Research Article

Seasonal Biodiversity of Braconidae (Hymenoptera) in Citrus Orchards of Sargodha, Pakistan

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Abstract | The present study was conducted to investigate the seasonal biodiversity of braconid parasitoids in citrus orchards of Sargodha, Pakistan. Surveys were done during January 2014 to December 2015 from various citrus growing localities. A total of 3,176 parasitoids belonging to five subfamilies (Alysiinae, Aphidiinae, Braconinae, Microgastrinae and Opiinae), 12 genera and 16 species were collected. Out of them, two genera and 12 species were recorded for the first time from this area. Aphidiinae contains 1,107 individuals which showed that it's a dominant braconid subfamily while Opiinae (209) was the least dominant. The richness of braconid parasitoids in different localities of Sargodha was also investigated. Braconid parasitoid populations were higher in the months of February to April and October to December, while less abundant in the remaining months of the year.

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Introduction

Biodiversity of natural enemies such as parasitoids in citrus orchards plays a very important role in the reduction of pest populations (Flint and Dreistadt, 1998). Braconid wasps are most plentiful and diverse among groups of parasitoids and they parasitize and finally kill their hosts (La Salle and Gauld, 1993; Shaw and Huddleston, 1991). Dipterous, coleopterous and lepidopterous larvae are the most common hosts of these wasps (Sharkey, 1993; Van Achterberg, 1993; Wharton, 1997). Braconids are also important parasitoids of aphids (Barahoei et al., 2012).

Braconid wasps have diverse biological and behavioural adaptations and they tend to attack on a very constricted host range, having good searching ability even at low host densities, which makes them an efficient group for biological control programs and also as indicators of ecosystem health (Avila et al., 2013; Campos, 2001; Chay-Hernandez et al., 2006; Delfin and Burgos, 2000; La Salle and Gauld, 1993; Shaw and Huddleston, 1991; Shaw, 1994; Wharton, 1993; Whitfield and Wagner, 1988).

Braconid parasitoids have been well explored in various parts of the world but little work has been done on these biological control agents in northern areas of Pakistan. Inayatullah and Karimullah, 1996 reported 12 subfamilies of Braconidae and gave illustrative keys to the subfamilies of braconid wasps of the North-West Frontier Province (NWFP) of Pakistan. Inayatullah and Naeem, 2004 recorded 18 subfamilies from NWFP. Among these 6 subfamilies were recorded first time from NWFP. Irshad, 2008 reported 66 species of Braconidae from Pakistan.

Bodlah et al. (2012a, b) described the genera *Binodoxys* Mackauer and *Praon* Haliday (Aphidiinae: Braconidae) from arid tracts of the Punjab Province of Pakistan. Inayatullah et al., 2013 recorded the Genus *Aphaereta* Foerster (Alysiinae: Braconidae) from the Khyber Pakhtunkhwa Province of Pakistan, with taxonomic and biological notes on *Aphaereta pallipes* (Say). Tahira et al., 2013 studied genera of the subfamily Euphorinae (Braconidae: Hymenoptera) from Khyber Pakhtunkhwa Province, 4 genera were recorded for the first time from this area. Fakhruddin and Inayatullah, 2015 recorded 23 species of Microgastrinae under 9 genera (*Apanteles* F., *Cotesia* C., *Dolichogenidea* V., *Diolcogaster* A., *Glyptapanteles* A., *Snellenius* W., *Microplitis* F., *Microgaster* L. and *Paroplitis* W.) from Khyber Pakhtunkhwa Province. Ullah et al., 2015 reported 8 genera of Aphidiinae parasitoids including *Aphidius*, *Binodoxys*, *Diaeretiella*, *Ephedrus*, *Monoctonus*, *Praon*, *Toxares* and *Trioxys* from the District Dera Ismail Khan.

However, no efforts have been made to explore the braconid parasitoids fauna of Sargodha. Hence, an extensive taxonomic survey of the Braconidae parasitoids was carried out in different citrus growing localities of Sargodha to determine their seasonal biodiversity in relation to temperature and relative humidity.

Research objectives

The objectives of the proposed study are as under:

1. Investigation of biodiversity of the braconid wasps in different citrus growing localities of Sargodha.
2. Determination of the most prevalent species of braconids in citrus growing localities of Sargodha in different seasons using suitable diversity index analysis.
3. Determination of effect of temperature and relative humidity in citrus growing localities of Sargodha on braconid biodiversity.
4. Molecular identification/DNA sequencing of closely related braconid species using Lep F and Lep R primers.

Materials and Methods

The present study was conducted during 2014-2015 to investigate the biodiversity of Braconidae (Hymenoptera) in citrus growing localities of Sargodha.

Study region and sampling locations

The adult parasitoid wasps were collected by using hand net and installing Malaise traps during the years 2014-2015 from six citrus growing localities (Bhalwal, Kot Momin, Sahiwal, Sargodha, Shahpur and Sillanwali) of Sargodha district, Pakistan. Three Malaise traps were installed in each locality, in total eighteen traps were installed throughout Sargodha district. The parasitoids collected with the help of sweeping net were killed in a poison bottle containing potassium cyanide while 70% ethanol was used for Malaise trap collections.

Mounting and preservation of braconid parasitoids

Collected specimen were brought to the lab, washed thoroughly with 5-10% soap solution, rinsed with distilled water to remove dust particles and wax material present on their bodies. These specimens were then dehydrated by passing through ascending grades of alcohol i.e. 70%, 80%, 90%, 95% and absolute by keeping them in each grade for about half an hour. Wings of these braconid wasps were spread by keeping these specimens in a drop of absolute ethanol and managed through insect pins. Specimens were mounted on microscopic slides by using Hoyer's medium (Distilled water 50cc, Gum arabic 30g, Chloral hydrate 200g, Glycerine 20cc and Glacial acetic acid 1-2cc).

Identification of braconid parasitoids

The parasitoids were identified up to the species level under a wild M_3B binocular microscope. The classification and terminology of family Braconidae has been followed by using Goulet and Huber, 1993; Van Achterberg, 1993; Kavallieratos et al., 2001; Tomanovic and Kavallieratos, 2002; Rakhshani et al., 2006 and Kos et al., 2012. Closely related species were also confirmed by DNA sequencing.

DNA sequencing for the confirmation of some braconid species

Braconid species were identified by using Lep F and Lep R primers for the amplification of target 658-bp fragment of cytochrome c-oxidase subunit I (COI) gene (Hajibabaei, 2006) in Earth and Life Institute, Université Catholique de Louvain, Belgium. The PCR reactions were performed in a final volume of 15 µl containing 1 µl of genomic DNA, 0.5 µM of each primer, 200 µM dNTP's, 1X buffer and 0.625 unit of Taq DNA polymerase (Roche). The thermocycling profile consisted of 94°C for 1 min; 6 cycles of 94°C

for 1 min, 45°C for 1 min and 30s, and 72°C for 1 min and 15s; followed by 36 cycles of 94°C for 1 min, 51°C for 1 min and 30s, and 72 °C for 1 min and 15s; with a final 5 min extension period of 72°C. Amplicons were sequenced by Macrogen Inc., Amsterdam. Insects were identified to species by comparing resulting COI sequence data to Gene Bank using BLAST.

Data analysis

The Simpson diversity index was conducted for the analysis of species richness and evenness in different citrus growing localities. In this diversity index, biodiversity and prevalence of braconid parasitoids were investigated in different citrus growing localities of Sargodha in different seasons. Temperature and relative humidity of the selected orchards were also correlated with braconid biodiversity.

$$D = 1 - \left(\frac{\sum n(n-1)}{N(N-1)}\right)$$

D= Simpson's diversity index; n=Total number of organisms of a particular species; N=Total number of organisms of all species; E (evenness)= $D/_{Dmax}$; D= Simpson's diversity index; D_{max} = ln(S) (D_{max} is the maximum possible value of D); S= Richness (total number of species in the community).

Results and Discussion

Present study regarding Braconidae fauna of Sargodha district of Pakistan has yielded 5 subfamilies (Alysiinae, Aphidiinae, Braconinae, Microgastrinae and Opiinae), 12 genera and 16 species from 3,176 collected specimens. Out of them, two genera (*Areotetes* and *Idiasta*) and 12 species (*Aphaereta basirufa* Granger, *Idiasta transiens* Samiuddin, Lipolexis *gracilis* Foerester, *L. scutellaris* Mackauer, *Cotesia flaviconchae* Riley, *C. vestalis* Haliday, *Diolcogaster andamanensis* Gupta and Fernandez-Triana, *Microplitis espinachi* Walker, *M. mediator* Haliday, *M. varicolor* Viereck, *Areotetes carinuliferus* Li and Van Achterberg and *Opius pallipes* Wesmael) were recorded for the first time from this area.

Subfamily alysiinae

A total of 680 parasitoids belonging to two species wit Lipolexi hin two genera (*Aphaereta* and *Idiasta*) were identified from various localities of District Sargodha during 2014-2015.

Key to the genera of Alysiinae parasitoids (based on adult females)

1. 1st sub marginal cell confluent with 1st discal cell, (RS+M) a absent (Figure 1a)....*Aphaereta* Foerster.
2. 1st sub marginal cell separated from 1st discal cell, (RS+M) a present; (Figure 2e).....*Idiasta* Foerster.

