ABSTRACT: In nature, different Aphidiinae parasites play a vital role in the reduction of aphid population which ultimately results in decreased fruit production and poor juice contents. The diversity of Aphidiines has never been surveyed in citrus growing localities of Sargodha region. So, their distribution, richness and dominance in the specified area have to be investigated for their bio-control efficiency. Surveys were done during January 2014 to December 2015 from various citrus growing localities using malaise traps yielded 1107 parasitoids belonging to 5 species under 4 genera. Out of them, 2 species (Lipolexis gracilis, L. scutellaris) were new records to the area. Aphidiinae population was abundant in citrus orchards during the months of Feb-April and Oct-Dec while less in remaining months of the year. So this study helps us in knowing the abundance, richness and dominance of Aphidiinae in citrus growing localities of Sargodha, Pakistan and also tells us that in which season or weather Aphidiinae parasites are abundant in nature to control aphid pests.

KEY WORDS: Braconidae, Hymenoptera, Parasitoids, Citrus orchards, Taxonomic keys

Citrus is one of the most important fruits and is grown in more than 52 countries around the world. Pakistan also occupies a prominent position in citrus production. It is mostly growing in Punjab, particularly in Sargodha region. It plays a vital role in Pakistan economy through its export (Anon, 2004). A large number of insect pests attack on citrus crop but aphid cause great damage to plants directly or indirectly which results in decreased fruit production and poor juice contents (Aslan et al., 2004; Kavallieratos et al., 2005, 2008a).

In nature, different natural enemies such as parasitoids play a vital role in the reduction of aphid population (Stary, 1970; Kavallieratos et al., 2001, 2004, 2008a and b; Aslan et al., 2004; Rakhshani et al., 2006; Alexandre et al., 2013). Majority of aphid parasites belongs to the subfamily Aphidiinae within the family Braconidae. They are specialized solitary endo-parasitoids of aphids (Stary, 1970; Kavallieratos et al., 2001, 2004; Rakhshani et al., 2007; Tomanovic et al., 2003b, 2004, 2008). About 400 species of Aphidiinae parasites belonging to 55 genera are described worldwide (Stary, 1988; Dolphine & Quick, 2001; Aslan et al., 2004; Rakhshani et al., 2007). The aim of the present study was to identify the biodiversity of aphid parasites in different localities of Sargodha region.

Raychaudhuri (1990) explored the Aphidiinae parasites of Northeast India. He reported 122 species of parasitoids under 20 genera infecting more than 100 aphid species. Stary et al. (2000) collected 49 species of subfamily Aphidiinae from Iran. Wei et al. (2005) reported 20 genera and 99 species of Aphidiinae from
China. Rakhshani et al. (2007) recorded genus *Praon* Haliday (Aphidiinae: Braconidae: Hymenoptera) with its host relationships in Iran. A tentative key for the species identification of this genus has also been provided. Rakhshani et al. (2008a) illustrated 17 species of genus *Aphidius* Nees in different parts of Iran along with their host relationships. For species identification of genus *Aphidius* they also gave an illustrated key. Rakhshani et al. (2008b) also reported 11 species of subfamily Aphidiinae attacking 7 species of wheat aphids in Iran. Stary et al. (2008) examined a new species, *Areopraon thailandicum* of Aphidiinae parasitoids of aphids and recorded *Aphidius autriquei* for the first time in Thailand. Stary & Havelka (2008) studied faunal relationships of Aphidiinae parasitoids and illustrated that they are significant biological control agents in the world.

Kazemzadeh et al. (2009) explored a new species of aphid parasitoid *Areopraon lepelleyi* (Aphidiinae: Braconidae: Hymenoptera) informing that a total of 59 species have been discovered from Iran up till now. Talebi et al. (2009) found 34 species of Aphidiinae parasitoids on medicinal plants and also provided a list of tritrophic associations of aphid hosts, parasitoids and the respective food plants along with identification key. Barahoei et al. (2010) described 5 species of genus *Praon* along with 18 tritrophic relationships from which 2 parasitoid species, 7 host aphids, 5 host plants and 6 host aphid host plant relations were new for Iran. Stary et al. (2010) reported 11 aphid parasitoids species belonging to 10 genera from Thailand. Mejias, Hanson & Stary (2010) recorded ten species of Aphidiinae parasitoids belonging to six genera in Central America.

Bodlah (2010) reported 30 species of aphid parasitoids from Pakistan, out of those 11 species were recorded from Potohar region of Punjab Province of Pakistan. Bodlah et al. (2012a) described genus *Binodoxys* Mackauer (Aphidiinae: Braconidae: Hymenoptera) and its five species from Punjab Province of Pakistan. *Binodoxys rubicula* and *Binodoxys angelicae* were reported for the first time from Punjab. Bodlah et al. (2012b) also reported genus *Proan* Haliday (Aphidiinae: Braconidae: Hymenoptera) for the first time from Punjab Province. Rakhshani et al. (2015) identified 16 aphid parasitoids in Malta. Two species, *Aphidius absinthii* and *Trioxys pallidus* are newly recorded from the Maltese fauna.

