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Occurrence of eulophid parasitoids on apple leaf miner *Lyonetia clerkella* Linn. (Lepidoptera: Lyonetiidae) in Kashmir

[*] Sumi ulah Rather, Abdul A. Buhroo, Abdul Lateef Khanday
Postgraduate Department of Zoology, University of Kashmir, Hazratbal, Srinagar, Jammu and Kashmir, India

Abstract
Ten eulophid parasitoids, viz., *Achrysocharoides* sp., *Baryscapus* sp., *Chrysocharis* sp., *Chrysonotomyia* sp., *Closterocerus* sp., *Minotetrastichus* sp., *Pnigalio* sp., *Quadrastichus* sp., *Stenomesius* sp. and *Sympiesis* sp. were recorded for the first time as parasitoids on the larvae of apple leaf miner, *Lyonetia clerkella* Linn. (Lepidoptera: Lyonetiidae). Among the eight sites (S1-S8) chosen for the study, the parasitism of leaf miner larvae was observed from June (2015) onwards upto October (2015) coinciding with the population of leaf miner larvae. Among the parasitoids, *Chrysocharis* sp. was observed dominant recording 47.8% parasitism followed by *Chrysonotomyia* sp. (19.65%), *Minotetrastichus* sp. (8.18%), *Pnigalio* sp. (8.8%), *Sympiesis* sp. (5.81%), *Stenomesius* sp. (4.4%), *Closterocerus* sp. (2.98%), *Quadrastichus* sp. (1.72%), *Achrysocharoides* sp. (0.78%) and *Baryscapus* sp. (0.15%) respectively.

Keywords: *lyonetia clerkella*, eulophid parasitoids, leaf miner larvae, lyonetiidae

1. Introduction
Apple leaf miner *Lyonetia clerkella* is a key pest particularly in the late spring and early summer in Kashmir where it causes severe damage to the apple trees followed by heavy defoliation prior to harvest [16]. It is also considered as a major insect pest in commercial peach orchards in Japan where it causes heavy defoliation prior to harvest [14, 15]. This pest occurs all over Europe, North-Western Siberia, the Far East, Northern Africa, the Middle East, Turkey, India and Japan and damages a wide variety of fruits including apple, pear, cherry, plum, peach, quince [2]. It is considered as one of the most destructive pests in East Asia where it causes defoliation even when the leaves are infested merely by few larvae [3].
In general, each mining insect consumes only a relatively small quantity of leaf substance during the course of its life. There are nevertheless a few species which must be considered as distinct pests. They occur as such either when they appear in excessive numbers, as in some years with *L. clerkella* when on some trees no single leaf is spared, or when they appear so early that substantial damage is caused to the development of the very young plants. Damage caused by excessive numbers is larger than might immediately be expected. The larvae of *L. clerkella* live and feed inside the leaves, consuming the mesophyll without damaging the leaf epidermis. Their feeding mines are externally visible in leaves as whitish or grey areas with variable lengths that range from narrow linear galleries to wide chambers [8]. The galleries excavated by the larvae of *L. clerkella* can reduce the photosynthetic capacity of leaves, cause premature leaf abscission, and permit pathogen entry into plant tissue [18, 12, 13].
One must consider not only the parts of the plant destroyed by feeding, which are no longer available for the synthesis and translocation of foodstuffs; far larger areas of the leaves are "ringed" by the activity of the miners; the vessels for conducting sap are severed, translocation is interrupted and nutrient materials from these areas can no longer serve the plant as a whole. Furthermore, large-scale damage is caused, even before desiccation processes commence. In years in which miners occur in large numbers, the results of their activity can be seen in a reduction in the growth of wood in the plant.
The current interest in the development of management systems of this serious pest requires information on its natural enemies and their role in pest population regulation. Hymenopterans parasitoids arc among the most important natural enemies of leaf miners and have, therefore, received much attention [21, 5, 1]. Among the parasitic Hymenoptera, the Ichneumonidae with their numerous species are found on mining larvae. Proctotrupidae are found less frequently in mines, while the Cynipidae, parasitic gall-wasps, are found most rarely of all and have been bred almost exclusively from species of *Phytomyza*. Parasitic fungi also play a part in the lives of mining insects. Stary [19] pointed out that the larvae of *L. clerkella* frequently succumb to muscardine.
To understand the changing scenario of any pest it is essential to study the diversity of natural enemies and the intensity of parasitism from time to time of the concerned pest. Hence, the objective of the present study was to document the species of parasitoids of apple leaf miner which may be advantageous in the development of a management for this pest using biological control agents.