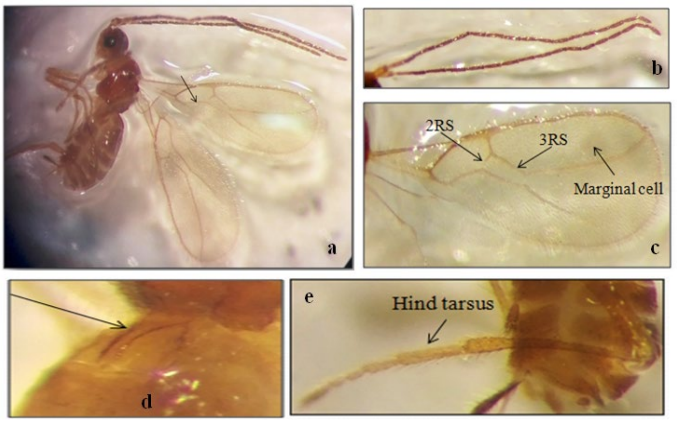

Figure 1: *Aphaereta basirufa Granger. a: External morphology; b: Antennae; c: Fore and hind wing; d: Propodeum; e: Hind tarsus.*

Genus *Aphaereta* Foerster

***Aphaereta basirufa* Granger:** (Figure 1a, b, c, d, e)**:** Head entirely smooth; antenna longer than body, 22–25 segmented (Figure 1a, b). Propodeum sculptured with long median longitudinal carina (Figure 1d). Length of fore wing 3 times its maximum width. Marginal cell reaching apex of wing; vein 3RS longer than 2RS (Figure 1c). 1st segment of hind tarsus (basitarsus) twice as long as 2nd segment (Figure 1e).

Material Examined: Kot Momin: 4♀ and 2♂, 6-ii-2014; 9♀ and 5♂, 6-iii-2014; 13♀ and 9♂, 21-iii-2014; 6♀ and 4♂, 6-iv-2014; 8♀ and 5♂, 21-xi-2014; 3♀ and 2♂, 6-xii-2014; 7♀ and 5♂, 21-ii-2015; 12♀ and 10♂, 6-iii-2015; 9♀ and 6♂, 6-iv-2015; 5♀ and 3♂, 21-iv-2015; 6♀ and 4♂, 21-ix-2015; 9♀ and 5♂, 6-xi-2015; 4♀ and 3♂, 21-xi-2015; **Sahiwal:** 5♀ and 2♂, 27-i-2014; 8♀ and 6♂, 27-ii-2014; 10♀ and 7♂, 12-iii-2014; 6♀ and 4♂, 27-iv-2014; 5♀ and 3♂, 27-ii-2015; 9♀ and 7♂, 12-iii-2015; 7♀ and 5♂, 27-iii-2015; 2♀ and 1♂, 12-ix-2015; 5♀ and 3♂, 12-xii-2015; **Sillanwali:** 4♀ and 3♂, 15-ii-2014; 11♀ and 9♂, 15-iii-2014; 5♀ and 3♂, 30-iv-2014; 6♀ and 4♂, 28-ii-2015; 10♀ and 8♂, 15-iii-2015; 8♀ and 6♂, 15-iv-2015; 2♀ and 2♂, 30-viii-2015

Genus *Idiasta Foerster*

***Idiasta transiens* samiuddin:** (Figure 2a, b, c, d, e): Head transverse dorsally; eyes large, clearly protruded; face pubescent, smooth and shiny; antenna 34 segmented (Figure 2a). Scutellum smooth and

shiny (Figure 2b). Wings slightly infuscate, veins and stigma brown, forewing vein 2RS somewhat longer than 3RS (Figure 2e). Ovipositor sheaths densely setose, extreme apex narrowed and sharply pointed (Figure 2c). Body yellowish-brown; apical metasoma brown to dark brown, subapical 6-10 segment whitish and rest of the segments brown (Figure 2d).

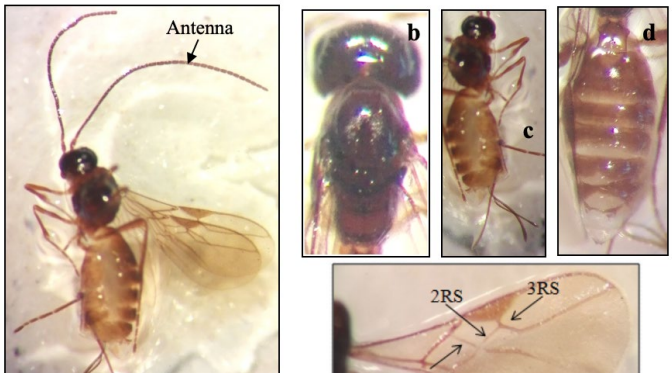

Figure 2: *Idiasta transiens Samiuddin a: External morphology; b: Scutellum; c: Ovipositor; d: Subapical 6-10 abdomonal segments; e: Fore wing.*

Material examined: Kot momin: 4♀ and 2♂, 6-ii-2014; 7♀ and 3♂, 6-iii-2014; 6♀ and 3♂, 21-iv-2014; 3♀ and 1♂, 21-i-2015; 5♀ and 2♂, 21-iii-2015; 2♀ and 1♂, 6-xi-2015; **Sahiwal:** 3♀ and 1♂, 12-iii-2014; 6♀ and 3♂, 27-iii-2014; 4♀ and 2♂, 27-iii-2015; 2♀ and 1♂, 12-iv-2015; **Sargodha:** 5♀ and 3♂, 1-ii-2014; 4♀ and 1♂, 16-ii-2014; 11♀ and 6♂, 1-iii-2014; 9♀ and 5♂, 16-iii-2014; 5♀ and 2♂, 16-iv-2014; 6♀ and 4♂, 1-xi-2014; 4♀ and 3♂, 16-ii-2015; 9♀ and 6♂, 1-iii-2015; 13♀ and 8♂, 16-iii-2015; 8♀ and 4♂, 1-iv-2015; 4♀ and 2♂, 1-ix-2015; 3♀ and 1♂, 16-xi-2015; **Shahpur:** 4♀ and 2♂, 24-i-2014; 3♀ and 1♂, 24-ii-2014; 9♀ and 5♂, 9-iii-2014; 12♀ and 9♂, 24-iii-2014; 7♀ and 4♂, 9-iv-2014; 2♀ and 1♂, 9-v-2014; 5♀ and 3♂, 24-x-2014;3♀ and 2♂, 24-ii-2015; 10♀ and 7♂, 9-iii-2015; 14♀ and 11♂, 24-iii-2015; 8♀ and 6♂, 9-iv-2015; 5♀ and 3♂, 24-iv-2015; 6♀ and 4♂, 9-x-2015; 4♀ and 2♂, 9-xi-2015; 4♀ and 3♂, 24-xi-2015.

Subfamily aphidiinae

As a result of extensive surveys conducted during January 2014 - December 2015 from various localities of District Sargodha, a total of 1,107 parasitoids belonging to five species within four genera (*Aphidius*, *Binodoxys*, *Diaeretiella* and Lipolexis) were identified.

Key to the genera of Aphidiinae parasitoids (based on adult females)

1. Last sternite modified into prongs, antennae 12 segmented (Figure 4a,b)....*Binodoxys* Mackauer
2. Last sternite not modified into prongs, antennae more than 11 segmented.....2
3. *Ovipositor sheath curved downwards*.....Lipolexis Forster
4. *Ovipositor sheath curved upwards*.....3
5. Wing venation extremely reduced, only radial vein developed, antennae 13-15 segmented (Figure 5b, c). ....*Diaeretiella* Stary
6. Both radial and median veins developed, antennae 15-22 segmented (Figure 3a, b). *Aphidius* Nees Genus *Aphidius* Nees

***Aphidius transcaspicus* Telenga:** (Figure 3 a, b, c, d, e) Antennae 15-17 segmented, F1 sub equal to F2 (Figure 3a, b). Maxillary and labial palpi 4 and 3 segmented respectively (Figure 3c). Propodeum with wide central areola which can be either complete, distinctly or indistinctly or partly incomplete (Figure 3e). Tergite-1 with antero lateral area costate (Figure 3d).

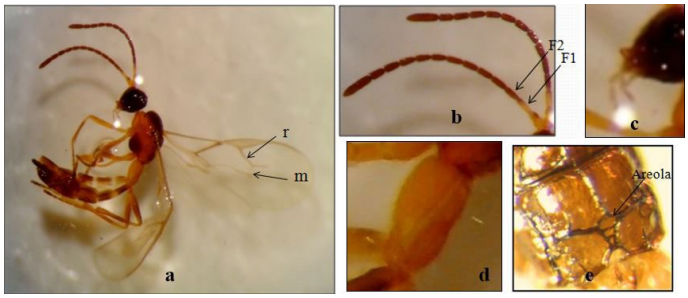

Figure 3: *Aphidius transcaspicus Telenga. a: External morphology; b: Antenna; c: Maxillary and Labial palpi d: Lateral view of tergite-1; e: Propodeum.*

Material Examined: Bhalwal: 4♀ and 2♂, 3-i-2014; 6♀ and 3♂, 3-ii-2014; 13♀ and 8♂, 18-iii-2014; 7♀ and 5♂, 3-iv-2014; 5♀ and 4♂, 3-x-2014; 7♀ and 3♂, 3-i-2015; 9♀ and 6♂, 18-iii-2015; 3♀ and 1♂,3-xii-2015; **Sahiwal:** 5♀ and 3♂, 27-i-2014; 14♀ and 11♂, 12-iii-2014; 19♀ and 16♂, 27-iii-2015;7♀ and 5♂, 12-iv-2015; 4♀ and 3♂, 12-ix-2015; **Sargodha:** 9♀ and 2♂, 16-ii-2014; 21♀ and 11♂, 1-iii-2014; 8♀ and 4♂, 16-iv-2014; 18♀ and 7♂, 1-iii-2015; 12♀ and 5♂, 16-iii-2015; 4♀ and 1♂, 1-xii-2015; **Shahpur:** 9♀ and 3♂, 24-iii-2014; 6♀ and 2♂, 9-iv-2014; 2♀ and 1♂, 24-xii-2014; 13♀ and 4♂, 9-iii-2015; 3♀ and 1♂, 9-xi-2015; 6♀ and 2♂, 24-xii-2015; **Sillanwali:** 11♀ and 2♂, 15-iii-2014; 5♀ and 1♂, 15-iv-2014; 14♀ and 3♂, 30-iii-2015; 3♀ and 1♂, 15-ix-2015.

