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Molecular characterization of *Trichogramma* spp. in sugarcane (*Saccharum officinarum*) ecosystem in Punjab

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Trichogramma species are useful for biological control of Lepidopteran insect pests all over the World, especially for the control of pests of agricultural crops, cash crops, orchards and forests (Yousuf *et al*. 2016). Till date, 28 species of *Trichogramma* have been recorded from India (Nagaraja and Mohanraj 2010). *T. chilonis* and *T. japonicum* Ashmead are the two predominant species which are distributed throughout India have been successfully used for augmentative biological control programmes (Chakraborty 2011). In Punjab, augmentative releases of *Trichogramma* have been done in various crops like sugarcane (*Saccharum officinarum* L.) (against early shoot-borer, top-borer and stalk-borer) (Singh *et al.* 2007), organic rice [against stem-borer, *Scirpophaga incertulas* (Walker) and leaf-folder, *Cnaphalocrocis medinalis* (Guenee)] (Kaur and Brar 2008) and maize [against maize borer, *Chilo partellus* (Swinhoe)] (Shera *et al.* 2017).

The identification and use of correct species of the parasitoids is an important step in biological control programs before its release in the field. Despite the substantial practical use of *Trichogramma* parasitoids, studies of members of this genus are complicated due to their complex taxonomy, minute size, intraspecific variations and presence of cryptic species (Querino and Zucchi 2002). Therefore, alternative identification methods such as reproductive compatibility studies (Pinto *et al*. 1991) and molecular methods have been developed. Sequence diversity in a 650 bp fragment of the mitochondrial gene cytochrome c oxidase I (cox1; also referred to as COI) are commonly used in DNA barcoding which provides species-level resolution (Monaghan *et al.* 2005). As *T. chilonis* is being widely used as a biocontrol agent for the last 20 years, there is a need to establish the species composition in the fields through modern day techniques for differentiation among the species/strains. This study was designed to identify the *Trichogramma* species associated with borer pests across all the sugarcane growing regions of Punjab.

Trichogramma populations were collected from 20 different locations in Punjab from 4 sugarcane fields/village (one acre each) during 2017–18 (Table 1).

Recording of natural parasitization by Trichogramma *spp.*: Sentinel cards having eggs of rice meal moth, *Corcyra cephalonica* (Stainton) were used for collection of *Trichogramma* spp. from different locations at fortnightly intervals. These cards were cut into 40 strips, each having approximately 100 eggs. These strips were stapled on the lower surface of the sugarcane leaves uniformly at 40 spots per acre to record natural parasitization by *Trichogramma* spp. The fields were surveyed during tillering stage of the crop. Sampling took place between mid-April to mid -June and July to October which targeted primarily the incidence of sugarcane early shoot-borer (C*hilo infuscatellus* Snellen) and sugarcane stalk-borer (*Chilo auricilius* Dudgeon) respectively.

Collection and maintenance of recovered Trichogramma *spp.*: These strips were removed after 24 hr from the fields and were brought to the laboratory. These were kept separately in the glass vials for adult emergence. The adults hatched from the collected parasitized host eggs were mass reared on laboratory host, C. *cephalonica* by following the methodology given by Sharma *et al.* (2016).

Preservation of recovered Trichogramma *spp.*: The population of recovered *Trichogramma* spp. thus maintained in the laboratory were kept in labeled vials with 95% ethyl alcohol and stored at –20˚C for molecular characterization.

DNA extraction: The total genomic DNA was isolated from 10 *Trichogramma* adult wasps from each population using NucleoSpin Tissue XS kit (Macherey Nagel) as per manufacturer's protocol. Horizontal agarose (1.2%) gel electrophoresis was used to determine the quality of isolated DNA. The DNA bands were visualized and photographed under a UV transilluminator in Ultra Cam Gel Documentation System.