2. Material and methods
Study sites
In order to study the occurrence of parasitoids on the apple leaf miner *Lyonetia clerkella* Linn. (Lepidoptera: Lyonetiidae) in the Kashmir Valley, preliminary field surveys were

conducted in June 2015. Eight sites (S1-S8) were selected in four study areas (Table 1). Spatial information regarding sample sites were recorded in the form of latitude and longitude with the help of handheld GPS (Garmin eTrex 10) and then file was exported to Esri ArcGIS 9.2.1 software. Subsequently data was coregistered with cartosat-1 satellite data to develop the layout (Figure 1).

Sampling procedure

Starting from June 2015, infested apple leaves were collected randomly at weekly intervals. A minimum of 20 infested leaves were collected every week from different sites (Table 1). Infested leaves were sectioned carefully and examined for healthy and parasitized larvae under stereomicroscope LEICA (M205). After observing the parasitized larvae, infested leaves were kept in glass bottles @ 10–15 leaves/bottle of 500 ml capacity covered with a thick muslin cloth and kept under lab conditions (temperature 20-30°C; relative humidity 80-90%). The bottles were observed daily for any parasitoid emergence. The adult parasitoids were isolated and preserved in 70% ethanol. The specimens were identified up to genus level through the standard key provided by Boucek [6].

The level of percent parasitism was calculated by the following formula.

$$\text{Percent Parasitism} = \frac{\text{No. of each species}}{\text{Total number of parasitoids}} \times 100$$

3. Results and discussion

During the present investigation, ten larval parasitoids namely, *Achrysocharoides* sp., *Baryscapus* sp., *Chrysocharis* sp., *Chrysonotomyia* sp., *Closterocerus* sp., *Minotetrastichus* sp., *Pnigalio* sp., *Quadrastichus* sp., *Stenomesius* sp. and *Sympiesis* sp. all belonging to Eulophidae have been recorded as larval parasitoids on apple leaf miner (Table 2 and Figures 2 & 3). This is the first report of ten eulophid species as parasitoids of *L. clerkella*. Among the recorded parasitoids, *Chrysocharis* spp. were earlier reported as endoparasitoids of leaf mining larvae of Gracillariidae and also of other insects belonging to Diptera, Lepidoptera, Coleoptera and Hymenoptera [23-24, 17]. Adachi [1] reported 19 species of parasitoids belonging to the families Eulophidae (17 species), Pteromalidae (1) & Braconidae (1) from larvae of the peach leaf miner *L. clerkella* in Japan with *Chrysocharis nitetis* as the most frequent parasitoid. Among the larval instars of apple leaf miner, the second and third instar larvae were generally parasitized. For many parasitoids, preference for different stages of hosts for parasitization was reported [7, 4]. Parasitism at the third instar stage may be economically important from the pest management point of view, since in apple orchards, a large portion of the damage and reduction of leaf area was found to be caused by the third instar leaf miner larva [9]. Similarly, preference of *Chrysocharis pentheus* for the third instar larvae of citrus leaf miner *Phyllocnistis citrella* and third instar of *Phytomyza ranunculi* for oviposition and feeding, respectively were documented [20, 11].

Among the parasitoids, *Chrysocharis* sp. was observed dominant recording 47.8% parasitism followed by *Chrysonotomyia* sp. (19.65%), *Minotetrastichus* sp. (8.18%), *Pnigalio* sp. (8.8%), *Sympiesis* sp. (5.81%), *Stenomesius* sp. (4.4%), *Closterocerus* sp. (2.98%), *Quadrastichus* sp. (1.72%), *Achrysocharoides* sp. (0.78%) and *Baryscapus* sp. (0.15%) respectively (Table 2). Parasitoids namely *Cirrospilus* sp., *Sympiesis* sp. (Eulophidae) and *Chelonus* sp. (Braconidae) were reported on cashew leaf miner in Karnataka and Kerala respectively, and a parasitism of 35 % by *Chelonus* sp. and 59 % by *Sympiesis* sp. were reported in those states [21, 5]. Vanitha [22] reported three eulophid parasitoids, viz., *Chrysocharis* sp., *Closterocerus* sp. and *Aprostocetus* sp. on the larvae of *Acrocercops syngramma* with *Chrysocharis* sp. as dominant recording 99.0 % abundance.

During the present investigation, the sudden decline in the population of this pest in the second half of the summer season could be mainly due to the biological control agents like insects, fungi and parasitoids that regulate the population of the leaf miners. Insects like beetles and spiders lay eggs on or into the developing miner caterpillars inside the mines and after hatching, the larvae of these insects consume the miner caterpillars [10]. The weather seems to play no role in the successive reduction of these miner microlepidopterans. However, it was seen that the degree of infestation is less in the insecticide sprayed apple orchards as compared to unsprayed ones [16].