Genus *Binodoxys Mackauer*

***Binodoxys indicus* Subba Rao and Sharma: (**Figure 4a, b, c**):** Antennae filiform, 11-12 segmented (Figure

4b). Wing metacarp broadly triangular, forewing radial vein longer than pterostigma (Figure 4a). Squarish propodeum with distinct pentagonal areola, upper areolae with 7-8 setae and lower with 1 seta only (Figure 4c).

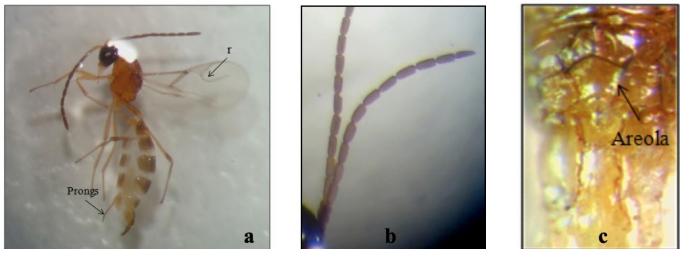

Figure 4: *Binodoxys indicus Subba Rao and Sharma. a: External morphology; b: Anntenna; c: Propodeum.*

Material Examined: Bhalwal: 11♀ and 9♂, 18-iii-2014; 5♀ and 4♂, 3-iv-2014; 12♀ and 5♂, 3-iii-2015; 4♀ and 3♂,3-x-2015; **Sahiwal:** 4♀ and 3♂, 27-ii-2014; 7♀ and 5♂, 12-iii-2014; 3♀ and 2♂,12-xi-2015; **Sargodha:** 10♀ and 8♂, 16-ii-2014; 6♀ and 5♂, 1-iv-2014; 16♀ and 14♂, 1-iii-2015; 8♀ and 7♂, 1-iv-2015; 5♀ and 4♂, 16-xii-2015; **Sillanwali:** 4♀ and 3♂, 30-i-2014; 11♀ and 9♂, 15-iii-2015; 2♀ and 2♂, 30-x-2015.

Genus *Diaeretiella Stary*

***Diaeretiella rapae* M'Intosh:** Wing venation extremely reduced, radial vein developed (Figure 5c). Antenna mostly 13-15 segmented, F1 and F2 of equal length (Figure 5a, b). Propodeum rectangular in shape with distinct pentagonal areola (Figure 5d). Highly variable body colouration ranging from yellow to black.

Figure 5: *Diaeretiella rapae M'Intosh. a: External morphology; b: Antenna; c: Fore wing; d: Propodeum.*

Material Examined: Bhalwal: 12♀ and 10♂, 3-iii-2014; 7♀ and 5♂, 18-iv-2014; 14♀ and 12♂, 18-ii-2015; 10♀ and 9♂, 3-ix-2015; **Kot Momin:** 8♀ and 6♂, 21-i-2014; 11♀ and 8♂, 21-iii-2014; 15♀ and 12♂, 6-ii-2015; 7♀ and 5♂, 21-x-2015; 10♀ and 8♂, 21-xi-2015; **Sahiwal:** 9♀ and 3♂, 12-ii-2014; 12♀ and 7♂, 27-iii-2015; 4♀ and 2♂, 12-v-2015; **Sargodha:** 8♀ and 5♂, 16-ii-2014; 13♀ and 12♂, 1-iv-2014; 17♀ and 15♂, 1-iii-2015; 9♀ and 7♂, 16-xii-2015; **Shahpur:** 5♀ and 3♂, 24-ii-2014; 9♀ and 5♂, 9-iv-2015; 3♀ and 2♂, 24-xi-2015; **Sillanwali:** 2♀ and 1♂, 30-iii-2014; 4♀ and 2♂, 28-ii-2015.

Genus Lipolexis *Foerster*

Lipolexis ***gracilis* Foerster: (**Figure 6a, b, c**):** Head sparsely to moderately hairy; eye sparsely pubescent; antenna with 12 segments, flagellar segments clothed with dense short hairs (Figure 6a, b). Forewing radius long, reaching almost wing margin (Figure 6c).

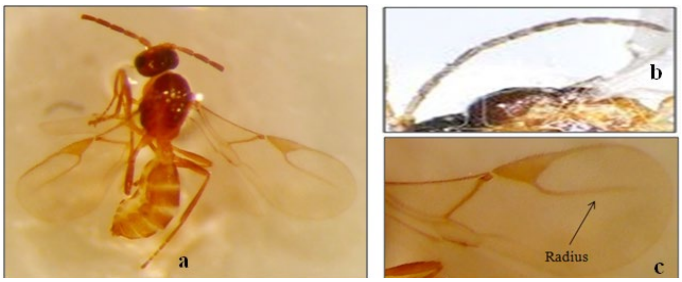

Figure 6: Lipolexis *gracilis Foerster. a: External morphology; b: Antenna; c: Fore wing.*

Material Examined: Kot Momin: 5♀ and 3♂, 21-ii-2014; 9♀ and 6♂, 6-iii-2014; 11♀ and 7♂, 21-iv-2014; 6♀ and 3♂, 21-ii-2015; 5♀ and 3♂, 6-iv-2015; 4♀ and 2♂, 21-viii-2015; **Sahiwal:** 4♀ and 2♂, 12-i-2014; 9♀ and 5♂, 27-iii-2015; 3♀ and 2♂, 12-xii-2015; **Sillanwali:** 3♀ and 1♂, 15-iii-2014; 2♀ and 1♂, 28-ii-2015.

Lipolexis ***scutellaris* Mackauer: (**Figure 7a, b, c**):** Head transverse to subquadrate, smooth, shiny and sparsely haired; eyes large, nearly hemispherical, sparsely haired, strongly prominent and convergent towards clypeus (Figure 7a, b). Forewing pterostigma triangular, twice as long as wide (Figure 7c).

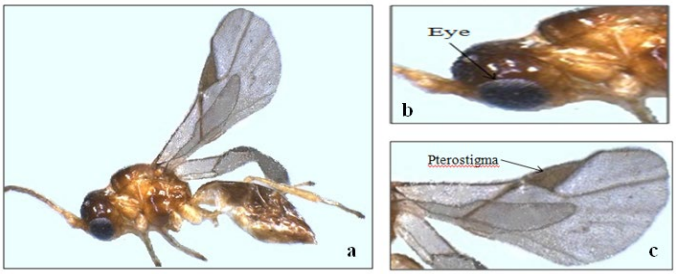

Figure 7: Lipolexis *scutellaris Mackauer. a: External morphology; b: Head; c: Fore wing.*

Material Examined: Bhalwal: 4♀ and 2♂, 18-i-2014; 7♀ and 3♂, 3-iii-2014; 5♀ and 2♂, 3-v-2014; 10♀ and 5♂, 18-iii-2015; 7♀ and 4♂, 3-x-2015;

Sargodha: 6♀ and 2♂, 16-i-2014; 8♀ and 5♂, 1-iii-2014; 5♀ and 3♂, 1-v-2014; 11♀ and 8♂, 16-ii-2015; 4♀ and 2♂, 1-iv-2015; 3♀ and 1♂, 16-iv-2015; 5♀ and 2♂, 16-xi-2015; **Sillanwali:** 5♀ and 3♂, 15-ii-2015; 2♀ and 1♂, 30-iii-2015.

Subfamily Braconinae

A total of 307 specimens belonging to one species in the Genus *Bracon* were identified.

Genus *Bracon Fabricius*

***Bracon hebetor* Say: (**Figure 8a, b, c, d**):** Antenna 20-23 segmented, sub moniliform, (Figure 8a, b). Veins l-SR and 3-SR short; marginal cell of fore wing medium sized, ending distinctly removed from apex of wing (Figure 8c). 2nd to 7th abdominal segments superficially granulate to (mainly) smooth, shiny (Figure 8d).

