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DIVERSITY AND ABUNDANCE OF PARASITOIDS OF *LIRIOMYZA TRIFOLII* IN NORTH-WESTERN HIMALAYAS, INDIA

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ABSTRACT
The serpentine leafminer, *Liriomyza trifolii* (Burgess) (Agromyzidae: Diptera) is an important polyphagous pest of a large number of vegetables, ornamentals, field crops and weeds. The present study was carried out to study the diversity of its parasitoids under different agro-climatic conditions of Himachal Pradesh. In total 15 species (11 Eulophids, 2 Braconids, 1 Pteromalid and 1ceraphronid) were recorded. Parasitoid diversity varied with agro-climatic conditions and agroclimatic zone II (sub-temperate, sub-humid mid-hills) was the richest in diversity having all the 15 species followed by zone I (sub-tropical low-hills) and IV (dry temperate high-hills) (8 species each) and zone III (wet temperate high-hills) (4 species). Percent parasitization of the miner varied from 10.1-36.5, 11.2-35.3, 8.2-29.1 and 6.4-14.7 in zone I, II, III and IV, respectively. Shannon diversity index, species richness, species evenness and species dominance varied from 0.99 to 1.58, 1.95 to 2.64, 0.51 to 0.60 and 0.40 to 0.49, respectively. The diversity and population density of the parasitoids present in the Himalayan region has great potential for effective management of the leafminer in the region.

INTRODUCTION

The serpentine leafminer, *Liriomyza trifolii* (Burgess) (Agromyzidae: Diptera) is an important polyphagous pest infesting over 70 host plants representing vegetables, ornamentals, field crops and weeds (Kapadia, 1994) and during peak period of activity it can cause up to 45.95 per cent infestation in tomato (Mandloi *et al*., 2015). Damage is mainly caused by the maggots which mine into the leaves resulting in drastic reduction in water contents and photosynthesis of the foliage (Johnson *et al*., 1983; Yildirim *et al*., 2010). Besides, feeding punctures and egg laying wounds made by insertion of ovipositor by the females also cause minor injury (Parrella *et al*., 1985) and serve as entry points for different pathogens (Durairaj *et al*., 2010). *L. trifolii* is also responsible for reducing the aesthetic value of a large number of ornamental plants. This pest is believed to be introduced accidently into India during 1990 (Viraktamath *et al*., 1993) and has now been spread to all parts of the country attaining pest status on various crops. For the management of this pest, farmers rely mainly on the application of chemical insecticides. Nevertheless, indiscriminate use of these chemicals, in general, leads to many environmental consequences like pest resurgence, secondary pest outbreaks, insecticide resistance, elimination of beneficial organisms especially predators and parasitoids from the ecosystem and pesticide residues. *L. trifolii* is also notorious for the development of insecticide resistance (Keil *et al*., 1985; Murphy and LaSalle, 1999; Gouda *et al*., 2010). Development of insecticide resistance often leads to the use, by the farmers, of higher doses of insecticides at a reduced time interval which further aggravates this problem. In nature leafminers are known to have rich natural enemy fauna throughout the world. Noyes (Noyes, 2015) listed over 300 species of agromyzid parasitoids, and over 80 species are known to attack various *Liriomyza* species. Eulophid wasps are the most common parasitoids recorded on leafminers worldwide (Murphy and LaSalle, 1999; Minkenberg and Van Lanteran, 1986; Waterhouse and Norris, 1987; Konishi, 1998; Mekhlif and Abdul, 2002; Reina and LaSalle 2003; Chen *et al*., 2003; Tran, 2009).

In India the parasitoid fauna of other agromyzids, especially *Chromatomyia horticola* (Goureau) has been studied extensively (Ahmad and Gupta, 1941; Narayanan *et al*., 1956; Odak *et al*., 1968; Kaurava *et al*., 1969; Mani, 1971; Dubey, 1974; Mukherjee, 1975; Gokulpure, 1972; Khan, 1983; Kumar, 1985; Kumar, 1989; Kumar, 1990; Singh and Kumar, 1985; Khan, 1985a; Khan, 1985b; Khan, 1995, Hussain & Khan 1986, Khan & Sushil 1998, Sureshan & Narendran 2002, Purwar et al. 2003; Khan *et al*., 2005; Bhat and Bhagat, 2009; Bhat and Bhagat, 2011; Mahendran and Agnihotri, 2013; Yadav and Patel 2015), however, very less information is available on the parasitoid fauna of *L. trifolii*. There are few reports (Viraktamath *et al*., 1993; Hansson and LaSalle, 1996; Kapadia and Parmar, 1997; Upadhyay *et al*., 2001; Reina and LaSalle, 2004; Bhat *et al*., 2009; Narendran *et al*., 2001; Sharma *et al*., 2011) on the parasitoids of *L. trifilii* from the country, but, none of them were specifically aimed at to document the complete list of the parasitoid fauna of *L. trifolii*. Since the pest is exotic in nature, it is important to study the adaptation of the native parasitoids against the pest and to determine their role in natural control of the pest. The present study was therefore aimed at to study the diversity and abundance of parasitoids of *L. trifolii* (Burgess) in North-western Himalayas where the agro-climatic conditions are different from rest of the country.

