Coccinellidae beetles (Coleoptera) fauna of district Layyah (Punjab), Pakistan

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Abstract
Beetles belonging to family Coccinellidae are predators of various sucking insect pests, some of them are phytophageous. A lot of work for the exploration of these beetles has been done in Pakistan. Layyah being a multi crop area was selected as study area for the exploration of Coccinellidae beetles. A survey was conducted for the collection of Coccinellidae adult specimens from the territory of Layyah district of southern Punjab, Pakistan, during 2018-19. A total 568 adult specimens were collected from seven localities. Out of which, ladybird beetles belonging to seven genera, with ten species namely; Coccinella septempunctata Linnaeus, 1758, Brumoides suturalis (Fabricius, 1798), Henosepilachna vigintioctopunctata (Fabricius, 1775, Henosepilachna elaterii (Rossi, 1794), Hippodamia variegata (Goeze, 1777), Scymnus (Pullus) quadrillum Motschulsky, 1858, Scymnus (Pullus) posticalis Sicard, 1913, Scymnus (Scymnus) nubilus Mulsant, 1850, Micraspis allardi (Mulsant, 1866), Pharoscymnus flexibilis (Mulsant, 1853), have been recorded with their prey and host plants for the first time from the surveyed area. The coccinellids distribution is also given and map was prepared by using ArcGIS map tool. Explored predatory beetles may be used as bio-control agents in the region for the management of various sucking insect pests of various crops, fruits vegetables etc.

Keywords: Ladybird beetles, Coccinellidae, Predators, Diversity, Taxonomy, Layyah, Punjab

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Introduction
The voracious and non-specialist predator, beetles belong to the family Coccinellidae, are the beneficial insects for having ample impacts on diverse insect pest species like aphids, mites, scale insects, whiteflies (Obrycki and Kring, 1998). The predatory behavior of the Coccinellidae varies within the species as they
have a positive impact by feeding on the insect pests and intraguild cannibalism included in their negative feeding behavior (Lucas, 2012). Most of the species are economically important predators, while some are also plant or being mycetophagous (Sutherland and Parrella, 2009). So, the predatory behavior of the Coccinellids includes different transitions throughout different trophic levels which covers primary carnivores to herbivorous insect. Similarly, the female Coccinellids are being proved more stronger predators than the male beetles (Chowdhury et al., 2008). Most of the Coccinellidae species adults are with bright and shining color patterns with patches or spots against the contrasting backgrounds. Similarly, these color patterns have been found defensive as signs of warning or danger or distasteful for their bird predators (Moreton, 1969). The farmers usually spray pesticides to kill pests extensively which kills both the pests and their natural enemies. The indiscriminate use of chemicals may affect lady bird beetles searching behavior, feeding behavior, mating behavior, number of eggs, and overall life span (Alinejad et al., 2014; Afza et al., 2020). However, ladybird beetles have great potential to survive the lethal effects of applied chemicals which makes them preferable for any integrated pest management (Mughal et al., 2017). According to the latest classifications, Coccinellinae Latreille, 1807 and Microweiseinae Leng, 1920, are now subfamilies of Coccinellidae family (Slipinski, 2007; Seago et al., 2011). Moreover, it is reclassified to superfamily Coccinelloidea (Coleoptera: Polyphaga: Cucujiformia) (Robertson et al., 2015). Overall, more than 6000 species with 360 genera have been reported worldwide. Additionally, more than 300 species have been reported from Indo-Pak subcontinent and more than 71 species specifically from Pakistan. (Fleming, 2000; Irshad, 2001; Boopathi et al., 2020). Most recently and locally, Irshad (2001) 71 species, Rafi et al. (2005) 75 species, Khan et al. (2007) 12 species, Ali et al. (2014a, 2014b, 2015) 29 species, Ahmed et al. (2017) 9 species, Iqbal et al., (2017, 2018, 2019a, 2019b, 2020) 5 species, described predatory ladybird beetles with their associated prey insect pests from different locations of provinces in Pakistan. However, till now there is still deficiency of information about this economically important biological control agent’s family from Pakistan. The current study was intended to explore the ambiguous species riches and distribution of Coccinellidae family from the district Layyah of Punjab, Pakistan.