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Table 1 Details of survey sites

District	Agro-climatic zone	Village	Geographical co-ordinates
Gurdaspur	Sub-mountain undulating zone	Bakhshiwal	32° 1' 21.936" N, 75°14' 34.584" E
		Sahowal	32° 4' 30.36" N, 75°28' 24.564" E
		Kahnuwan	31° 54' 34.128" N, 75°26' 33.684" E
		Rai Chak	31° 53' 7.944" N, 75°18' 24.012" E
Amritsar	Central Plain zone	Bhilowal	31° 40' 49.26" N, 75°5' 55.428" E
		Mannawalla	31° 35' 30.876" N, 74°58' 21.468" E
		Gumanpura	31° 36' 40.032" N, 74° 45' 55.8" E
		Saidopura	31° 41' 49.128" N, 74° 46' 19.056" E
Shaheed Bhagat Singh Nagar	Undulating Plain zone	Mahalon	31° 8' 43.116" N, 76° 6' 3.24" E
		Barnala Kalan	31° 7' 25.5" N, 76° 8' 45.24" E
		Kariha	31° 8' 33.612" N, 76° 3' 45.9" E
		Amargarh	31° 7' 41.376" N, 76° 4' 20.46" E
Patiala	Central Plain zone	Kheri Jattan	30° 28' 49.368" N, 76° 17' 48.228" E
		Rauni	30° 20' 41.928" N, 76° 20' 20.184" E
		Ghanaur	30° 19' 54.048" N, 76° 36' 40.068" E
		Sahauli	30° 27' 12.024" N, 76° 13' 1.776" E
Fazilka	Western Plain zone	Sureshwala	30° 22' 36.912" N, 74° 0' 38.376" E
		Korianwali	30° 23' 0.42" N, 74° 3' 39.78" E
		Karni Khera	30° 22' 23.16" N, 73° 58' 41.124" E
		Diwan Khera	30° 6' 54.252" N, 74° 0' 21.852" E

Amplification of mtCoI: A fragment of the mitochondrial COI gene was amplified using the specific universal primers: forward (5'-GGTCAACAAATCATAAAGATATTGG-3') and reverse primers (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.* 1994).

PCR reaction were executed in 50 µl reaction, each containing 10 ng template DNA solution (5 µl), 1 mM dNTPs mix (10 µl), Primers (2 µl each), Taq polymerase 5 units/µl (1 µl), 15 mM $MgCl_2$ in 10X Taq reaction buffer (5 µl) and sterile Mili-Q H_2O (25 µl). All PCR-amplifications were done in a programmable DNA thermocycler (Mastercycler Gradient-eppendorf ^{TM}). The PCR conditions were as follows: 95°C for 5 min; 35 cycles at 95°C for 1 min, 52°C for 1 min, 72°C for 1 min; and a final elongation step at 72°C for 10 min and stored at 4°C until used.

The PCR mixture was subjected to agarose gel (0.75%) electrophoresis and UV-Gel Documentation System (UltraLum) was used to visualize the amplification profile. The agarose block containing the specific amplified DNA band was excised from the gel with a clean, sharp scalpel blade and transferred to a 1.5 mL microcentrifuge tube. 'FavorPrep ^{TM} GEL/PCR purification Kit' was used to purify the DNA fragments as per the gel purification protocol. The purified product was sent to Xcleris labs Ltd, Ahmedabad, India for determination of nucleotide sequences in both directions.

Analysis of sequence data: All the nucleotide sequences for mtCOI were edited using CLC Sequence Viewer software for any misread using forward and reverse strands. The edited nucleotide sequences were blasted in 'Blastn' function of 'National Centre for Biotechnology Information' and identified based on the derived taxonomy report which was generated on the basis of maximum sequence homology/ query coverage in database from other species.

Phylogenetic analysis: The mtCOI sequences available from other countries, i.e. Pakistan, China, Japan, Veitnam, other parts of India were downloaded from NCBI database. All the sequences were aligned using Clustal W programme and phylogenetic analysis was done with MEGA programme by using maximum likelihood method.