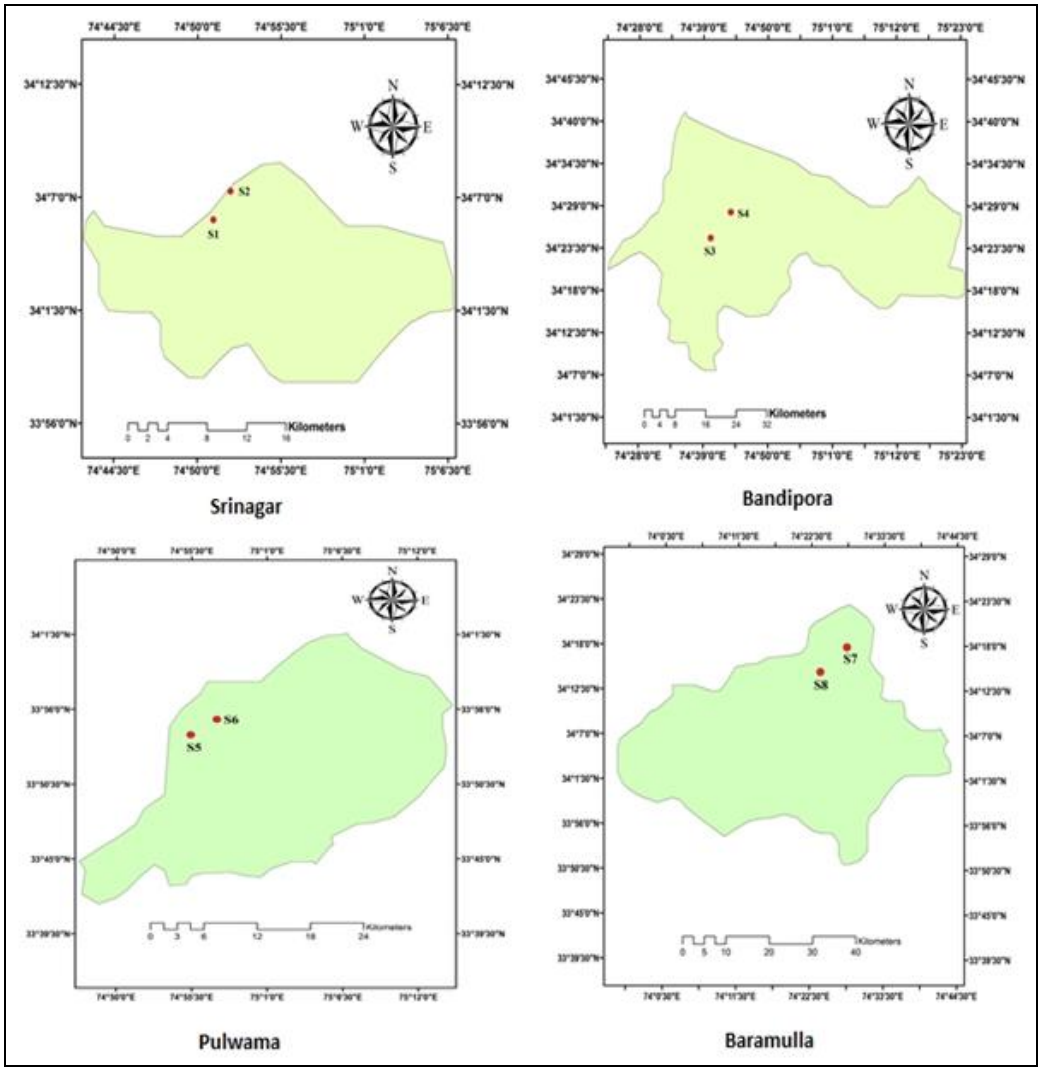

Fig 1: Map showing the sampling sites

Fig 2: Parasitoid larva (p) on the larva of *Lyonetia clerkella* (L). Both of them were extracted from the mine when Parasitoid larva had been observed consuming the larva of *L. clerkella* externally in the mine.

