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Species composition and population dynamics of some arthropod pests in cotton fields of irrigated and semi-arid regions of Punjab, Pakistan

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ABSTRACT

The present study aimed to record seasonal dynamics and diversity of different insect pests in cotton agroecosystems. Two well-known cotton growing districts i.e. district Layyah and Vehari were selected for the study from the cotton belt of Punjab, Pakistan. Sampling was done bi-weekly for two consecutive years from July to October during 2018 and 2019. Sweep netting, visual counting, hand picking, wet finger method, beat sheets, aspirator and pitfall trapping methods were used for collection. Shannon-Wiener and Simpson indices were used to compute diversity while Menhinick and Margalef indices were used for the estimation of species richness. A total of 94,343 individuals representing 43 species, 40 genera, 28 families and 6 orders were recorded. Family Aleyrodidae dominated over other pest families. *Bemisia tabaci* (Gennadius, 1889) of family Aleyrodidae was the dominant species with 39.68% share among all pest species. Estimated species richness of all arthropod pest species belonging to both districts were about 94%. The densities of pests fluctuated with time. The peaks of sucking pest densities were observed in July and August while densities of chewing pests peaked in late September or early October each year. Population densities of jassids *Amrasca biguttula* (Ishida), armyworm *Spodoptera litura* (Fabricius), and pink bollworm *Pectinophora gossypiella* (Saunders), showed strong negative correlation with temperature, humidity and rainfall while thrips population density showed positive correlation with these parameters. Minor loss due to pests are acceptable everywhere, but it is only possible when their populations remain below their economic threshold levels. Present study will aid to design pest management strategies in cotton growing areas round the globe.

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1. Introduction

Cotton being a major cash crop, not only provides livelihood to farmers but also provides raw material to textile industry and earns foreign exchange for Pakistan (Saleem et al., 2018). Pakistan is the fourth largest cotton producer country of the world (Ashraf et al., 2018) and produced 10.671, 11.935 million bales in 2016–2017, 2017–2018 million bales in 2016–2017, 2017–2018 cropping seasons (Government of Pakistan 2017, 2018). However, during the 2018–2019, 2019–2020, and 2020–2021 cropping seasons, Pak-

istan faced a 17.5 %, 7.2 %, and 22.8 % decline in annual cotton production and yielded 9.861, 9.148, and 7.064 million bales of cotton, respectively (Government of Pakistan 2019,2020,2021). This decline in cotton production ended in the current cropping season i.e. 2021–2022 in which a 17.9 % increase in cotton production is observed as compared to last year's production gains, with a yield of 8.329 million bales (Government of Pakistan 2022). However, during 2018–2019 cropping season, Pakistan faced 17.5 % decline in annual cotton production and yielded 9.861 million bales of cotton (Government of Pakistan 2019). Cotton seeds are used for the production of edible oil while its fiber is used by the textile industry of the country (Shuli et al., 2018).

Cotton crop is attacked by a wide range of insect pests at its various phenological stages (Sahito et al., 2017). Diverse pest fauna has been reported from cotton fields by different researchers (Yunus and Yousuf 1979; Sarwar and Sattar 2016). All these pests cause serious damage to the cotton crop either by sucking its sap or by chewing its different tender parts or by transmitting serious diseases to the plant (Abou-Elhagag 1998; Sahito et al., 2017). For

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decades, chewing pests were considered as major threat to the cotton crop, but after the induction of *Bt* cotton and its acceptance and cultivation on large scale worldwide, the whole damaging scenario is shifted to sucking insect pests (Ellsworth and Martinez-Carrillo, 2001). Pest-resistant transgenic *Bt* crops are widely cultivated throughout the world (James, 2017). The induction of *Bt* gene into cotton plant enables it to produce endotoxins which enables cotton plants to show resistance against many chewing insect pests (Arshad and Sohail, 2011). This factor indirectly helps sucking insect pests to flourish (Frutos et al., 1999). Moreover, limited use of insecticide sprays on *Bt* cotton also provides opportunity to some sucking pests to proliferate (Men et al., 2003).

In Pakistan, Jassid, whitefly and thrips are major sucking pests of cotton and are responsible for major yield loss (Arshad and Sohail, 2011). These sucking pests make the plant leaves wilt by sucking sap from the plant leaves (Abro et al., 2004). Different cotton pests collectively cause 5–10 % yield loss but under conditions favorable for them, they may cause a damage to crop yield up to 35–50 % (Masood et al., 2011). These pests act as a serious threat to economy as it is estimated that annually they, all alone, are responsible for 20–40 % loss of agricultural crops globally (Raman, 2017). *Bt* cotton provides better yield along with reduced production cost as compared to traditional non-*Bt* cotton (Kouser and Qaim, 2011). With the passage of time, pests showed resistance against *Bt* gene (Tabashnik et al., 2012, 2013). Intensive usage of different chemicals is a key factor for bringing sudden decline in different insect populations (Bruhl and Zaller, 2019) but adding more financial and economic burden to its growers (Kouser and Qaim, 2011). Their injudicious usage is also responsible for eutrophication which again contributes towards economic loss of the farmer (Villalobos-Jimenez et al., 2016).