Material and Methods

Area surveyed for collection
Multiple surveys were carried out in the district Layyah during 2018-19 to collect ladybird beetles, which covers an area of 6,291 km² with its geographical presence as 30°58’15.2”N and 70°56’39.6”E. The major cereal crops are (wheat, millet, barley, rice), oil seed crops (sunflower, linseed and rapeseed), pulses (gram, mash, masoor, moong), fodder (maize, millet, Alfalfa, barley), fruits (citrus, date palm, guava, jamun, mango) and vegetables (potato, radish, carrot, cauliflower, onion, garlic, green pepper) are grown in district Layyah.

Collection and identification
The ladybird beetle specimens were collected in the morning (8:00-10:00 am) and afternoon (2:00 to 4:00 pm) from different intended areas of district Layyah as shown in figure 1. The ladybird beetle’s distribution map is prepared by using ArcGIS map tool. The adult ladybird beetles were collected through hand-picking and net sweep method and killed by using cyanide insect killing glass bottle (Iqbal et al., 2017). The killed specimens were brought to the insect taxonomy laboratory of Fareed Biodiversity Conservation Centre, Department of Agricultural Engineering, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Punjab, Pakistan, and preserved in wooden boxes.

Figure-1. Distribution map of collected coccinellid specimens from Layyah district of Punjab, Pakistan.

Each specimen was pinned and mounted on small triangular plastic card tips with the labelling information of their locality, date of collection, host plants etc. Before placing specimens, the wooden
boxes were treated with Coopex Powder (®) and naphthalene tablets were placed to deter the other insects feeding on preserved insects. The morphological and taxonomic characters of ladybird beetles were studied on the basis of available published literature and checklists by Poorani, (2002), Rafi et al. (2005), Ali et al. (2015, 2018), Ashfaq et al. (2015) and other website links like NBAII, (2009) under the microscope ZMS 2000 compound microscope. However, Photographs of the adult specimens were taken using Nikon Digital camera (SMZ 1500), which was attached to the stereo microscope and photographs were processed by using Helicon focus 6.7.1 and Adobe Photoshop CS 6.0.

**Results**

*Coccinella septempunctata* Linnaeus, 1758 (Fig. 2a)

**Material examined**
Karor Lal Esan, 7♀ and 6♂, Chowk Azam, 12♀ and 14♂; Jaman Shah, 9♀ and 5♂; Kot Sultan, 14 and 3♂; Ladhana, 7♀ and 13♂, Layyah, 6♀ and 2♂

**World distribution**
Indo-Pak, Indo-China subcontinents, New Zealand, Indonesia, Australia, Japan, Nepal, (Poorani 2002).

**Prey species with host plants**
*Aphis gossypii* (Glover), *Brevicoryne brassicae* (L), *Schizaphis graminum* (Rondani), *Myzus persicae* (Sulzer), *Phenacoccus solenopsis* (Tinsley) and *Amrasca biguttula biguttula* (Ishida) on cotton, mustard, cabbage, potato, okra, wheat and rose plants respectively.

**General comments**
*Coccinella septempunctata* found similar with the given diagnostic characters description by Ali et al. (2018) and first time reported from Layyah district.

*Bromoides suturalis* (Fabricius, 1798) (Fig. 2b)

**Material examined**
Karor Lal Esan, 4♀ and 2♂, Chowk Azam, 11♀ and 13♂; Jaman Shah, 10♀ and 3♂; Kot Sultan, 4 and 5♂; Ladhana, 2♀ and 7♂, Hafiz Abad, 2♀ and 2♂

**World distribution**
Bhutan, Pakistan, Sri Lanka, Nepal, India, Bangladesh (Poorani, 2002).

**Prey species with host plants**

**General comments**
*Bromoides suturalis* found similar with given diagnostic characters description by Ali et al. (2018) and first time reported from Layyah district.