Genetic variability was determined amongst different populations of *Trichogramma* spp. collected from Punjab using mtCOI gene. The DNA was isolated from adult wasps of *Trichogramma* and was subjected to PCR amplification by mtCOI specific primers. The primers amplified ~700 bp region of the genomic DNA fragment of the samples by PCR (Fig 1). The nucleotide sequences for all the samples were edited and subjected to BLAST (Basic Local Alignment Search Tool) with the database of NCBI.

Taxonomic analysis of the mt COI sequences: The nucleotide sequences of the collected *Trichogramma* populations were blasted with available sequences in the GeneBank (Table 2). The retrieved sequences were submitted to NCBI (Accession no. OQ921616-OQ921620). The *Trichogramma* spp. collected from all the locations showed maximum identity with *Trichogramma chilonis* voucher CUTC 01-A1 (Query coverage 100%, identity

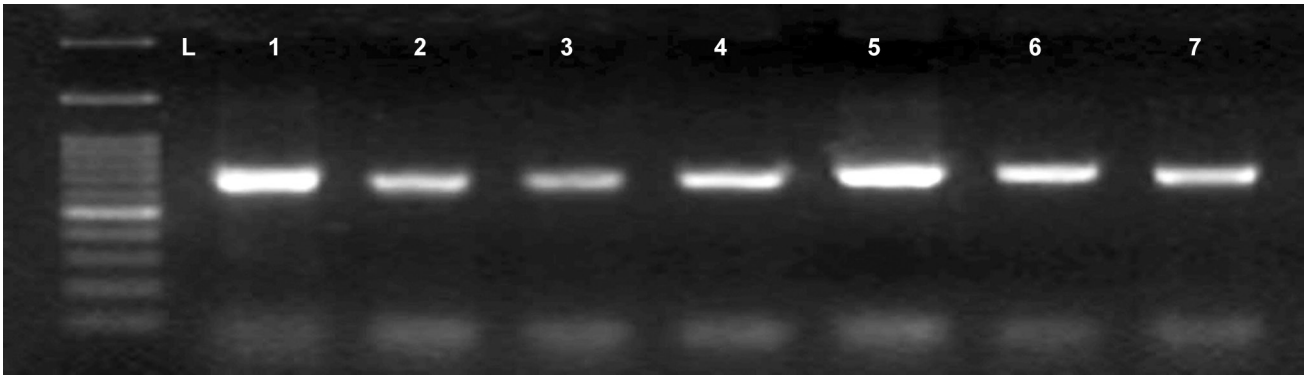

Fig 1 PCR amplification of genomic DNA of *Trichogramma* populations with Lep A primer 1-Gurdaspur, 2-SBS Nagar, 3-Patiala, 4-Fazilka, 5-Ludhiana, 6-Gurdaspur-2, 7-Amritsar.

Table 2 Sequence homology of mt COI region of *Trichogramma* collected from different locations with available sequences in Gene Bank database