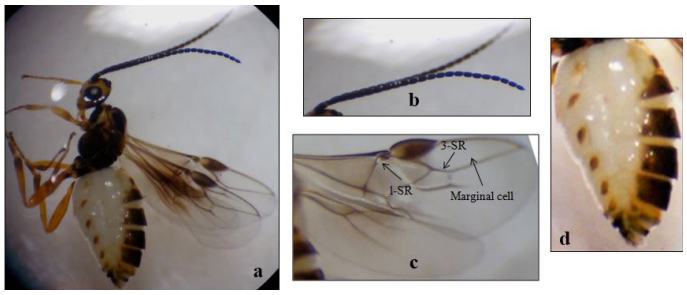

Figure 8: *Bracon hebetor Say. a: External morphology; b: Antenna; C: Fore wing; d: Abdomen.*

Material Examined: Bhalwal: : 4♀ and 2♂, 18-i-2014; 7♀ and 5♂, 3-ii-2014; 12♀ and 9♂, 18-iii-2014; 6♀ and 4♂, 3-v-2014; 1♀ and 1♂, 3-vi-2014; 5♀ and 2♂, 18-x-2014; 4♀ and 3♂, 18-i-2015; 8♀ and 6♂, 3-iii-2015; 5♀ and 3♂, 18-iv-2015; 2♀ and 1♂, 3-xii-2015; **Kot Momin:** 3♀ and 1♂, 21-i-2014; 8♀ and 5♂, 6-iii-2014; 13♀ and 9♂, 21-iii-2014; 6♀ and 3♂, 6-xi-2014; 7♀ and 4♂, 6-ii-2015; 10♀ and 6♂, 21-iii-2015; 8♀ and 4♂, 21-iv-2015; 4♀ and 2♂, 21-xii-2015; **Sargodha:** 5♀ and 3♂, 1-ii-2014; 7♀ and 4♂, 16-iii-2014; 9♀ and 6♂, 1-iv-2014; 2♀ and 1♂, 16-ix-2014;4♀ and 3♂, 16-i-2015; 11♀ and 9♂, 1-iii-2015; 8♀ and 6♂, 16-iii-2015; **Sillanwali:** 6♀ and 4♂, 30-i-2014; 10♀ and 8♂, 15-iii-2014; 7♀ and 5♂, 15-iii-2015; 4♀ and 2♂, 30-xi-2015.

Subfamily Microgastrinae

A total of 873 parasitoids belonging to six species within three genera (*Cotesia*, *Diolcogaster* and *Microplitis*) were identified.

Key to genera of Microgastrinae parasitoids (based on adult females)

- Forewing areolet absent (Figure 9, 10a)..... *Cotesia* Cameron
- Forewing areolet present, varying in size from large to small, sometimes slit like or spot like.2
- Forewing areolet triangular or quadrangular (Figure 12a, 13, 14c); 1CU-1 never more than about half length of 2CU-1 (Figure 13c)..... *Microplitis* Foerster
- Forewing areolet small and slit-like; 1CU-1 often equal in length to 2CU-1, sometimes less than half its length (Figure 11b).....*Diolcogaster* Ashmead

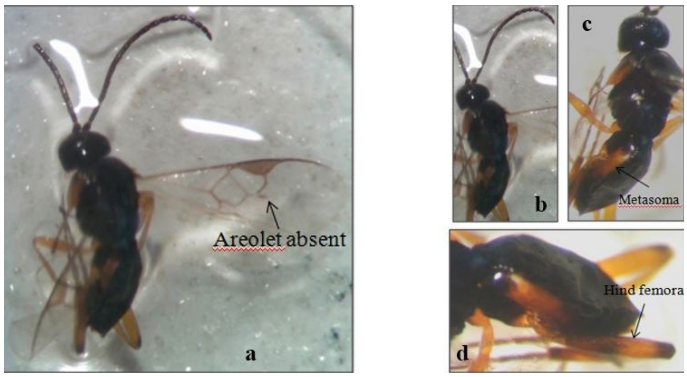

Figure 9: *Cotesia vestalis Haliday. a: External morphology; b: Antenna; c: Latero tergites of metasomal segments; d: Hind femora.*

Genus *Cotesia Cameron*

***Cotesia vestalis* Haliday: (**Figure 9a, b, c, d**):** Antennae long, reaching apex of metasoma or almost so (Figure 9a, b). Hind coxa mostly dark brown to black; hind femora mostly yellowish or orangish, sometimes darker at base or tip (Figure 9d). Latero tergites of first two metasomal segments mostly pale yellowish to orangish (Figure 9c). This species has a close resemblance to other species of *Cotesia* due to some characters on wings and thorax that's why this species was confirmed after DNA sequencing.

Sequence

GTTAAAGTTTAATCTTGGGATTATAGATCGGGATATTAGGATTTTCAATAAGTTTAATTATTCGTTT
AGAATTAGGAATACCTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCA
TGCATTTATTATAATTTTTTTTATAGTTATACCAGTAATAATTGGTGGATTTGGAAATTGATTAATT
CCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAATATAAGTTTTTGATTATTAA
TTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACAGT
TTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTAC
ATTTGGCTGGTGCTTCTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTC
TAATTTATTTAATATAGATAAAATATCTTTATTTTCTTGATCAGTATTTATTACAGCAATTTTATTAT
TATTATCTTTGCCTGTTTTAGCTGGTGCTATCACTATGTTATTAACTGATCGTAATATAAATACAAG
ATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTTGATTTTTTGGACAT
CAAGAAAGATTAAAATAGGGAAAGGATTAAAAGATTTTTTATT.

Material Examined: Bhalwal: 4♀ and 3♂, 18-ii-2014; 6♀ and 4♂, 3-iii-2014; 3♀ and 2♂, 3-v-2014; 7♀ and 5♂, 3-iv-2015; 3♀ and 2♂, 18-x-2015; **Kot**

Momin: 3♀ and 1♂, 6-ii-2014; 8♀ and 5♂, 21-iii-2014; 6♀ and 3♂, 21-ii-2015; 7♀ and 4♂, 6-iv-2015; 4♀ and 2♂, 21-xi-2015; **Sahiwal:** 4♀ and 2♂, 12-iii-2014; 8♀ and 5♂, 27-iii-2015; 6♀ and 2♂, 12-iv-2015; **Shahpur:** 2♀ and 1♂, 24-i-2014; 4♀ and 2♂, 9-iii-2014; 4♀ and 2♂, 24-iii-2015; 3♀ and 1♂, 24-iv-2015.

***Cotesia flaviconchae* Riley: (**Figure 10 a, b, c, d**):** Female antennae long, reaching apex of metasoma or almost so (Figure 10b). Fore wing not smoky over most of area (Figure 10a). Hind coxa mostly dark brown to black (Figure 10d). Hypopygium sharp acutely angled apically or blunt, obtusely or right angled apically (Figure 10c).

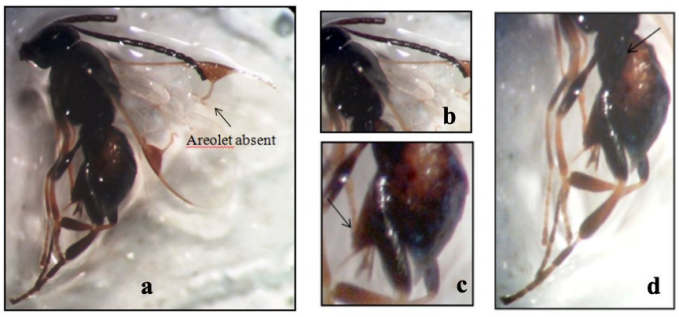

Figure 10: *Cotesia flaviconchae Riley. a: External morphology; b: Antenna; c: Hypopygium; d: Hind coxa.*

Material Examined: Kot Momin: 4♀ and 1♂, 21-i-2014; 9♀ and 4♂, 6-iii-2014; 6♀ and 3♂, 6-x-2014; 8♀ and 4♂, 21-iii-2015; 5♀ and 2♂, 6-ix-2015; 4♀ and 2♂, 21-xi-2015; **Sargodha:** 3♀ and 1♂, 16-i-2014; 5♀ and 2♂, 1-iii-2014; 8♀ and 5♂, 16-iii-2014; 4♀ and 2♂, 1-ii-2015; 6♀ and 3♂, 1-iv-2015; 3♀ and 2♂, 16-viiii-2015; **Shahpur:** 2♀ and 3♂, 24-ii-2014; 7♀ and 5♂, 24-iii-2014; 9♀ and 6♂, 9-iii-2015; 5♀ and 3♂, 9-iv-2015; 5♀ and 2♂, 24-xi-2015; 4♀ and 2♂, 9-xii-2015.

Genus *Diolcogaster Ashmead*

***Diolcogaster andamanensis* Gupta and Fernandez-Triana: (**Figure 11 a, b, c, d**):** Antenna dark brown, 17-18 segmented (Figure 11a). T1, T2 and anterior half of T3 yellow, T1-T3 occupying more than half of metasoma (Figure 11c). Hind femur dark brown except yellowish brown extreme base (Figure 11d). Fore wing areolet small, triangular, slit like (Figure 11b).

Material Examined: Sargodha: 3♀ and 2♂, 1-ii-2014; 5♀ and 3♂, 1-iii-2014; 10♀ and 6♂, 16-iii-2014; 4♀ and 3♂, 1-x-2014; 7♀ and 4♂, 16-iii-2015; 6♀ and 3♂, 1-iv-2015; 4♀ and 2♂, 16-ix-2015; 2♀ and 1♂, 16-xi-2015; **Shahpur:** 5♀ and 3♂, 9-ii-2014; 9♀ and 4♂, 24-iii-2014; 6♀ and 3♂, 9-iii-2015; 5♀ and 2♂, 24-iv-2015; 4♀ and 2♂, 9-xii-2015.