MATERIALS AND METHODS

Study area

The present study was carried out in the Himachal Pradesh which is a mountainous state of India situated in North-west Himalayas having an area of 55673 km^2 between 350 and 7000 m amsl at 30°22′ 40′′ to 33°12′ 40′′ N latitude and 75° 45′ 55′′ to 79°04′ 20′′ E longitude. Due to huge variations in altitude, agro-climatic conditions also vary greatly at different parts of the state. Climate varies from hot and sub-humid-sub-tropical in the southern tracts to cold, alpine and glacial in the northern and eastern mountain ranges. Himachal Pradesh also varies greatly in rainfall and has areas like Dharamshala which receives as high annual rainfall as about 3400 mm, as well as Lauhal and Spiti that are cold deserts and almost rainless. Depending upon the agro-climatic conditions, Himachal Pradesh is broadly divided into four zones. Zone-I is sub-tropical sub-montane region comprising of low hills and valley areas up to an elevation of 914 m amsl. Zone-II is sub-temperate sub- humid mid hills with an altitude ranging from 915 to 1523 m amsl. This region is characterized by moderate to heavy monsoon rains. Zone-III on the other hand, is a wet temperate zone with high hills spanning between an altitude of 1524 and 2472 m amsl. Zone-IV of the state is dry temperate high hills representing areas beyond 2472 m amsl. This zone is characterized by very low rainfall during summer (less than 50mm) and heavy snow fall (3-5 m) during winter.

Collection of parasitoids

For the collection of parasitoids, field surveys were conducted in all the four agro climatic zones of Himachal Pradesh. The details of the locations surveyed are as under:

Zone	Location	Altitude(m amsl)	Latitude	Longitude
I	Nurpur	640	32.30° N	75.90° E
	Ghumarwin	670	31.45° N	76.68° E
II	Solan	1580	30.92° N	77.12° E
	Sarahan	1550	30.72°N	77.18°E
III	Rohru	1690	31.13°N	77.45° E
	Chopal	2190	30.95° N	77.58°E
IV	Sharbo	2290	31.54° N	78.27° E
	Pooh	2660	31.77°N	78.60° E

Leafminer infested leaves were collected periodically at random from three strata (top, middle and bottom) of tomato, cucumber and French bean plants from 5-6 different sites from each location which were then pooled to get a representative sample. These leaves were brought to the laboratory and examined under stereo zoom microscope for live, empty, dead and parasitized mines. The numbers of these mines (except dead mines) were added to get total mines. Collected mines were kept in plastic jars or glass vials in the laboratory at 25±0.5°C temperature and 70±5 per cent relative humidity for the emergence of parasitoids and/or adult flies of the miners. In order to study the overall diversity and abundance of parasitoids, all the samples for a particular agroclimtic zone were pooled. The samples were also pooled host plant wise to study the effect of host plants on the parasitoid diversity and abundance. Freshly emerged specimens of parasitoids were used to prepare permanent and temporary mounts for further identification.

Preparation of slides

For detailed microscopic observations on taxonomic characters, temporary and permanent mounts of specimens were prepared using standard procedures given by Willoughby and Koszrtarab (1974), Noyes (1982) and Khan *et al*. (2005).

Identification of parasitoids

The mounted specimens were examined under phase-contrast compound microscope (Olympus CX 41) and identified as per the keys and/ or morphological characters described by Mani (1971), Sureshan and Narendran (2002), Reina and LaSalle (2004), Khan *et al*. (2005) and Fisher *et al*. (2008). The specimens were also sent to National Bureau of Agricultural Insect Resources, Bangaluru, India for identification or confirmation of their identity.