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

### MATERIALS AND METHODS

The present study was conducted to investigate or explore the biodiversity of Aphidiinae (Braconidae: Hymenoptera) in Sargodha region with particular reference to citrus orchards for the duration of two years.

**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 the six citrus growing localities (Bhalwal, Kot Momin, Sahiwal, Sargodha, Shahpur and Sillanwali) of Sargodha. Three malaise traps were installed in each district, total eighteen traps were installed.
The parasitoids collected with the help of net sweeping were killed in a poison bottle containing potassium cyanide while 70% ethanol was used for malaise trap collections.

**Mounting and preservation of Aphidiinae parasitoids**

In the laboratory, collected specimens were thoroughly washed with dilute (5-10%) soap solution and then rinsed with distilled water to remove any dust or waxy material on them. 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. The spreading of wings was managed through needles by keeping the specimens in a drop of absolute ethanol. After that, they were mounted from dorso-lateral side by sticking them with water based seccotine glue on triangular card points which were held by entomological pin no. 16. Small specimens were mounted on microscopic slides by using Hoyer's medium (Distilled water 50cc, Gum arabic 30g, Chloral hydrate 200g, Glycerin 20cc, Glacial acetic acid 1-2cc).

Each specimen was properly labeled and then stored in insect wooden collection boxes. The stored specimens were protected from ants and dermestid beetles by keeping naphthalene balls on pins and coopex powder in small containers in the collection boxes.

**Identification of Aphidiinae parasitoids**

The parasitoids were then identified under Wild M3B binocular microscope having three magnifications of 10X x 6.4X, 10X x 16X and 10X x 40X up to the species level. The illustrations were drawn by using the line drawings and camera lucida. The classification and terminology of sub family Aphidiinae have been partly followed as given by Chou (1981), Sureshan & Narendran (2000), Kavallieratos et al. (2001), Tomanovic & Kavallieratos (2002), Kavallieratos & Lykouressis (2004), Rakhshani et al. (2006) and Kos et al. (2012).

The temperature and relative humidity of all the selected localities were also been appended.

**Data analysis**

Diversity index (Simpson diversity index) was conducted for the analysis of species richness and evenness in different citrus growing areas of Sargodha. In this diversity index biodiversity and prevalence of Aphidiinae 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 parasitoids biodiversity.

**RESULTS AND DISCUSSION**

As a result of extensive surveys done during January 2014 - December 2015 from various localities of Sargodha districts, a total of 1107 parasitoids belonging to 5 species under 4 genera were identified. Out of them, 2 species (Lipolexis gracilis, L. scutellaris) were new records to the area.

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

1. Last sternite modified into prongs (Fig. 2), antennae 11 segmented..............................................**Binodoxys** Mackauer
2. Ovipositor sheath curved downwards (Figs. 4, 5)..............................................................................**Lipolexis** Forster
3. Wing venation extremely reduced, only radial vein developed, antennae 13-15 segmented 140 (Fig. 3)..............................................................................................................................**Diaeretiella** Starý
The results (Table 1) regarding the richness of braconid parasitoids in different localities of Sargodha showed that species richness was more in Sillanwali (5) while less in Kot Momin (2) and Shahpur (2) so Sillanwali was the
richest locality with a higher number of braconid species. 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 2), *Aphidius transcaucasicus* was a dominant species with 367 individuals while *Lipolexis gracilis* (96) was least dominant. Aphidiinae parasitoids *Aphidius transcaucasicus* (102), *Lipolexis scutellaris* (65) and *Binodoxys indicus* (83) were collected in higher number from Sargodha while population of *Aphidius* and *Lipolexis* was less in Sillanwali (40, 11) and *Binodoxys* in Sahiwal (24). In Kot Momin, *Diaeretiella rapae* (90) and *Lipolexis gracilis* (64) population was higher while less in Sillanwali (9, 7). Among localities Sargodha was a dominant locality as having 336 Aphidiinae individuals while Shahpur (79) was least dominant.

The results of table 3 revealed that the diversity of Aphidiinae (0.54) species was highest in Shahpur whereas in Sargodha Aphidiinae (0.25) parasitoids were less diverse.

The results shown in figs. 6-11 depicted that Aphidiinae population in various citrus growing localities of Sargodha was positively correlated with relative humidity while negatively correlated with temperature, as temperature increases Aphidiinae population decreases. The results also showed that Feb-April and Oct-Dec were favorable months for the activity of Aphidiines due to environmental conditions and availability of their hosts in citrus orchards. As the population of aphids was more on citrus crop during these months so Aphidiinae population was also higher in order to control them.