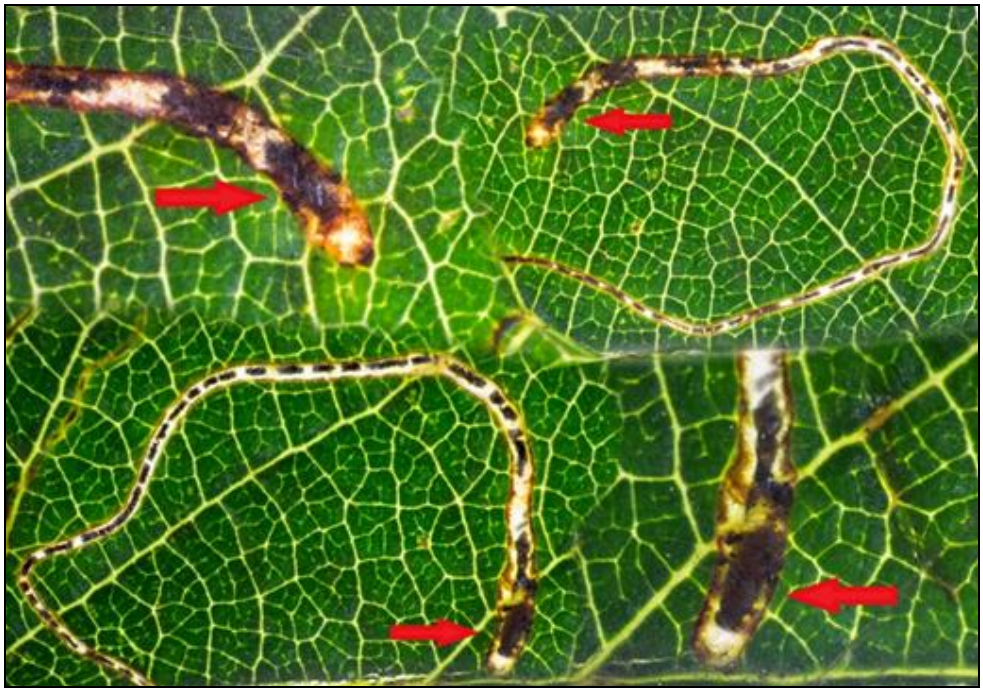

Fig 3: The pupae of parasitoids (arrows) at the mine terminals. They have consumed the caterpillars of *Lyonetia clerkella* during their larval periods and are now passing their pupal phase at the mine terminals where the caterpillars of *L. clerkella* were previously been located before being consumed by the parasitoid larvae.

Table 1: Sampling sites (S1-S8) chosen for the collection of *L. clerkella* parasitoids

Study area	Sample sites	GPS location
Srinagar	Kashmir University Campus (S1)	34.1290794^0 N, 74.8440675^0 E
	Zakura (S2)	34.1659407^0 N, 74.8303291^0 E
Bandipora	Gundpora (S3)	34.3946476^0 N, 74.6650689^0 E
	Koil Muqaam (S4)	34.4400112^0 N, 74.6286946^0 E
Pulwama	Ratnipora (S5)	33.9208324^0 N, 74.9551653^0 E
	Gudoora (S6)	33.9056965^0 N, 74.9070736^0 E
Baramulla	Zehanpora (S7)	34.1389672^0 N, 74.2342870^0 E
	Sopore (S8)	34.2875940^0 N, 74.4672050^0 E

Table 2: The number of specimens collected from four districts and percent parasitization of *L. clerkella* parasitoids

District		Srinagar		Bandipora		Pulwama		Baramullah		
S. NO.	Genus	T S (S1+S2)	% P	T S (S3+S4)	% P	T S (S5+S6)	% P	T S (S7+S8)	% P	Total % P
1	*Achrysocharoides sp.*	2	0.6	2	1.28	0	0.00	1	1.51	0.78
2	*Baryscapus sp.*	1	0.3	0	0.0	0	0.00	0	0.00	0.15
3	*Chrysocharis sp.*	140	46.5	80	51.3	58	51.32	24	36.36	47.48
4	*Chrysonotomyia sp.*	61	20.3	28	17.94	22	19.46	14	21.21	19.65
5	*Closterocerus sp.*	19	6.3	0	0	0	0.00	0	0.00	2.98
6	*Minotetrastichus sp.*	38	12.6	9	5.78	5	4.42	0	0.00	8.18
7	*Pnigalio sp.*	7	2.3	20	12.82	17	15.04	12	18.18	8.8
8	*Quadrastichus sp.*	2	0.6	3	1.92	2	1.76	4	6.06	1.72
9	*Stenomesius sp.*	27	9	1	0.64	0	0.00	0	0.00	4.4
10	*Sympiesis sp.*	4	1.3	13	8.33	9	7.96	11	16.66	5.81

T S - Total species, % P - Percent parasitization, S1-S8- Study sites

4. Conclusions

The destructive phase of this microlepidopteran is the larval stage. The larvae form mines in the leaves and these mines often cross the midrib and block the circulation of sap and other vital nutrients. This causes the leaves to dry which ultimately minimizes the process of photosynthesis and therefore reduces the yield. During the field trips, it was observed that the orchards having apple trees when sprayed with insecticides, showed least degree of infestation. However, the unsprayed orchards displayed the abundance of mines and were initially severely affected and later managed by natural enemies especially parasitoids. Therefore identifying parasitoid species and investigating the relative abundance of both the leaf miner and the parasitoids in various localities could provide information on effective biological agents for future management decisions and there would be no need of insecticidal spray to manage leaf miner unless required.

5. References

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