Description	Max score	Total score	Query cover	E value	Identity	Gene Bank accession no.
Trichogramma spp. collected from Gurdaspur						
Trichogramma chilonis voucher CUTC 01-A1	1188	1188	100%	0.0	99%	KP994546.1
Trichogramma chilonis voucher BIOUG02442-A01	1136	1136	96%	0.0	99%	KY836727.1
Trichogramma chilonis	1136	1136	95%	0.0	99%	KJ911023.1
Trichogramma chilonis	1125	1125	99%	0.0	98%	KU575095.1
Trichogramma chilonis voucher AIN29Tc001BLR	1114	1114	94%	0.0	99%	KF234137.1
Trichogramma spp. collected from SBS Nagar						
Trichogramma chilonis voucher CUTC 01-A1	1199	1199	100%	0.0	99%	KP994546.1
Trichogramma chilonis voucher BIOUG02442-A01	1147	1147	96%	0.0	99%	KY836727.1
Trichogramma chilonis	1147	1147	95%	0.0	100%	KJ911023.1
Trichogramma chilonis	1136	1136	99%	0.0	98%	KU575095.1
Trichogrammachilonis voucher AIN29Tc001BLR	1125	1125	94%	0.0	99%	KF234137.1
Trichogramma spp. collected from Patiala						
Trichogramma chilonis voucher CUTC 01-A1	1199	1199	100%	0.0	99%	KP994546.1
Trichogramma chilonis voucher BIOUG02442-A01	1147	1147	96%	0.0	99%	KY836727.1
Trichogramma chilonis	1147	1147	95%	0.0	100%	KJ911023.1
Trichogramma chilonis	1136	1136	99%	0.0	98%	KU575095.1
Trichogramma chilonis voucher AIN29Tc001BLR	1125	1125	94%	0.0	99%	KF234137.1
Trichogramma spp. collected from Fazilka						
Trichogramma chilonis voucher CUTC 01-A1	1199	1199	100%	0.0	99%	KP994546.1
Trichogramma chilonis voucher BIOUG02442-A01	1147	1147	96%	0.0	99%	KY836727.1
Trichogramma chilonis	1147	1147	95%	0.0	100%	KJ911023.1
Trichogramma chilonis	1136	1136	99%	0.0	98%	KU575095.1
Trichogramma chilonis voucher AIN29Tc001BLR	1125	1125	94%	0.0	99%	KF234137.1
Trichogramma spp. collected from Amritsar						
Trichogramma chilonis voucher CUTC 01-A1	1199	1199	100%	0.0	99%	KP994546.1
Trichogramma chilonis voucher BIOUG02442-A01	1147	1147	96%	0.0	99%	KY836727.1
Trichogramma chilonis	1147	1147	95%	0.0	100%	KJ911023.1
Trichogramma chilonis	1136	1136	99%	0.0	98%	KU575095.1
Trichogramma chilonis voucher AIN29Tc001BLR	1125	1125	94%	0.0	99%	KF234137.1

99%, max score 1188) followed by *Trichogramma chilonis* voucher BIOUG02442-A01, *Trichogramma chilonis* and *Trichogramma chilonis* voucher AIN29Tc001BLR.

Multiple alignments of mt COI sequences using maximum likelihood method formed one cluster of Punjab populations and other *T. chilonis* populations from New Delhi, Pakistan, Calicut, and Bengaluru. The populations from Hawaii, China, Taiwan, Japan, France and La Reunion are separately clustered (Fig 2).

Trichogramma populations collected from sugarcane from different districts of Punjab have been characterized based on mt COI gene. All the populations showed 99% similarity with *T. chilonis*. Our results corroborate with Rijesh *et al.* (2012) who studied the genetic diversity in *Trichogramma* populations from different agro-climatic zones of India (sugarcane ecosystems), i.e Uttar Pradesh, Punjab, Haryana, Maharashtra, Karnataka, Andhra Pradesh, Orissa and Tamil Nadu based on ITS 2 sequence analysis and

