and pale after emergence which later turn white.

The puparia of *P*. *bondari* can be identified in being pale and in having a row of submarginal fine setae, one pair of large agglomerate wax secreting pore on

Table 1. Gen Bank Accession numbers of mtCOI-I sequences for whiteflies infesting sword bean in India.

S. No.	Accession No.	Whitefly species	Matched sequences	Coverage	Similarity (%)
1	PP748526	*Paraleyrodes bondari*	MK343480.1	100	99.85
2	PP748528	*Paraleyrodes bondari*	MK343480.1	100	99.70
3	PP758520	*Paraleyrodes bondari*	MK343480.1	100	99.24
4	PP748524	*Tetraleurodes acaciae*	OP692696.1	100	100
5	PP748529	*Tetraleurodes acaciae*	OP692696.1	100	100
6	PP758524	*Tetraleurodes acaciae*	OP692696.1	100	100
7	PP750859	*Aleurodicus dispersus*	MN022652.1	100	100
8	PP750860	*Aleurodicus dispersus*	MN022652.1	100	100

Fig. 1. Whitefly infestation on sword bean: (A) *P. bondari* infestation on leaves; (B) colonies on lower surface; (C) and upper surface of leaves; (D), closeup of infestation of *P. bondari*; (E & F) leaves showing Infestation of *T. acaciae* with white (E) and pale (F) morphotypes; (G) mixed infestation of *A. dispersus with P. bondari (G)* and *T. acaciae* (H).

Fig. 2. Habitus, puparium of *P. bondari* and *T. acaciae*.

cephalic region and six pairs on abdomen (anterior two are smaller in size than remainders), meso- and metathoracic setae and exposed lingula with four setae (Fig. 3). Puparia of *A. dispersus* have five pairs of large subdorsal compound pores, four of which are located on the abdomen and lingua tongue-like. The slide mounted puparia of the whitefly, *T. acaciae* differs from the other two species in this study. The former is black, and have a submarginal ridge, row of tubercles on the submargin, meso- and metathoracic setae, median tubercles on anterior abdominal segments and elevated vasiform orifice (Fig. 3).

Although mixed infection of the three species were seen on leaves, *population of P. bondari were observed higher* on matured and older leaves, and whitefly colonies were seen on both the lower and upper surfaces of the leaves (Fig. 1). The population of *T. acaciae* was seen more on younger

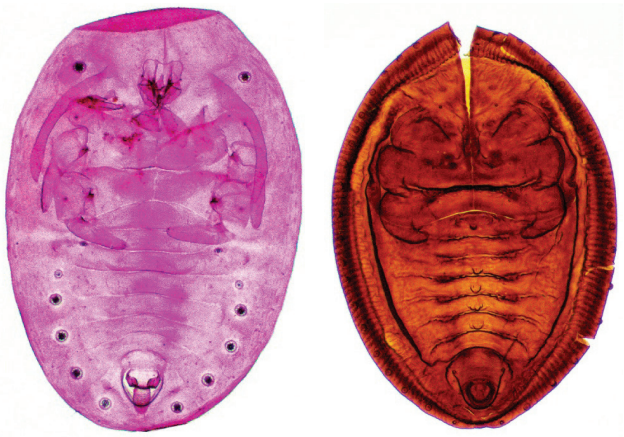

Fig. 3. Slide-mounted puparium of *P. bondari* and *T. acacia* (right).

leaves and mostly confined to lower surface of leaves. Populations of *A. dispersus* were negligible compared to *T. acacia* and *P. bondari*. Dubey (4) did not consider *T. acaciae* as a pest due to its occurrence on non-commercial crops, however, it is found severely infesting legume crop in this study. This is the first report on the occurrence of whiteflies, *T. acaciae* and *P. bondari on sword bean. T acaciae* is known to infest mainly leguminous plants, it has been reported breeding on legumanacious tree, *Leucaena leucocephala* in Bangalore, India (Sundararaj and Vimala, 17). In India, *P. bondari* was first reported as an invasive pest on coconut from Kerala and found co-existing with *A. rugioperculatus* Martin, 2004 on coconut palms (Josephrajkumar *et al*., 9); *P. bondari* feeds on coconut, ficus, cassava, *Morinda citrifolia*, banana, guava and *Thespesia populnea* (Selvaraj *et al*., 14). However, *P. bondari* was not reported from any legume host plants earlier. Severe incidence of invasive whitefly pests like, *P. bondari* and *T. acaciae* on sword bean could be a cause of concern, and need monitoring on legume crops.

Although sword bean is generally known for its resistance to insect pests and diseases, our observations in India revealed severe infestations by *T. acaciae*, *P. bondari*, and *A. dispersus*. Notably, *P. bondari* and *T. acaciae* pose a potential threat to other leguminous hosts as well, as demonstrated by their impact on sword bean in this study. Therefore, regular monitoring is essential to prevent possible outbreaks in such crops.

AUTHORS' CONTRIBUTION

Conceptualization of research (KC); Management of field experiments (SS); Execution of lab experiments (AKD & AV); Preparation of the manuscript and Editing (All).

DECLARATION

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENTS

This work resulted from institutional project of ICAR-IARI. Authors K.C, A.V, S.S. and A.K. are thankful Indian Council of Agriculture Research for funding, and AKD is thankful to MOEFCC, New Delhi for the in-house project on the invasive whiteflies.

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(Received : October, 2024; Revised : June, 2025; Accepted : June, 2025)