*Henosepilachna vigintioctopunctata* (Fabricius, 1775) (Fig. 2c)

**Material examined**
Karor Lal Esan, 2♀ and 3♂, Chowk Azam, 5♀ and 14♂; Layyah, 3♀ and 5♂ Jaman Shah, 8♀ and 3♂; Kot Sultan, 8 and 2♂.

**World distribution**
Reported from different European, African and Asian countries of the world. (Katoh et al., 2014)

**Host plants**
Being a phytophagous pest from coccinellid family, was found on Solanaceous and Cucurbitaceae family vegetables including tomato, potato, bitter gourds and pumpkins.

**General comments**
This species also named as Hadda beetle. *Henosepilachna vigintioctopunctata* found similar with given diagnostic characters description by Ahmed et al. (2017). This species first time reported from district Layyah.

*Henosepilachna elaterii* (Rossi, 1794) (Fig. 2d)

**Material examined**
Karor Lal Esan, 5♀ and 6♀, Layyah, 7♀ and 2♂ Jaman Shah, 9♀ and 6♂; Kot Sultan, 4 and 5♂

**World distribution**
Middle East, Madagascar, Africa, South Europe and Asian countries. (Katoh et al., 2014).
Host plants
It’s a phytophagous invasive pest of Cucurbitaceae family vegetables like pumpkins.

General comments
Henosepilachna elaterii also known as Melon ladybird beetle first time reported from Layyah district and found similar with given diagnostic characters description by Ahmed et al. (2017).

Hippodamia variegata (Goeze, 1777) (Fig. 2e).

Material examined
Karor Lal Esan, 4♀ and 2, Chowk Azam, 11♀ and 12♂; Jaman Shah, 10♀ and 3♂; Kot Sultan, 4♀ and 5♂; Ladhana, 2♀ and 7♂, Hafiz Abad, 2♀ and 2♂.

World distribution
Afghanistan, India, China, Pakistan, Africa, Nepal (Poorani, 2002).

Prey species with host plants
Aphis punicae Passerini, Lipaphis erysimi (Kaltenbach), Aphis gossypii (Glover), Schizaphis graminum (Rondani), Brevicoryne brassicae (L.), Hyadaphis coriandri (Das), Myzus persicae (Sulzer), Amrasca biguttula biguttula (Ishida) and Bemisia tabaci (Gennadius) on different plant hosts like lucern, potato, turnip, brinjal, okra, cotton, wheat mustard and weeds.

General comments
Hippodamia variegata found similar with given diagnostic characters description by Ali et al. (2018) and first time reported from Layyah district.

Scymnus (Pullus) quadrillum Motschulsky, 1858 (Fig. 2f)

Material examined
Karor Lal Esan, 16♀ and 4♂, Chowk Azam, 5♀ and 2♂, Jaman Shah, 6♀ and 3♂; Kot Sultan, 2♀ and 4♂.

World distribution
Mugfghanistan, India, China, Pakistan, Africa, Nepal.

Prey species with host plants
Brevicoryne brassicae (L.), Aphis gossypii (Glover), and Aphis punicae Passerini on different crops and fruit plants.

General comments
Scymnus (Pullus) quadrillum found similar with given diagnostic characters description by Ali et al. (2015) and first time reported from Layyah district.

Scymnus (Pullus) posticalis Sicard, 1913 (Fig. 2g).

Material examined
Layyah, 3♀ and 4♂, Chowk Azam, 12♀ and 7♂, Jaman Shah, 7♀ and 5♂; Kot Sultan, 13♀ and 14♂.

World distribution
Africa, Europe and very common in Asian countries like India, Nepal, Pakistan. (Ashfaque et al., 2015).

Prey species with host plants
Generally, it feeds on aphid species like Aphis punicae Passerini, Myzus persicae (Sulzer), Aphis craccivora Koch, Brevicoryne brassicae (L.), Aphis gossypii (Glover), phytophagous mites and scale insects on different weeds and fruit plants like grapes, apples.

General comments
Scymnus (Pullus) posticalis found similar with given Diagnostic characters description by Ashfaque et al. (2015) and first time reported from Layyah district.

Scymnus (Scymnus) nubilus Mulsant, 1850 (Fig. 2h).