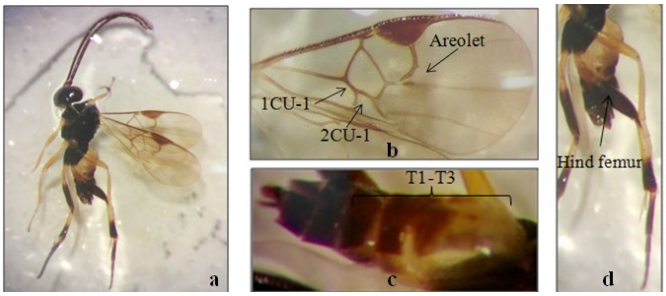

Figure 11: *Diolcogaster andamanensis Gupta and Fernandez-Triana. a: External morphology; b: Fore wing; c: Metasoma; d: Hind femur.*

Genus *Microplitis Foerster*

***Microplitis varicolor* Viereck: (**Figure 12a**):** This species has a close resemblance to *Microplistis espinachi* due to some confusing characters such as forewing areolet large, vein 3RS as long as vein r-m; metatarsus brown to black (Figure 12a) therefore, this species was confirmed by DNA sequencing.

Sequence

AAAAAATTTTATTTTTGGATTTAGATCGGGATATTAGGATTTTCAATAAGTTTAATTATTCGATTAG
AATTAGGAATACCCGGAAGTTTAATTGGAAATGAACAAATTTATAATAGAATTGTAACTTCTCATG
CATTTATTATAATTTTTTTTATAGTAATACCAGTTATAATTGGGGGATTTGGAAATTGATTAATTCC
ATTAATATTAGGATCTCCAGATATAGCATTTCCACGAATAAATAATATAAGATTTTGATTATTAAT
TCCTTCATTATTTTTATTAATTTTAAGAAGATTTATTAATGTAGGTGTTGGAACAGGATGAACTGTC
TATCCTCCATTATCTTTAATTTTAGGTCATGGTGGGTTATCTGTTGATATAAGAATTTTTGCTTTAC
ATTTAGCAGGTGCATCATCTATTATAGGTGCAGTTAATTTTATTACAACTATTTATAATATACGATC
GAATTATTTAGATATAGATAAAATTTCTTTATTTACTTGATCGGTATTAATTACAGCAATTTTATTA
TTATTGTCTTTACCAGTTTTAGCAGGTGCAATTACTATATTATTAACTGATCGAAATTTAAATACAA
GATTTTTTGATCCTTCAGGAGGAGGTGATCCAATTTTATATCAGCATTTATTTTGATTTTTTGGACA
TCGGGAAAATTTAAAATTTTAAAATGGGTGGGATTTGGGGGGT.

Figure 12: *Microplitis varicolor Viereck. a: External morphology.*

Material Examined: Bhalwal: 2♀ and 1♂, 18-i-2014; 5♀ and 2♂, 3-ii-2014; 9♀ and 5♂, 18-iii-2014; 6♀ and 3♂, 3-xi-2014; 4♀ and 2♂, 18-ii-2015; 7♀ and 3♂, 18-iii-2015; 4♀ and 1♂, 3-x-2015; **Kot Momin:** 4♀ and 2♂, 6-ii-2014; 11♀ and 6♂, 21-iii-2014; 7♀ and 4♂, 6-iv-2014; 5♀ and 3♂, 21-xii-2014; 8♀ and 4♂, 6-iii-2015; 3♀ and 1♂, 6-v-2015; 5♀ and 3♂, 21-ix-2015; **Sahiwal:** 3♀ and 1♂, 27-i-2014; 7♀ and 4♂, 12-iii-2014; 5♀ and 3♂, 27-iii-2015; 3♀ and 2♂, 27-iv-2015; 4♀ and 3♂, 12-viii-2015; **Sargodha:** 3♀ and 1♂, 16-ii-2014; 5♀ and 2♂, 1-iii-2014; 6♀ and 3♂, 1-iv-2014; 4♀ and 1♂, 16-iii-2015; 2♀ and 1♂, 16-xii-2015.

***Microplitis espinachi* Walker:** (Figure 13a, b, c): Fore wing areolet relatively larger, vein 3RS as long as vein r-m; 1CU-1 never more than about half length of 2CU-1 (Figure 13c). Metatibial spurs yellow and metatarsus brown to black (Figure 13a, b).

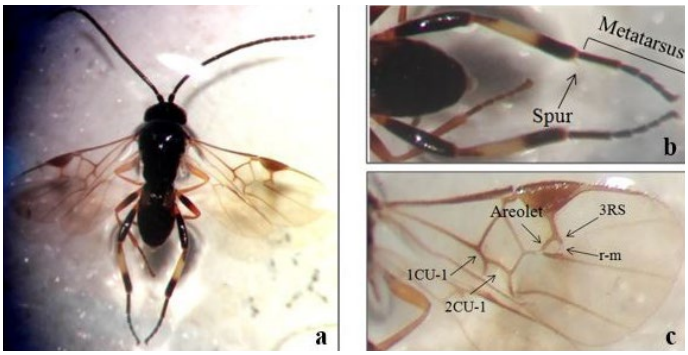

Figure 13: Microplitis espinachi Walker. *a: External morphology; b: Meta leg; c: Fore wing.*

Material Examined: Sahiwal: 4♀ and 3♂, 12-ii-2014; 9♀ and 5♂, 12-iii-2014; 7♀ and 4♂, 27-iv-2014; 5♀ and 3♂, 12-xi-2014; 10♀ and 7♂, 27-ii-2015; 8♀ and 5♂, 12-iii-2015; 3♀ and 1♂, 12-v-2015; 4♀ and 2♂, 27-ix-2015; 6♀ and 3♂, 27-xi-2015; 5♀ and 2♂, 12-xii-2015; **Shahpur:** 3♀ and 1♂, 24-i-2014; 7♀ and 3♂, 9-iii-2014; 8♀ and 5♂, 24-iii-2014; 5♀ and 2♂, 9-iv-2014; 4♀ and 2♂, 24-xi-2014; 2♀ and 1♂, 24-ii-2015; 5♀ and 3♂, 9-iii-2015; 8♀ and 4♂, 24-iii-2015; 6♀ and 3♂, 24-iv-2015.

***Microplitis mediator* Haliday: (**Figure 14a, b, c**):** Antenna often all black, long as the body; legs yellowish brown or red including posterior femur, tarsus dark (Figure 14a). Pterostigma often with basal yellow dot (Figure 14c). 2-3 abdominal tergites often unclear; tergite-1 narrow and almost all sculpted with a shiny bulge at the apex (Figure 14b).

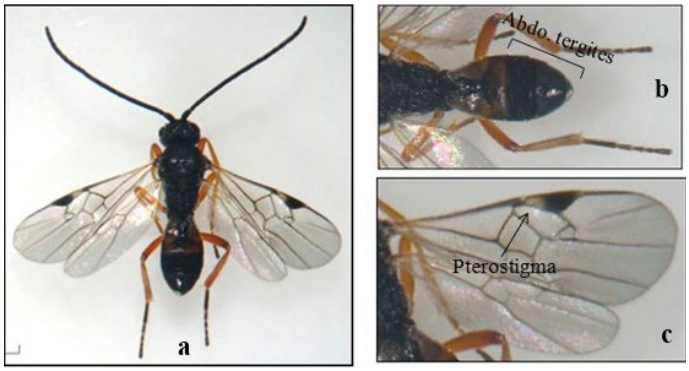

Figure 14: Microplitis mediator *Haliday. a: External morphology; b: Abdomen; c:* Fore wing.

Material Examined: Bhalwal: 3♀ and 2♂, 18-i-2014; 5♀ and 3♂, 3-iii-2014; 4♀ and 2♂, 3-xi-2014; 7♀ and 3♂, 18-iii-2015; 6♀ and 3♂, 3-iv-2015; 3♀ and 1♂, 3-xii-2015; **Kot Momin:** 5♀ and 3♂, 6-ii-2014; 8♀ and 6♂, 21-iii-2014; 6♀ and 4♂, 6-iv-2014; 4♀ and 2♂, 6-iii-2015; 6♀ and 4♂, 21-iii-2015; 5♀ and 3♂, 21-x-2015; **Sargodha:** 4♀ and 2♂, 16-ii-2014; 6♀ and 4♂, 1-iii-2014; 7♀ and 4♂, 16-iii-2015; 5♀ and 3♂, 1-iv-2015; 3♀ and 1♂, 1-xi-2015.

Subfamily opiinae

A total of 209 parasitoids belonging to two species within two genera (*Areotetes* and *Opius*) were identified.

Genus *Areotetes Li and Van Achterberg*

***Areotetes carinuliferus* Li and Van Achterberg: (**Figure 15 a, b, c**):** Antenna with 21 segments; face smooth (Figure 15a). Forewing pterostigma nearly elliptical; 1-R1 reaching wing apex; first subdiscal cell closed; CU1b short; vein 3-SR of fore wing slightly curved and twice as long as vein 2-SR (Figure 15b). Setae of hind femur long and of tibia moderately long; hind tibia with a long nearly straight carinula basally (Figure 15c).

