

Short communication**Sentinel egg card studies for resident egg-parasitoids harbouring horticultural crops of IARI, New Delhi campus****Sachin S. Suroshe*, K. Shankarganesh, Bishwajeet Paul and N.S. Chandra Bose**

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ABSTRACT

One thousand three hundred and sixty sentinel egg cards were prepared from eggs of rice grain moth. These were fixed for parasitisation and ultimately for evaluation of the diversity of resident egg-parasitoids among the twenty three horticultural crops planted in IARI campus. DNA barcode studies were also carried out to confirm the identity of recovered egg-parasitoids. Egg parasitoids could be reared only from seven crops i.e. guava, cabbage, cauliflower, broccoli, knol-khol, lettuce, and tomato. Among the fruit crops, egg-parasitoid, *Trichogrammatoidea* sp. was found in guava. While in vegetable crops, *Trichogramma chilonis* was observed in tomato, lettuce, broccoli and cauliflower. Egg-parasitoid, *Trichogramma achaeae* was recorded in tomato, knol-khol and cabbage.

Key words: Sentinel egg cards, *Trichogrammatids*, parasitoids, DNA barcode.

The egg-parasitoids, *Trichogramma* spp. are minute parasitic wasps that are used worldwide to control Lepidopteran pests (Nagarkatti, and Nagaraja, 5; Hassan, 2). The genus *Trichogramma* is one of 80 genera in the family *Trichogrammatidae*. All members of this family are parasites of insect eggs. *Trichogrammatidae* includes the smallest of insects, ranging in size from 0.2 to 1.5 mm. Within the genus *Trichogramma*, there are 145 described species worldwide; 30 species have been identified from North America and an estimated 20 to 30 species remain to be described (Olkowski and Zhang, 6). Today, *Trichogramma* species are the most widely used insect natural enemy in the world (Ying, 4), partly because they are easy to mass rear and they attack many important crop insect pests. Nine species of *Trichogramma* are reared in private or government owned insectaries around the world and released annually on an estimated 80 million acres of agricultural crops and forests in 30 countries (Ying, 4; Olkowski and Zhang, 6). *Trichogramma* are released to control some 28 different caterpillar pests attacking corn, rice, sugarcane, cotton, vegetables, sugar beets and fruit crops (Hassan, 3). Though, the successful use of *Trichogramma* has been witnessed in some of the vegetables crops like tomato, it is need of hour to intensify this safe option in other horticultural crops. Therefore, the current studies were carried out to find out the efficient and locally adaptable species of egg-parasitoids, for their mass multiplication and release in augmentative biological control programmes of horticultural crops.

Field studies were carried out in IARI campus using sentinel egg card prepared from eggs of rice grain moth for knowing the diversity of resident egg-parasitoids among the horticultural crops, viz., pomegranate, Kinnow, papaya, Kagzi lime, grape fruit, ber, grape, guava, phalsa, mango, bael, banana, cauliflower, cabbage, cabbage (red), broccoli, knol-khol, lettuce, kale, brinjal, tomato, okra and potato. Egg cards were attached to respective horticultural crops planted in research field/blocks during 2013-2014. Due care was taken while attaching the cards in the field; border rows were avoided and the middle rows were selected. After attaching the cards on respective crops, they were left in the field for 48 h, so that the local egg-parasitoids lay their eggs in the eggs pasted on the sentinel egg cards. Then, sentinel egg cards were brought to the laboratory and kept in BODs at 27°C and observed daily under binocular microscope for blackening of eggs. Those cards in which some of the eggs starts getting black were separated and kept in test tube and secured with cotton plug. After emergence, the samples of egg-parasitoids were stored in 70% ethyl alcohol for identification in homeopathy vials having labels containing details of crop and date of tagging egg cards in the field. All the samples were numbered date and crop wise and handed over to National Pusa Collection (NPC), Division of Entomology, IARI, Pusa, New Delhi for identification. The samples of egg parasitoids were identified by *Trichogramma* expert at ICAR-National Bureau of Agriculturally Important Insects, Bengaluru, Karnataka (Table 1).

DNA barcode studies were also carried out to confirm the identity of resident egg-parasitoids

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Table 1. Identification of egg-parasitoids of IARI, Pusa campus.

Code	Name of crop	Date of collection	Egg parasitoid species recovered
1	Guava	07/10/13	<i>Trichogrammatoidea</i> sp.
2	Tomato	10/12/13	<i>Trichogramma chilonis</i>
3	Tomato	17/12/13	<i>Trichogramma achaeae</i>
4	Lettuce	10/12/13	<i>Trichogramma chilonis</i>
5	Broccoli	07/12/13	<i>Trichogramma chilonis</i>
11	Cauliflower	23/11/13	<i>Trichogramma chilonis</i>
12	Cauliflower	27/11/13	<i>Trichogramma chilonis</i>
13	Cauliflower	01/12/13	<i>Trichogramma chilonis</i>
14	Knol-khol	10/12/13	<i>Trichogramma achaeae</i>
15	Cabbage	07/12/13	<i>Trichogramma achaeae</i>