**CONCLUSION**

This study helps us in knowing the abundance, richness and dominance of Aphidiinae in citrus growing localities of Sargodha, Pakistan. Aphidiinae population is abundant in citrus orchards during the months of Feb-April and Oct-Dec. So there is a need to conserve their population during these months by avoiding excessive use of chemical insecticides in citrus orchards.

**ACKNOWLEDGEMENTS**

I express gratitude to my worthy supervisor Prof. Dr. Muhammad Afzal, my teachers and colleagues Dr. Muhammad Anjum Aqueel, Prof. Thierry Hance, Dr. Abu Bakar Muhammad Raza, Muhammad Sajjad Khalil, Farhama Khalil, Petr Stary and Jose Fernandez who always prayed, encouraged, extend their support and provide every possible comfort for the successful accomplishment of this project.

**LITERATURE CITED**


Table 1. An overview of presence and absence of Aphidiinae parasitoids in various localities of Sargodha collected during 1-1-2014 to 30-12-2015.

<table>
<thead>
<tr>
<th>Species</th>
<th>Localities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bhalwal</td>
</tr>
<tr>
<td>Aphidius transcaspicus</td>
<td>+</td>
</tr>
<tr>
<td>Binodoxys indicus</td>
<td>+</td>
</tr>
<tr>
<td>Diaeretiella rapae</td>
<td>+</td>
</tr>
<tr>
<td>Lipolexis gracilis</td>
<td>-</td>
</tr>
<tr>
<td>Lipolexis scutellaris</td>
<td>+</td>
</tr>
<tr>
<td><strong>Total Species (Richness)</strong></td>
<td><strong>4</strong></td>
</tr>
</tbody>
</table>

Table 2. Sex ratio of Aphidiinae parasitoids collected during 1-1-2014 to 30-12-2015.

<table>
<thead>
<tr>
<th>Subfamilies</th>
<th>Localities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bhalwal</td>
</tr>
<tr>
<td>Aphidius transcaspicus</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>54♂</td>
</tr>
<tr>
<td></td>
<td>32♂</td>
</tr>
<tr>
<td>Binodoxys indicus</td>
<td>53</td>
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<tr>
<td></td>
<td>32♀</td>
</tr>
<tr>
<td></td>
<td>21♀</td>
</tr>
<tr>
<td>Diaeretiella rapae</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>43♂</td>
</tr>
<tr>
<td></td>
<td>36♀</td>
</tr>
<tr>
<td>Lipolexis gracilis</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Lipolexis scutellaris</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>33♀</td>
</tr>
<tr>
<td></td>
<td>16♂</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>267</td>
</tr>
</tbody>
</table>

Table 3. Diversity Index (Simpson).

<table>
<thead>
<tr>
<th>Localities</th>
<th>Aphidiiinae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bhalwal</td>
<td>0.26</td>
</tr>
<tr>
<td>Kot Momin</td>
<td>0.51</td>
</tr>
<tr>
<td>Sahiwal</td>
<td>0.33</td>
</tr>
<tr>
<td>Sargodha</td>
<td>0.25</td>
</tr>
<tr>
<td>Shahpur</td>
<td>0.54</td>
</tr>
<tr>
<td>Sillanwali</td>
<td>0.29</td>
</tr>
</tbody>
</table>
Figure 1. *Aphidius transcaspicus* Telenga. a: External Morphology; b: F1 and F2; c: Lateral view of tergite-1; d: Propodeum; e: Forewing; f: Labial palpi.

Figure 2. *Binodoxys indicus* Subba Rao and Sharma. a: External Morphology; b: F1 and F2; c: Propodeum; d: Forewing.
Figure 3. *Diaeretiella rapae* M’Intosh. a: External Morphology; b: F1 and F2; c: Lateral view of tergite-1; d: Forewing; e: Propodeum.

Figure 4. *Lipolexis gracilis* Foerster. a: External Morphology; b: F1 and F2; c: Genitalia; d: Forewing.

Figure 5. *Lipolexis scutellaris* Mackauer. a: External Morphology; b: F1 and F2.
Figure 6. Monthly population of Aphidiinae parasitoids in relation to temperature and relative humidity in Bhalwal.

Figure 7. Monthly population of Aphidiinae parasitoids in relation to temperature and relative humidity in Kot Momin.
Figure 8. Monthly population of Aphidiinae parasitoids in relation to temperature and relative humidity in Sahiwal.

Figure 9. Monthly population of Aphidiinae parasitoids in relation to temperature and relative humidity in Sargodha.
Figure 10. Monthly population of Aphidiinae parasitoids in relation to temperature and relative humidity in Shahpur.

Figure 11. Monthly population of Aphidiinae parasitoids in relation to temperature and relative humidity in Sillanwali.