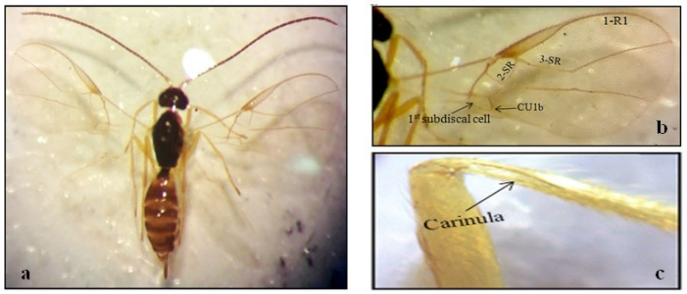

Figure 15: *Areotetes carinuliferus Li and Van Achterberg. a: External morphology; b: Fore wing; c: Hind femur and tibia.*

Material Examined: Kot Momin: 5♀ and 3♂, 21-iii-2014; 6♀ and 4♂, 6-iii-2015; **Sargodha:** 3♀ and 1♂, 16-ii-2014; 9♀ and 3♂, 16-iii-2014; 5♀ and 2♂,

1-iv-2014; 4♀ and 1♂, 16-xi-2014; 8♀ and 3♂, 1-iii-2015; 6♀ and 2♂, 16-iii-2015;4♀ and 1♂, 1-x-2015; **Shahpur:** 4♀ and 1♂, 9-ii-2014; 8♀ and 2♂, 24-iii-2014; 6♀ and 2♂, 9-iv-2014; 7♀ and 3♂, 9-iii-2015; 5♀ and 1♂, 24-iii-2015; 2♀ and 1♂, 9-ix-2015; 4♀ and 1♂, 24-xi-2015; **Sillanwali:** 2♀ and 1♂, 15-ii-2014; 3♀ and 2♂, 30-iii-2014; 1♀ and 1♂, 30-xi-2014; 3♀ and 1♂, 15-iii-2015.

Genus *Opius Wesmael*

***Opius pallipes* Wesmael: (**Figure 16 a, b, c**):** Fore wing with three sub marginal cells; marginal cell closed; second sub marginal cell relatively elongate (Figure 16a, c). Metasomal tergites separated through a fine suture, tergites not fused to form carapace (Figure 16b).

Figure 16: *Opius pallipes Wesmael. a: External morphology; b: Metasomal tergites; c: Fore wing.*

Material Examined: Bhalwal: 4♀ and 1♂, 18-ii-2014; 7♀ and 3♂, 3-iii-2014; 6♀ and 2♂, 3-iv-2014; 2♀ and 1♂, 18-viii-2014; 5♀ and 2♂, 3-iii-2015; 6♀ and 1♂, 18-iii-2015; 3♀ and 1♂, 18-iv-2015; **Kot Momin:** 2♀ and 1♂, 21-iii-2014; 1♀ and 1♂, 6-iii-2015; 2♀ and 1♂, 6-xii-2015; **Sargodha:** 2♀ and 1♂, 1-ii-2014; 3♀ and 2♂, 16-iii-2014; 1♀ and 1♂, 16-ii-2015; 2♀ and 1♂, 1-iii-2015; 3♀ and 1♂, 1-iv-2015; **Sillanwali:** 3♀ and 1♂, 15-iii-2014; 4♀ and 1♂, 30-iii-2014.

The results (Table 1 and Figure 17) reveal that out of 3,176 collected specimens, 1,107 individuals belong to Aphidiinae which showed that it's a dominant braconid subfamily in the region, while Opiinae (209) was the least dominant subfamily. Out of six citrus growing localities, Sargodha was the richest locality with total number of 785 specimen collected in 5 subfamilies followed by Kot Momin with total count of 687 specimen collected in 5 subfamilies while Sillanwali was the one with fewer individuals (248) of braconid species. Braconid parasitoid populations were higher in the months of February to April and October to December (Figure 18) while less in the remaining months of the year. In Bhalwal and Kot Momin, Braconinae parasitoids were higher in the months of February to April while in the other localities Alysiinae had the highest populations.

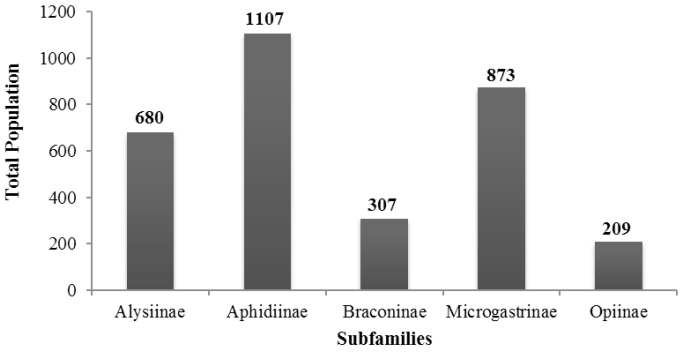

Figure 17: *Total number of braconid parasitoids collected from various localities of Sargodha during January 2014 to December 2015.*

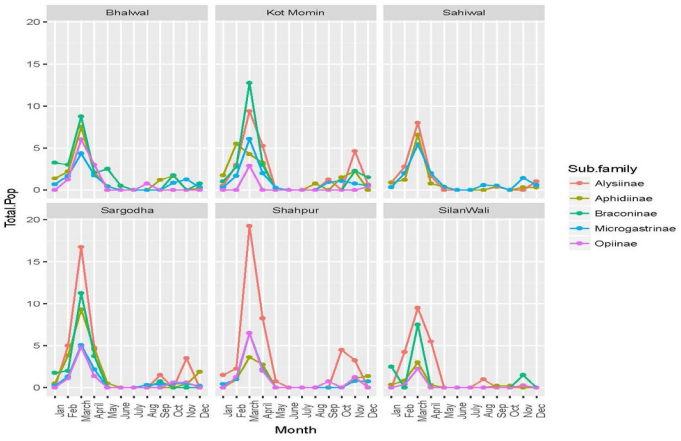

Figure 18: *Month wise distribution of Braconidae subfamilies from various localities of Sargodha during January 2014 to December 2015.*

The results (Table 2) regarding the richness of braconid parasitoids in different localities of Sargodha showed that species richness was highest in Sargodha (12) and lowest in Shahpur (8). Only one species, *Diaeretiella rapae* was found in all citrus growing localities of Sargodha. Among all the species collected from different citrus growing areas of Sargodha (Table 3), *Aphidius transcaspicus* was a dominant species with 367 individuals while *Opius pallipes* (78) was least dominant showing that aphid attack was more on citrus orchards that's why *Aphidius transcaspicus* was collected in higher number to biologically control this pest. The results regarding population of Alysiinae parasitoids exhibited that maximum population (158) of *Aphaereta basirufa* was recorded in Kot Momin while minimum (81) from Sillanwali. *Idiasta transiens* maximum population (159) was found in Shahpur while minimum (22) in Sahiwal.

Table 1: *Number of braconid parasitoids collected from various localities of Sargodha during January 2014 to December 2015.*

Subfamilies	Localities						Total
	Bhalwal	Kot Momin	Sahiwal	Sargodha	Shahpur	Sillanwali	
Alysiinae	--	197	117	126	159	81	680
Aphidiinae	267	154	173	336	79	98	1107
Braconinae	90	93	--	78	--	46	307
Microgastrinae	135	217	158	176	187	--	873
Opiinae	44	26	--	69	47	23	209
Total Subfamilies	4	5	3	5	4	4	-
Total Individuals	536	687	448	785	472	248	3176

Table 2: *An overview of presence and absence of braconid parasitoids in various localities of Sargodha collected during January 2014 to December 2015.*

Species	Localities					
	Bhalwal	Kot Momin	Sahiwal	Sargodha	Shahpur	Sillanwali
Aphaereta basirufa	-	+	+	-	-	+
Aphidius transcaspicus	+	-	+	+	+	+
Areotetes carinuliferus	-	+	-	+	+	+
Binodoxys indicus	+	-	+	+	-	+
Bracon hebetor	+	+	-	+	-	+
Cotesia flaviconchae	-	+	-	+	+	-
Cotesia vestalis	+	+	+	-	+	-
Diaeretiella rapae	+	+	+	+	+	+
Diolcogaster andamanensis	-	-	-	+	+	-
Idiasta transiens	-	+	+	+	+	-
Lipolexis gracilis	-	+	+	-	-	+
Lipolexis scutellaris	+	-	-	+	-	+
Microplitis espinachi	-	-	+	-	+	-
Microplitis mediator	+	+	-	+	-	-
Microplitis varicolor	+	+	+	+	-	-
Opius pallipes	+	+	-	+	-	+
Total Species (Richness)	9	11	9	12	8	9

Aphidiinae parasitoids *Aphidius transcaspicus* (102), Lipolexis *scutellaris* (65) and *Binodoxys indicus* (83) were collected in higher number from Sargodha while population of *Aphidius* and Lipolexis were less in Sillanwali (40, 11) and *Binodoxys* in Sahiwal (24). In Kot Momin, *Diaeretiella rapae* (90) and Lipolexis *gracilis* (64) populations were higher while less in Sillanwali (9, 7). *Bracon hebetor,* a Braconinae parasitoid, was found most abundant (93 specimens) in Kot Momin while less abundant (46) in Sillanwali.

The microgastrinae species *Cotesia flaviconchae* was recorded in highest numbers (53) from Shahpur and lowest (44) from Sargodha.

Maximum number of individuals (43) of *Cotesia vestalis* were collected from Kot Momin while minimum (19) from Shahpur. *Diolcogaster andamanensis* (65) in Sargodha and *Microplitis espinachi* (96) population in Sahiwal was higher while population of both species was lower in Shahpur (43, 72). *Microplitis mediator* (56) and *Microplitis varicolor* (66) individuals were collected in higher number from Kot Momin while less were found in Sargodha (39, 28).