recovered from IARI campus. The DNA was extracted using Qiagen's QIAamp® DNA Investigator Kit from single adult specimen of trichogrammatids according to the manufacturer protocol. The isolated DNA was then stored at -20°C for further studies. The extracted DNA from each sample was amplified through PCR using universal primers LCO1490: 5'-gggtcaacaaatcataaagatattgg-3'; HCO2198:5'-taaacttcagggtgaccaaataatca-3' for targeting the mitochondrial cytochrome oxidase subunit-I (COI) gene (Folmer *et al.*, 1). PCR was performed with a reaction mixture of 25 µl; heated at 94°C for 4 min. followed by 35 cycles of 30 sec at 94°C for denaturing, 60 sec at 47°C for annealing, 50 sec at 72°C for extension and a final extension at 72°C for 8 min. in a C1000™ Thermal cycler. The reactions were combined (as described by KOD FX puregene™ manufacturer protocol) of DNA template (4 µl), 2x PCR buffer (12.5 µl), 2mM dNTP (10 µl), *Taq* (KOD FX) enzyme (1 unit), forward and reverse primers were 0.3 µM each at final concentration to the reaction. The amplified

products were run on 2% agarose gel and observed under UV using Alphaview® software version 1.2.0.1 (Fig. 1). The amplified products were then sequenced at Scigenom Pvt. Lab., India. The sequenced PCR products were then edited by BioEdit version 7.0.0. The aligned sequences of *T. chilonis* and *T. achaeae* (Fig. 2) were then deposited in NCBI Genbank for getting the accession numbers. DNA barcode studies also confirm the identity of resident Trichogrammatids as identified by taxonomist (Table 2).

Among the fruit crops, pomegranate, Kinnow, papaya, Kagzi lime, grape fruit, *ber*, grape, guava, *phalsa*, mango, *bael* and banana surveyed for resident egg-parasitoids through sentinel egg cards, only guava reported egg-parasitoids, *i.e.*, *Trichogrammatoidea* sp. It could not be identified up to species level because samples were without males, and that is mandatory for identification of Trichogrammatids. Total 688 sentinel cards were attached on the fruit crops and 672 cards were attached in the vegetable crops during the course of investigation. Among the vegetable crops, cauliflower, cabbage, cabbage (red), broccoli, knol khol, lettuce, kale, brinjal, tomato, okra and potato. However, only six crops *i.e.*, tomato, lettuce, broccoli, cauliflower, knol-khol and cabbage could recover some resident egg parasitoid.

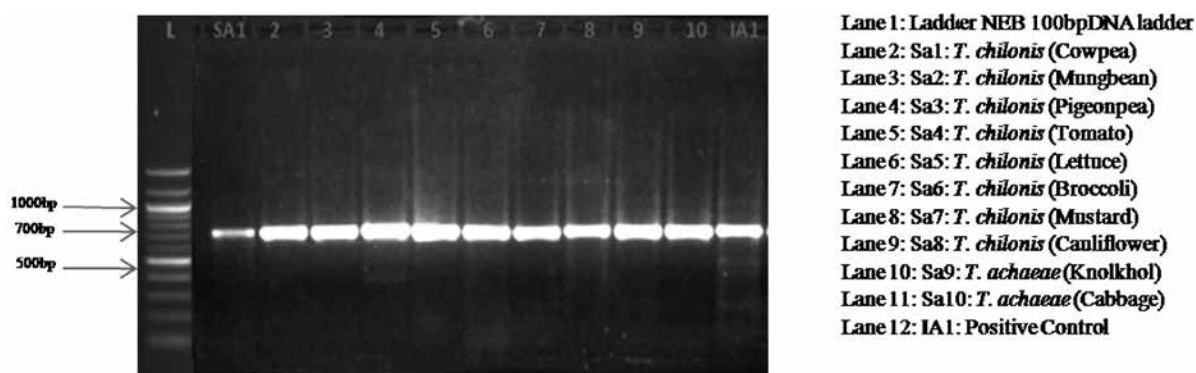
**Fig. 2.** Egg parasitoids a) *T. Chilonis* b) *T. achaeae*.**Fig. 1.** Mt COI amplified products of *T. chilonis* and *T. achaeae* from different horticultural crops.

Table 2. DNA Barcode of native *Trichogrammatids*.

Species	Source crop	NCBI Gen Bank Accession No.	Location
<i>Trichogramma chilonis</i>	Cowpea	KM501046	IARI, New Delhi
<i>Trichogramma chilonis</i>	Mungbean	KM501047	IARI, New Delhi
<i>Trichogramma chilonis</i>	Pigeonpea	KM501048	IARI, New Delhi
<i>Trichogramma chilonis</i>	Tomato	KM501049	IARI, New Delhi
<i>Trichogramma chilonis</i>	Lettuce	KM501050	IARI, New Delhi
<i>Trichogramma chilonis</i>	Broccoli	KM501051	IARI, New Delhi
<i>Trichogramma chilonis</i>	Mustard	KM501052	IARI, New Delhi
<i>Trichogramma chilonis</i>	Cauliflower	KM501053	IARI, New Delhi
<i>Trichogramma achaeae</i>	Knolkhol	KM501054	IARI, New Delhi
<i>Trichogramma achaeae</i>	Cabbage	KM501055	IARI, New Delhi

The present investigation throws light on the available species of egg-parasitoids in the fruit and vegetable crop ecosystems of IARI, New Delhi. These species could be used though augmentative biological control for the management of insect pests without using introduced species.

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