Statistical analysis

Percent parasitization

The percentage of total parasitization and parasitization by each species was calculated using following formulae

$$\text{Total Parasitization (\%)} = \frac{\text{Total number of all the parasitoids obtained}}{\text{Total number of mines collected}}$$

$$\text{Parasitization by ith species} = \frac{\text{Total number of individuals of ith species}}{\text{Total number of mines}} \times 100$$

$$\text{Relative proportion of ith species} = \frac{\text{Total number of individuals of ith species}}{\text{Total number of all the parasitoids}} \times 100$$

Diversity indices: Diversity indices like Shannon diversity index, maximum diversity, species evenness and species dominance were calculated as per procedure given by Shannon (1948) which is described as under

Shannon diversity index (H) = $-\sum pi \log_e pi$; pi = fraction of ith species

Species richness/ maximum diversity ($H_{max.}$) = $\log_e k$; k = total number of species

Species evenness (J) = H/H_{max}

Species dominance (D) = 1 - J

RESULTS AND DISCUSSION

Diversity and abundance of parasitoids

During the present study, 15 species of hymenopteran parasitoids *viz*. *Diglyphus horticola* khan, *Diglyphus isaea* (Walker), *Cirrospilus* sp., *Hemiptarsenus varicornis* (Girault), *Pnigalio* sp., *Quadrastichus plaquoi* (Reina and La Salle), *Asecodes delucchii* (Boucek), *Asecodes erxias* (Walker), *Neochrysocharis formosa* (Westwood), *Chrysocharis* sp, *Chrysocharis indicus* Khan, (Eulophidae), *Opius exiguus* (Wesmael), *Dacnusa* sp. (Braconidae), *Cyrtogaster* sp., (Pteromalidae), and an unidentified ceraphronid were recorded from *L. trifolii* infesting tomato, cucumber and French bean (Table 1). Of these, *D. isaea, D. horticola, A. delucchii, A. erxias, C. indicus*, *Pnigalio* sp., *Cyrtogaster* sp., and Ceraphronid are new parasitoids of *L. trifolii* from India. Nevertheless, parasitoids like *Chrysonotomyia* sp, *Chirosilus*

Table1: diversity and abundance of parasitoids of *L. trifolii* in different agro-climatic zones

Parasitoid species	Zone I Parasitization (%)	Relative proportion(%)	Zone II Parasitization (%)	Relative proportion(%)	Zone III Parasitization (%)	Relative proportion(%)	Zone IV Parasitization (%)	Relative proportion(%)
Neochrysocharis formosa	14.26(7.3-22.3)	69.72	8.74(5.4-19.8)	54.65	10.69(3.5-12.7)	54.63	0.35(0-1.8)	4.00
Diglyphus isaea	2.91(0.4-14.2)	14.22	3.16(0.2-6.53)	19.79	5.43(0.1-9.4)	27.78	5.06(2.2-7.09)	57.60
Diglyphus horticola	0.09(0-1.2)	0.46	0.18(0-1.2)	1.12	0.72(0-1.5)	3.70	2.46(1.1-6.6)	28.00
Chrysocharis indicus	1.03(0-3.3)	5.05	0.83(0-4.7)	5.17	-	-	-	-
Chrysocharis sp	0.47(0-4.5)	2.29	0.82(0-4.5)	5.10	-	-	0.14(0-0.5)	1.60
Asecodes deluchhi	-	-	0.04(0-0.2)	0.22	-	-	-	-
Asecodes erxias	-	-	0.01(0-0.2)	0.07	-	-	-	-
Cirrospilus sp	0.09(0-0.3)	0.46	0.01(0-0.2)	0.07	-	-	-	-
Hemiptarsenus varicornis	0.47(0-1.4)	2.29	0.01(0-0.2)	0.07	-	-	-	-
Pnigalio sp	-	-	0.02(0-0.1)	0.15	-	-	0.21(0-1.1)	2.40
Quadrastichus plaquoi	-	-	0.66(0.2-4.8)	4.12	-	-	0.42(0-1.2)	4.80
Cyrtogaster sp	-	-	0.01(0-0.2)	0.07	-	-	-	-
Ceraphronid	-	-	0.08(0-1.6)	0.52	-	-	0.07(0-0.23)	0.80
Opius exiguus	1.13(0-6)	5.50	1.400.2-10.5)	8.77	2.72(0.2-4.4)	13.89	0.07(0-0.3)	0.80
Dacnusa sp	-	-	0.01(0-0.2)	0.07	-	-	-	-
Total	20.45(10.1-36.5)	100	15.19(11.2-35.3)	100	19.57(8.2-29.1)	100	8.78(6.4-14.7)	100