The opiinae parasitoid *Areotetes carinuliferus* maximum population (52) was collected from

Table 3: *Sex ratio (localities wise) and total number of Braconidae subfamilies parasitoids in various localities of Sargodha collected during January 2014 to December 2015.*

Subfamilies	Species	Localities						Total
		Bhalwal	Kot Momin	Sahiwal	Sargodha	Shahpur	Sillanwali	
Alysiinae	*Aphaereta basirufa*	-	158	95	-	-	81	334
		-	95♀ 63♂	57♀ 38♂	-	-	46♀ 35♂	198♀ 136♂
	Idiasta transiens	-	39	22	126	159	-	346
		-	27♀ 12♂	15♀ 7♂	81♀ 45♂	96♀ 63♂	-	219♀ 127♂
Aphidiinae	*Aphidius transcaspicus*	86	-	87	102	52	40	367
		54♀ 32♂	-	49♀ 38♂	72♀ 30♂	39♀ 13♂	33♀ 7♂	247♀ 120♂
	Binodoxys indicus	53	-	24	83	-	31	191
		32♀ 21♂	-	14♀ 10♂	45♀ 38♂	-	17♀ 14♂	108♀ 83♂
	Diaeretiella rapae	79	90	37	86	27	9	328
		43♀ 36♂	51♀ 39♂	25♀ 12♂	47♀ 39♂	17♀ 10♂	6♀ 3♂	189♀ 139♂
	Lipolexis gracilis	-	64	25	-	-	7	96
		-	40♀ 24♂	16♀ 9♂	-	-	5♀ 2♂	61♀ 35♂
	Lipolexis scutellaris	49	-	-	65	-	11	125
		33♀ 16♂	-	-	42♀ 23♂	-	7♀ 4♂	82♀ 43♂
Braconinae	*Bracon hebetor*	90	93	-	78	-	46	307
		54♀ 36♂	59♀ 34♂	-	46♀ 32♂	-	27♀ 19♂	186♀ 121♂
Microgastrinae	*Cotesia flaviconchae*	-	52	-	44	53	-	149
		-	36♀ 16♂	-	29♀ 15♂	32♀ 21♂	-	97♀ 52♂
	Cotesia vestalis	39	43	27	-	19	-	128
		23♀ 16♂	28♀ 15♂	18♀ 9♂	-	13♀ 6♂	-	82♀ 46♂
	Diolcogaster andamanensis	-	-	-	65	43	-	108
		-	-	-	41♀ 24♂	29♀ 14♂	-	70♀ 38♂
	Microplitis espinachi	-	-	96	-	72	-	168
		-	-	61♀ 35♂	-	48♀ 24♂	-	109♀ 59♂
	Microplitis mediator	42	56	-	39	-	-	137
		28♀ 14♂	34♀ 22♂	-	25♀ 14♂	-	-	87♀ 50♂
	Microplitis varicolor	54	66	35	28	-	-	183
		37♀ 17♂	43♀ 23♂	22♀ 13♂	20♀ 8♂	-	-	122♀ 61♂
Opiinae	*Areotetes carinuliferus*	-	18	-	52	47	14	131
		-	11♀ 7♂	-	39♀ 13♂	36♀ 11♂	9♀ 5♂	95♀ 36♂
	Opius pallipes	44	8	-	17	-	9	78
		33♀ 11♂	5♀ 3♂	-	11♀ 6♂	-	7♀ 2♂	56♀ 22♂
Total Subfamilies		4	5	3	5	4	4	-
Total Individuals		536	687	448	785	472	248	3176

Sargodha and minimum (14) from Sillanwali. In Bhalwal *Opius pallipes* individuals were found in higher number (44) whereas less (8) in Kot Momin.

The analysis of variance (ANOVA) of the data presented in Table 4(A) regarding braconid parasitoids in various localities of Sargodha showed highly significant differences among localities, species, months and also depicted significant differences in interactional responses between species and months. The results of Table 4(B) revealed that maximum braconid population was recorded from Sargodha vicinity (1.35) which did not differ greatly from other localities, except for Sillanwali (0.57) that showed minimum braconid parasitoids population. The results presented in Table 4(C) and Figure 19 regarding braconid species population in different months of the year showed that their population was higher in

the month of March (6.50) while in June (0.01) only few braconid individuals were encountered whereas in July (0.00), no braconid wasp was encountered.

Table 4: *Analysis of variance and comparison of means of the data regarding braconid parasitoids in various localities of Sargodha collected during January 2014 to December 2015.*

Table 4 (A): *Analysis of variance.*

S.O.V.	D.F.	S.S.	M.S.	F Value	Pr(>F)
Localities	5	190	37.9	3.675	0.00259 **
Species	15	746	49.8	4.820	< 0.001 ***
Months	11	8550	777.3	75.309	< 0.001 ***
Localities:Species	37	456	12.3	1.193	0.19757
Localities:Months	55	544	9.9	0.959	0.56084
Species:Months	165	2684	16.3	1.576	< 0.001 ***
Localities:Species:Months	407	2739	6.7	0.652	1.00000
Residuals	2088	21551	10.3		

*** = *Highly significant at P ≤ 0.01; Signif, Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 .*

Table 4 (B): *Comparison of means of braconid parasitoids (localities wise).*

Localities	Means ± S.E.
Bhalwal	1.24±0.18 a
Kot Momin	1.30±0.17 a
Sahiwal	1.04±0.17 a
Sargodha	1.35±0.18 a
Shahpur	1.23±0.18 a
Sillanwali	0.57±0.12 b

Means sharing similar letters are not significantly different by HSD Test at P = 0.05.

Table 4 (C): *Comparison of means of braconid parasitoids (month wise).*

Months	Means ± S.E.
Jan	0.62±0.13 cd
Feb	1.88±0.28 b
March	6.50±0.52 a
April	2.22±0.29 b
May	0.20±0.08 de
June	0.01±0.01 e
July	0.00±0.00 e
Aug	0.11±0.05 de
Sep	0.35± 0.12 cde
Oct	0.46± 0.13 cde
Nov	0.84± 0.17 c
Dec	0.47± 0.12 cde

Means sharing similar letters are not significantly different by HSD Test at P = 0.05.

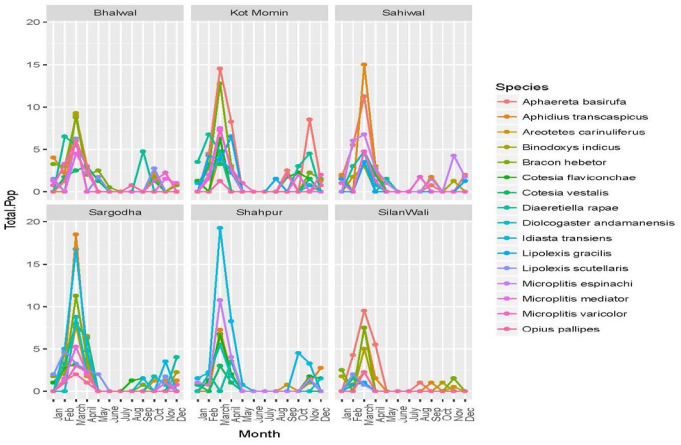

Figure 19: *Monthwise species distribution of Braconidae subfamilies from various localities of Sargodha during January 2014 to December 2015.*

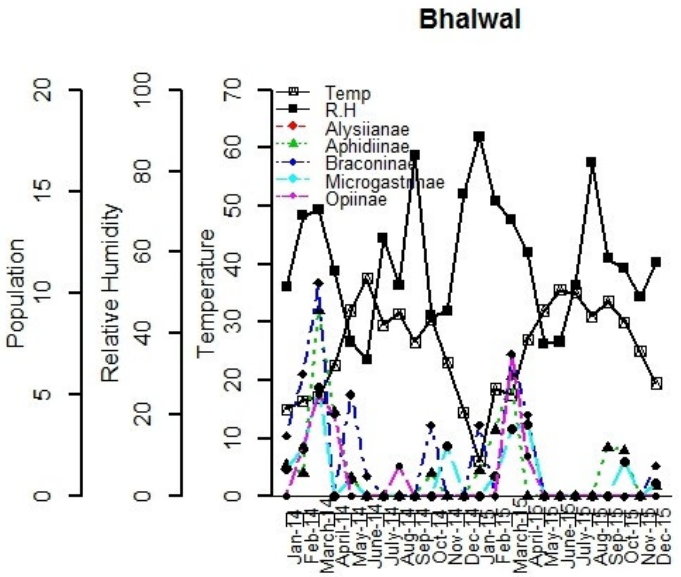

Figure 20: *Braconid parasitoids population in relation to Temperature and R.H. in Bhalwal.*

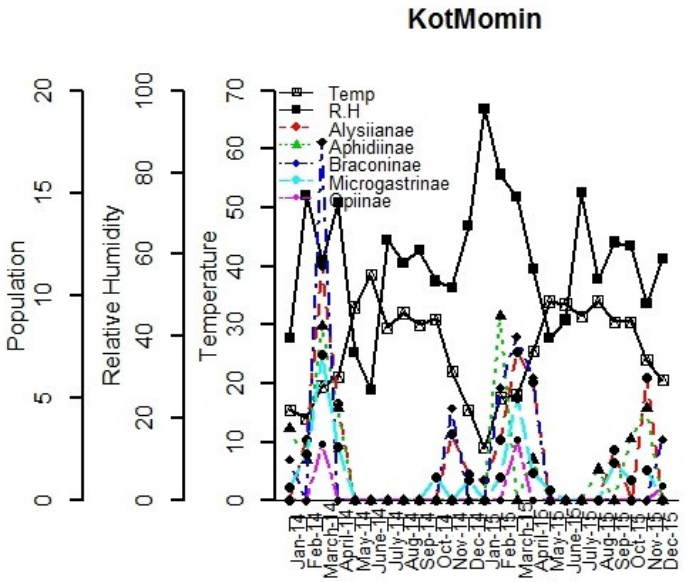

Figure 21: *Braconid parasitoids population in relation to Temperature and R.H. in Kot Momin.*