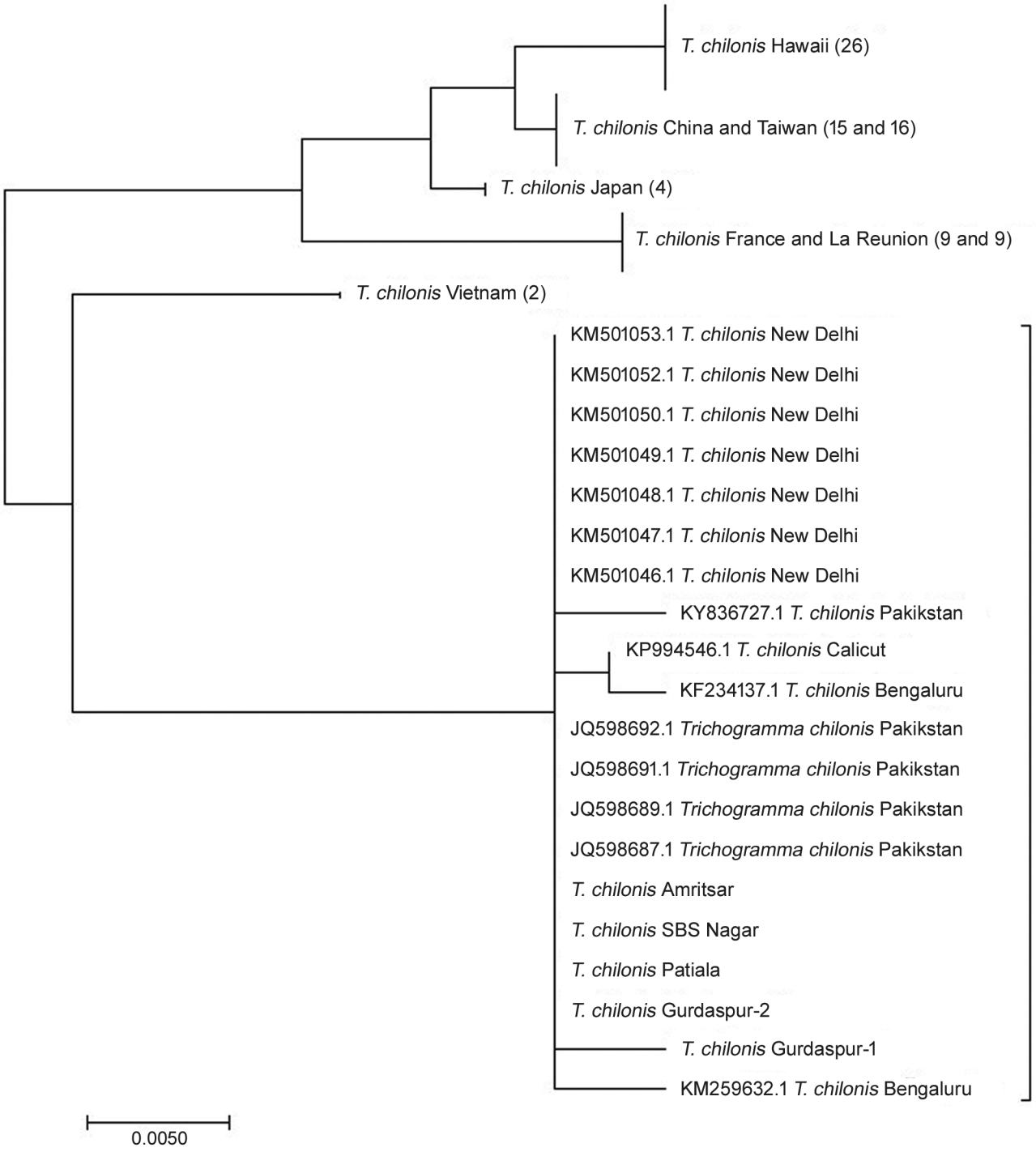

Fig 2 Molecular Phylogenetic analysis by maximum likelihood method for different *T. chilonis* from India and other countries.

RAPD markers**.** The BLASTN of the ITS 2 region revealed all the populations as *T. chilonis* except the population from Andhra Pradesh ecosystem. Yousuf *et al.* (2016) identified ten different species of *Trichogramma* out of total of 176 specimens collected from five Agro-climatic zones of Punjab. The maximum no. of specimens was of *T. chilonis* and minimum of *T. flandersi*. Based upon internal transcribed spacer 2 (ITS2) region of the rDNA Yu-di *et al.* (2017) did molecular identification of *Trichogramma* species from the parasitized eggs of lepidopteran pests from 21 sampling sites in East Asia and South-East Asia. They identified six *Trichogramma* species; *T. chilonis, T. evanescens*, *T. ostriniae, T. embryophagum, T. dendrolimi* and *T. japonicum*.

The molecular techniques represent a valuable alternative to the taxonomy of *Trichogramma*. As the correct identification of the natural enemy in a biological control program is important for successful pest control. Our results confirm the successful establishment of *T. chilonis* in sugarcane ecosystem under Punjab conditions. The generated data will be beneficial for the potential commercial use of these beneficial insects and the execution of conservation biocontrol strategies to promote them.