Figures in parantheses represent the range

variegatus Mani, *Chirispilus ambiguus*, *Asecodes* sp, *Closterocerus indica* Khan, Agnihotri and Sushil, *N. formosa, H. varicornis*, *Q. plaquoi, Quadrastichus* sp., *Diglyphus* sp., *Chrysocharis* sp., *Dacnusa* sp, *Opius* sp and *O. exiguus* were earlier reported from India attacking *L. trifolii* (Viraktamath *et al*., 1993; Hansson and LaSalle, 1996; Kapadia and Parmar, 1997; Kaushik, 1999; Upadhyay *et al*., 2001; Reina and LaSalle, 2004; Bhat *et al*., 2009; Narendran *et al*., 2001; Sharma *et al*., 2011). Parasitoid diversity varied with the agro-climatic conditions and the zone II which is characterized by sub-temperate, sub-humid mid-hill conditions was the richest in diversity having all the 15 species (Table 1). Agroclimatic zone III (Wet temperate high-hills), on the other hand, was the least diverse having only four species namely *D. isaea*, *D. horticola*, *N. formosa,* and *O. exiguus*. From zone I (sub-tropical low-hills) and IV (dry temperate high-hills) eight species each were recorded. Among them *D. horticola, D. isaea, N. formosa, Chrysocharis* sp, *O. exiguus*, *C. indicus, H. varicornis* and *Cirrospilus* sp. were active in zone I and *D. isaea*, *D. horticola*, *Chrysocharis* sp., *Sphegigaster* sp., *Q. plaquoi*, *N. formosa*, *Pnigalio* sp., *O. exiguus* and an unidentified ceraphronid were obtained from zone IV (Table 1). Diversity indices calculated as per Shannon (1948) reveal highest species richness (H_{max}) (2.64) for zone II followed by zone I and IV (2.08 each) and zone III (1.95) (Table2). Though the species richness of zone I and IV was same, zone IV could be considered slightly better in parasitoid diversity than zone I as the Shannon diversity index (H) for this zone was slightly higher (1.18) than zone I (1.06). Species evenness for zone I, II, III and IV was 0.51, 0.60, 0.51 and 0.57, respectively, indicating that 51, 60, 51 and 57 per cent of the recorded species in respective zones were evenly distributed. The parasitoid community of *L. trifolii* was dominated by *N. formosa* except in agroclimatic zone IV, where *D. isaea* and *D. horticola* emerged as the dominant parasitoids. The relative share of *N. formosa* in the total parasitoid community was 69.72, 54.65, 54.63 and 4.0 per cent in different agro-climatic zones (from I to IV, respectively) of the state (Table 1). The reason for the failure of *N. formosa* to outnumber other parasitoids in zone IV could be the dry temperate conditions of the region. N.formosa, perhaps, likes relatively warmer agro-climatic conditions as it has also been reported as the most dominant parasitoid of *L. trifolii* in mid hill conditions of Himachal Pradesh (Kaushik, 1999; Sharma *et al*., 2011). After *N.* formosa, *D. isaea, D. horticola* and *O. exiguus* were the common and widely distributed parasitoids present in all the zones of the state. Earlier, Kaushik (1999) reported *H. varicornis* as the second most dominant species after *N. formosa* in mid-hills of the state, however, in the present study it was recorded as very low in abundance. With time, *H. varicornis* might have gotten a stiff competition from other parasitoids especially *N. formosa* and might has been displaced competitively by *N. formosa*. Baigeng (2016) reported the occurrence of *H. varicornis*, *gronotoma* sp., *Opius* sp. and *Neochrysocharis* sp on *Liriomyza sativae* with dominance of H. *varicornis*. Association of *D. isaea, Diglyphus crassinervis* Erdos, *Pediobius metalicus* (Nees), *N. Formosa, Cirrospilus vittatus* walker, *Halticoptera circulus* (Walker), *Opius* sp. and *Ratzeburgiola incomplete* Boucek with *Liriomyza* spp. Has been reported from Iraq (Rassoul and Saffar, 2014). In a similar study, Hernandez *et al*. (2010) recorded 20 species belonging

Table 2: Indices of parasitoid diversity in different agro-climatic zones

Index	Zone I	Zone II	Zone III	Zone IV
Species richness/maximum diversity (H_{max})	2.08	2.64	1.95	2.08
Shannon index (H)	1.06	1.58	0.99	1.18
Species evenness (J)	0.51	0.60	0.51	0.57
Species dominance (D)	0.49	0.40	0.49	0.43

Table 3: Effect of host plants on the diversity and of parasitoids of *L. trifolii*