Table 5: *Diversity Index (Simpson).*

Localities	Alysiinae (S) (E)	Aphidiinae (S) (E)	Braconinae (S) (E)	Microgastrinae (S) (E)	Opiinae(S) (E)	Richness
	Simpson	Simpson	Simpson	Simpson	Simpson	
Bhalwal	------	0.2616 (4) (0.43)	1 (1)	0.3353 (3) (0.70)	1 (1)	9
Kot Momin	0.6808 (2) (2.26)	0.5111 (2) (1.70)	1 (1)	0.2523 (4) (0.42)	0.5569 (2) (1.85)	11
Sahiwal	0.692 (2) (2.29)	0.3349 (4) (0.56)	------	0.4439 (3) (0.93)	------	9
Sargodha	1 (1)	0.2539 (4) (0.42)	1 (1)	0.2591 (4) (0.43)	0.6232 (2) (2.07)	12
Shahpur	1 (1)	0.5443 (2) (1.81)	------	0.288 (4) (0.48)	1 (1)	8
Sillanwali	1 (1)	0.2855 (5) (0.41)	1 (1)	------	0.502 (2) (1.67)	9

S = *Richness (total number of species in the community);* **E** = *Evenness (relative abundance of the different species making up the richness of an area).*

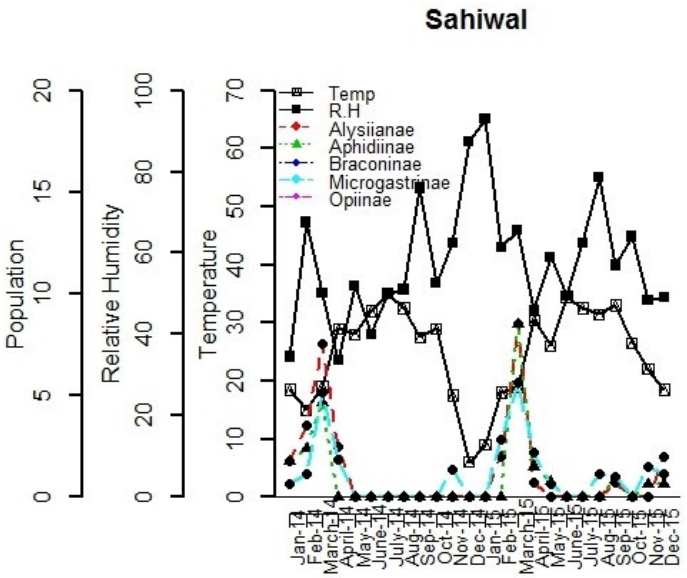

Figure 22: *Braconid parasitoids population in relation to Temperature and R.H. in Sahiwal.*

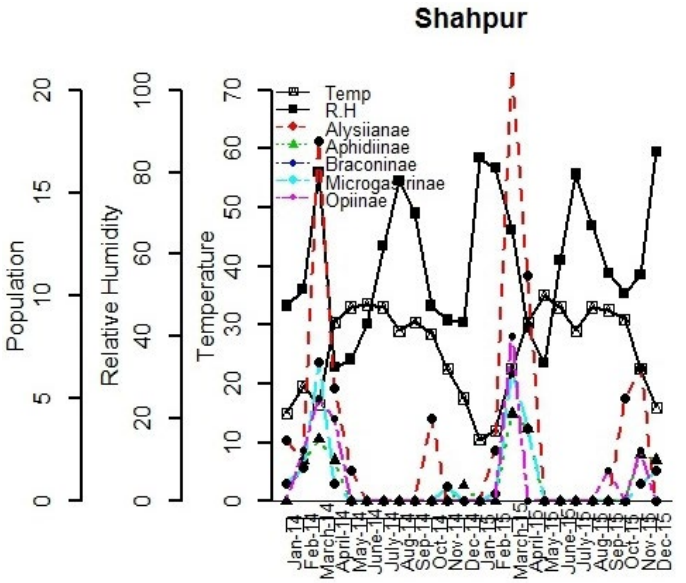

Figure 24: *Braconid parasitoids population in relation to Temperature and R.H. in Shahpur.*

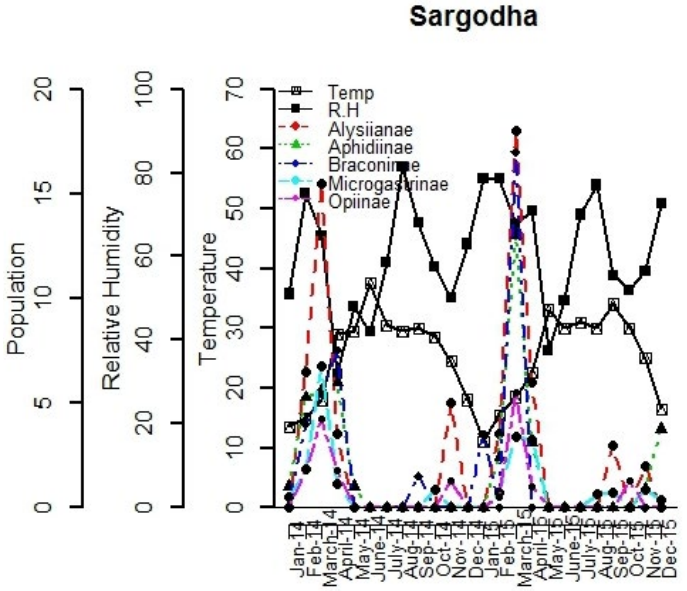

Figure 23: *Braconid parasitoids population in relation to Temperature and R.H. in Sargodha.*

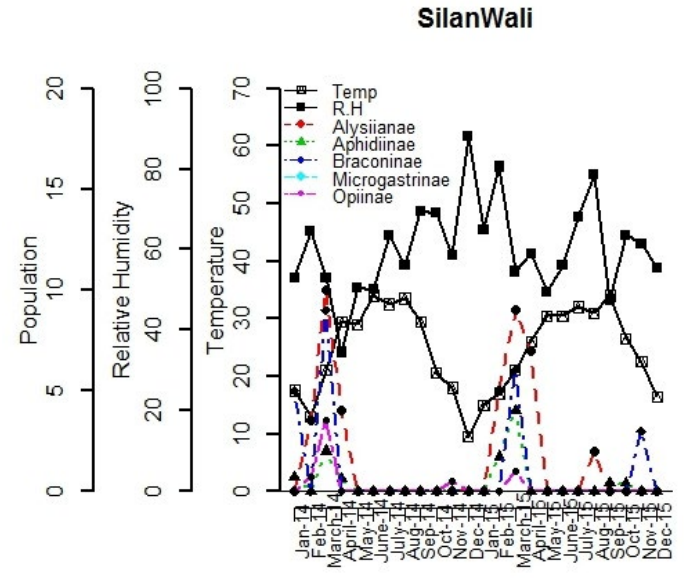

Figure 25: *Braconid parasitoids population in relation to Temperature and R.H. in Sillanwali.*

The results of Table 5 illustrated that in Bhalwal the Microgastrinae (0.3353) subfamily was most diverse while Braconinae and Opiinae showed no diversity as only one species of both subfamilies were collected.

In Kot Momin (0.6808) and Sahiwal (0.692) the diversity of Alysiinae species was highest whereas Braconinae was not found in Kot Momin and in Sahiwal Aphidiinae (0.3349) parasitoids was less diverse. In Sargodha (0.6232) and Sillanwali (0.502) Opiinae subfamily was mainly diverse because its species were evenly distributed in these localities while Alysiinae and Braconinae had low diversity as only one species of both was collected from Sargodha and Sillanwali. The results also revealed that the diversity of Aphidiinae (0.5443) species was highest in Shahpur whereas both Alysiinae and Opiinae had only one species which showed no diversity in Shahpur. So in Bhalwal Microgastrinae, in Kot Momin and Sahiwal Alysiinae, in Sargodha and Sillanwali Opiinae and in Shahpur Aphidiinae species showed more diversity as these species was evenly distributed in these localities.

The results shown in Figure 20, 21, 22, 23, 24 and 25 depicted that the braconid populations in various citrus growing localities of Sargodha were positively correlated with relative humidity while negatively correlated with temperature, as temperature increases braconid population decreases. The results also showed that February to April and October to December was favourable months for the activity of braconid parasitoids due to environmental conditions and availability of their hosts in citrus orchards. We speculate that, as the population of insect pests was more on citrus crop during these months so braconid population was also higher in order to control these pests.

Conclusions and Recommendations

This study helps us in knowing the abundance, richness and dominance of braconids fauna in citrus growing localities of Sargodha, Pakistan. Braconids population is abundant in citrus orchards during the months of February to April and October to December. So there is a need to conserve braconids population during these months by avoiding excessive use of chemical insecticides in citrus orchards.

Author's Contribution

Huma Khalil did data recording, data analysis and manuscript writing as a main author for this manuscript. Muhammad Afzal, Muhammad Anjum Aqueel and Abu Bakar Muhammad Raza supervised the research and manuscript writing. Muhammad Sajjad Khalil, Farghama Khalil and Hafiz Khurram Shurjeel helped in research, reviewing manuscript and correspondence.

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