Material examined
Karor Lal Esan, 13♀ and 6♂, Layyah, 2♀ and 3♂, Chowk Azam, 5♀ and 2♂, Jaman Shah, 6♀ and 3♂; Kot Sultan, 2♀ and 4♂.

World distribution
Pakistan, Myanmar, Nepal, Bangladesh, China, Sri Lanka, India (Ashfaque et al., 2015).

Prey species with host plants
This species feed on different life stages of insect pests like, Myzus persicae (Sulzer), Aphis craccivora Koch, Brevicoryne brassicae (L.), Aphis gossypii (Glover), Lipaphis erysimi (Kaltenbach), Drosicha mangiferae (Green) and Bemisia tabaci (Gennadius) on different corps, fruit, flowering and weed plants.

General comments
Scymnus (Scymnus) nubilus found similar with given diagnostic characters description by Ashfaque et al. (2015) and first time reported from Layyah district.
Material examined
Layyah, 11♀ and 6♂; Jaman Shah, 4♀ and 2♂; Kot Sultan, 5 and 7♂; Ladhana, 7♀ and 7♂, Hafiz Abad, 4♀ and 7♂.

World distribution
USA, Pakistan, Brazil, India, (Poorani 2002; Ali et al., 2018).

Prey species with host plants
*Schizaphis graminum* (Rondani), *Aphis craccivora* Koch, *Siassetia nigra* (Nietn) and *Aspidiotus destructor* Sign, on host plants like lucern, wheat, mustard, cotton, grams.

General comments
*Pharoscymnus flexibilis* found similar with given diagnostic characters description by Ali et al. (2018) and first time reported from Layyah district.

Discussion
The current surveys were anticipated to explore the indigenous Coccinellidae fauna from southern Punjab district Layyah. A total of 10 species belonging to 7 genera with their prey, associated host plants and distribution were recorded. Previously Poorani (2002) from India, Ali et al. (2015, 2018) from Sindh province, Ashfaque et al. (2015) from Northern area of Pakistan, Ahmed et al. (2017) from Sargodha, Gilani (1976) from Faisalabad and Rafi et al. (2005) from different localities of Pakistan reported these species. Other than these species, Shah (1985) reported about sixteen ladybird species with their distribution and pests host plants from Peshawar. Hussain et al. (2018) described four species from rainfed and irrigated localities of Gujrat. Din (2002) reported similar species from Chitral and Rafi et al. (2005) surveyed Districts of Azad Kashmir areas and reported similar seventy-five predatory ladybird beetles. Whereas, according to Abbas et al. (2013), there are about 91 species have been reported from Pakistan. Irshad (2001) studied the biotic potential, ecology, distribution and host of Coccinellids from Pakistan. Moreover, Coccinellidae distribution and diversity from District Dir Lower Pakistan have been studied by Rahatullah et al., 2011. Coccinellidae regional distribution record of Gilgit-Baltistan was given by Ashfaqe et al. (2013). Similarly, different predacious Coccinellids have been reported from different
Muhammad Adnan Bodlah et al.

districts of Khyber Pakhtunkhwa province of Pakistan like Nowshera, Mardan and Sawabi with species richness, diversity and dominance by Urooj and Ali, 2016. The persuasion for higher crops production has been directed towards intensive farming systems. That, however, become the reason for high pest populations followed by high intensity pesticides usage and other pest control techniques. The current study will be supportive in mass production of ladybird beetles against pests, reduction in number of insecticide applications and ultimately helping in reduction of environmental pollution.

Conclusion

The current study results contribute to a baseline to study ladybird beetle’s ecology, biology, phytophagy, and predatory behavior against different pests. The current reported species of Coccinellidae family were diversified and abundant. These species may be utilized as potential biological control agents after evaluating their predatory potentials. Finally, it leads the researchers towards the conservatory biological control strategies for the endemic plant protection programs.

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**Contribution of Authors**

Bodlah MA: Collected the samples and wrote the paper
Bodlah I: Planned the research
Rasheed MT: Species mapping and identification
Fareen AGE: Species mapping and identification
Ikram K: Reviewed the manuscript
Iqbal Z: Species Photography
Zada R: Provided the guidelines