SUMMARY

Trichogramma chilonis (Ishii) is of great importance to biological control of many harmful lepidopteran pests of crops. There is a need to establish the species composition in the fields for differentiation among the species/strains in order to enhance efficiency of their practical application. This study aimed to catalogue the species of *Trichogramma* available in sugarcane in Punjab. The populations were collected from different districts of Punjab, viz. Gurdaspur, Shaheed Bhagat Singh Nagar, Patiala, Amritsar and Fazilka during 2017–18. The mtCOI gene of *Trichogramma* was amplified and sequenced for identification of species and assessing the genetic diversity. Nucleotide sequences of *Trichogramma* populations collected from different sugarcane growing districts of Punjab showed 99% homology with *T. chilonis*. Multiple alignments of mtCOI sequences showed three branches with Punjab populations similar to New Delhi, Bengaluru and Pakistan. This study confirms the successful establishment of *T. chilonis* in sugarcane ecosystem in Punjab.

REFERENCES

Chakraborty K. 2011. Report on egg parasitoids of *Scirpophaga incertulas* from Hemtabad, West Bengal. *Annals of Plant Protection Sciences* **19**: 213–14.

Folmer O, Black M, Hoeh W, Lutz R and Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**: 294–99.

Kaur R and Brar K S. 2008. Evaluation of different doses of *Trichogramma* species for the management of leaf folder and stem borer on basmati rice. *Journal of Biological Control* **22**: 131–35.

Monaghan M T, Balke M, Gregory T R and Vogler A P. 2005. DNA-based species delineation in tropical beetles using mitochondrial and nuclear markers. *Philosophical Transactions of the Royal Society of London Biological Sciences* **360**: 1925–33.

Nagaraja H and Mohanraj P. 2010. Three new species of *Trichogramma* (Hymenoptera: Trichogrammatidae) from southern India. *Journal of Biological Control* **24**: 203–09.

Pinto J D, Stouthamer R, Platner G R and Oatman E R. 1991. Variation in reproductive compatibility in *Trichogramma* and its taxonomic significance (Hymenoptera: Trichogrammatidae). *Annals of the Entomological Society of America* **84**: 37–46.

Querino R B and Zucchi R A. 2002. Intraspecific variation in *Trichogramma bruni* Nagaraja, 1983 (Hymenoptera: Trichogrammatidae) associated with different hosts. *Brazilian Journal of Biology* **62**: 665–69.

Rijesh K, Puja R K, Hegde R, Joseph A, Jalali S K and Kush A. 2012. Genetic diversity estimates in *Trichogramma* populations from sugarcane cropping systems in India *Karnataka Journal of Agricultural Sciences* **25**: 68–71.

Singh S, Shenhmar M, Brar K S and Jalali S K. 2007. Evaluation of different strains of *Trichogramma chilonis* Ishii for the suppression of sugarcane early shoot borer, *Chilo infuscatellus* Snellen. *Journal of Biological Control Control* **21**: 247–53.

Sharma S, Shera P S, Kaur R and Sangha K S .2016. Standardization of egg dosages for mass production of *Corcyra cephalonica* (Stainton). *Phytoparasitica* **44**: 459–64.

Shera P S, Sharma S, Jindal J, Bons M, Singh G, Kaul A, Kaur R and Sangha K S. 2017. On-farm impact of egg parasitoid, *Trichogramma chilonis* against maize stem borer, *Chilo partellus* in Punjab. *Indian Journal of Agricultural Sciences* **87**: 1412–15.

Yousuf M, Khan S, Ikram M and Singh K P .2016. Diversity of biological control agents *Trichogramma* from Punjab (India) and its importance in forestry and agroforestry systems. *Journal of Environment and Bio-Sciences* **30**: 541–47.

Yu-Di L, Mao-Lin H and Song K. 2017. Molecular identification of *Trichogramma* species from South and South-East Asia and natural Wolbachia infection. *Entomologica Fennica* **28**: 57–66.