Parasitoid species	Host plants					
	Tomato		Cucumber		French bean	
	Parasitization (%)	Relative proportion(%)	Parasitization (%)	Relative proportion(%)	Parasitization (%)	Relative proportion(%)
Diglyphus isaea	3.40 (0.5-3.7)	20.45	1.57 (0-2.8)	12.50	6.31 (1-9.2)	36.54
Neochrysocharis formosa	9.19 (8.5-20)	55.30	6.27 (2.4-18)	50.00	5.87 (1.6-7.3)	33.97
Quadrastichus plaquoi	0.55 (0-1.9)	3.33	-	-	0.11 (0-1.1)	0.64
Opius exiguus	1.25 (0.6-10.5)	7.53	1.83 (0.2-12.2)	14.58	2.66 (0.8-15.4)	15.38
Chrysocharis sp.	0.80 (0.2-3.5)	4.80	-	-	0.22 (0-5.2)	1.28
Asecodes delucchii	0.02 (0-0.2)	0.13	-	-	0.11 (0-2.1)	0.64
Diglyphus horticola	0.44 (0-1.2)	2.66	-	-	1.66 (0-3.4)	9.62
Chrysocharis indicus	0.71 (0.2-4.7)	4.26	2.87 (1.7-7.4)	22.92	0.22 (0-5.3)	1.28
Dacnusa sp.	0.01 (0-0.2)	0.07	-	-	-	-
Cirrospilus sp.	0.02 (0-0.2)	0.13	-	-	-	-
Hemiptarsenus varicornis	0.07 (0-0.2)	0.40	-	-	-	-
Ceraphronid	0.09 (0-1.6)	0.53	-	-	-	-
Asecodes erxias	0.01 (0-0.2)	0.07	-	-	-	-
Pnigalio sp.	0.06 (0-0.2)	0.33	-	-	-	-
Cyrtogaster sp.	-	-	-	-	0.11 (0-0.4)	0.64
Total	16.63(13.9-35.3)	100	12.53 (9.3-28.6)	100	17.28 (14.2-36.2)	100
Shannon index (H)	1.43	1.22	1.46			
$H_{max.}$	2.64	1.39	2.20			
Evenness (J)	0.54	0.88	0.66			
Dominance (D)	0.46	0.12	0.34			

Figures in parentheses represent the range

to Eulophidae, Braconidae, figitidae and Pteromalidae from *Liriomyza* spp with the *N. formosa* as the most dominant. Pooling of data collected from different locations representing all the four agroclimatic zones of Himachal Pradesh reveal that the parasitization of *L. trifolii* ranged between 6.4 and 36.5 per cent with a mean of 16.48 per cent (Table 1). The results obtained during the present investigation agree with the results of Kaushik (1999) and Sharma *et al*. (2011) who reported 4.26 to 35.71 and 6.7 to 28.6 per cent parasitization of *L. trifolii* on tomato under mid-hill conditions of Himachal Pradesh.

Effect of host plant on parasitoid diversity and abundance: Three host plants namely tomato, cucumber and French bean were selected to study the effect of host plants on the diversity of parasitoids of *L. trifolii* and the results reveal that tomato was the most attractive host plant attracting 14 species namely *D. isaea*, *N. formosa*, *Q. plaquoi*, *O. exiguus*, *Chrysocharis* sp., *A. delucchii*, *D. horticola*, *C. indicus*, *Dacnusa* sp., *Cirrospilus* sp., *H. varicornis*, *A. erxias*, *Pnigalio* sp., *Cyrtogaster* sp. and an unidentified Ceraphronid to parasitize *L. trifolii*. Cucumber on the other hand was the least attractive host plant harbouring only four parasitoids *viz. D. isaea*, *N. formosa*, *O. exiguus* and *C. indicus*. On French bean, nine species of parasitoids namely *D. isaea*, *N. formosa*, *Q. plaquoi*, *O. exiguus*, *Chrysocharis* sp., *A. delucchii*, *D. horticola*, *C. indicus*, and *Cyrtogaster* sp. were associated with *L. trifolii* (Table 3). The total parasitization of *L. trifolii* on tomato, cucumber and French bean was 16.63, 12.53 and 17.28 per cent, respectively (Table 3). In a similar study, Foba *et al.* (2015) reported that the diversity and abundance of the parasitoids of *Liriomyza* spp vary with elevation, season and the host plant. These workers reported that tomato and beans were more attractive host plants for the parasitoids than other host plants which support the present results. Since the parasitoid diversity varied, though slightly, with host plant, it is pertinent to sample all the host plants to harness maximum diversity of the parasitoids, yet, the tomato remains as the key host plant for the collection of parasitoids of *L. trifolii* as it had attracted even the less abundant parasitoids. The variations in the attraction of parasitoids among different host plants could be attributed to the differences in semiochemicals, especially kairomones present in different host plants, nevertheless, a detailed study on this aspect is required.

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