Humans and insects have intertwined faith, especially through the agroecosystems (Samways et al., 2020). To have an updated knowledge about pest biodiversity of cotton fields and their predatory fauna is the need of time as many of these pests are natural food source for many beneficial arthropods. Many researchers are of the point of view that the sudden loss of some species from the intricate web of life may have serious consequences for all of us though those may not be clear to us at this point of time (Hallmann et al., 2017, 2020; Gaston, 2018; Seibold et al., 2019). So, maintaining population of these pests below their economic threshold levels (ETL) is the target of growers, without disturbing the food web of many other depending species which are necessary for the maintenance of a balanced and sustainable ecosystem (Hallmann et al., 2017, 2020). Complex biodiversity offers many services to mankind and even to the whole life forms existing around directly or indirectly (Hallmann et al., 2017, 2020; Cardoso et al., 2020).

Objectives: Present study aims at to develop a first-hand knowledge along with cataloguing the major insect pests from the cotton crop fields of Punjab, Pakistan. By using this information, a sustainable approach may be adopted in order to achieve better pest management and to reduce the insecticide usage as an integral part of any future devised IPM strategy.

Hypothesis: Different species of arthropod pests will be found from different locations of cotton growing regions.

Question: Is there any species complex exists in irrigated and semi-arid regions of cotton growing fields?

2. Materials and methods

2.1. Study areas

The study was conducted during 2018 and 2019 in two main cotton growing districts i.e. Vehari (29.9719° N, 72.4258° E) and

Layyah (31.0998° N, 71.0022° E) of province Punjab, Pakistan. Three study sites (from each district) were shortlisted to record the data. In each district each study sites' area (8094 m²) was further divided into five equal plots (1618 m²) so that we had fifteen plots per district. From each plot twenty-five plants were randomly selected during each sampling date and thoroughly observed to obtain the data of different pests and predators. Average distance between the selected study sites of district Layyah was about 20–25 Km. However, average distance between two sites of district Vehari was about 25–30 Km but one site was a little nearer i.e. 10 Km apart. Sowing was completed from 14th of May to 17th of May 2018 and 2019. After 34–40 days from the date of sowing, first square was observed at all locations. Standard agronomic practices (irrigation, fertilizers, weeding) were practiced as per requirement at all locations. To observe diverse pest fauna in the cotton fields, different sampling methods were used in both selected districts during two consecutive cotton cropping seasons. During the period of study, the data regarding abiotic factors (temperature, humidity and rainfall) was obtained from Pakistan Metrological Department.

2.2. Collection and estimation of Arthropod's abundance

Pests in cotton fields are mostly found everywhere in the cotton fields as many were ground dwelling, some reside at the lower parts of plants or in the middle and even at the top of plant near canopy. So different methods like sweep netting, visual counting, hand picking, wet finger, aspirator, beat-sheets and pitfall trapping methods were used for collection of arthropods during different phenological stages of the cotton crop, i.e. vegetative, flowering and boll formation stages.

2.2.1. Sweep netting

Sweep nets (self-made frame 8 × 10 in.) were used for capturing of flying arthropod pests, predators or pollinators etc. (Kharboutli and Allen, 2000). It was done by taking all possible care for establishing an idea of their relative abundance among other flying arthropods present in the cotton fields. Thirty vigorous sweeps of the net were made, from side to side in almost 180-degree arc, per session while moving in the selected field plots in '8' fashion. All the captured arthropods belonging to different species were then identified upto Order, Family and Genera level and counted for recording their abundance. It is believed for sure, that some more species of flying pests or predators were also there which were skipped as we were unable to observe them during our field visits.

2.2.2. Visual counting

Visual counting method was used for estimating the total number and relative abundance of different sucking insect pests (Garcia et al., 1982). From each selected cotton field plot, twenty-five plants were observed following random complete block design (RCBD) format. The counting was done from the leaf (upper, middle and lower) of the selected plants either with naked eye or by using a magnifying glass (4X). Many active fliers quickly flew away on approach, so many of such active fliers were calculated visually and due to this fact visual sampling/counting was clearly declared superior by Deutscher et al. (2003). Some pests like grey weevils and red cotton bugs were easily observable with naked eye. For mealybug counting, one branch from each infected plant was selected and counted their number from the top 10 in. of that branch.

2.2.3. Hand picking

For sampling and estimation of different chewing pests all randomly selected plants were thoroughly observed for the infestation. Every infested boll was slightly pressed by fingers and if it

was found soft, then it was opened and a number of larvae present in it were counted. Hand picking method was also practiced for most of the chewing pests including larvae of different species of bollworms and cut worms.

2.2.4. Wet finger method

Wet-finger method was used for the collection of small and delicate insects. A small sized open container of alcohol (70 %) was held in one hand and the tip of the index finger of the other hand was dipped into the alcohol, withdrawn and moved to touch and pick up the insect which was then transferred directly into the alcohol container by dipping the index finger again into the alcohol (Upton et al., 2010).

2.2.5. Beat-sheets method

Beatsheets were also used to collect maximum arthropod samples from the cotton plants as this method is more reliable for developing diversity estimates of different arthropod species as compared to other methods like visual counting or sampling through suction devices (Deutscher et al., 2003; Wade et al., 2006). For this purpose, a plastic sheet of bright yellow color of 1.5 × 2 m size was placed below the selected cotton plant and then the plant was vigorously shaken for 10 times with one-meter long stick moving from lower to upper canopy of the plant. The fallen arthropods were then collected and labelled after their general identification up to family or order level (Whitehouse et al., 2007; Williams et al., 2011).

2.2.6. Aspirator

Both mouth operated and battery charged (Model: WIN-601 12 V China) aspirators were used in the field for the collection of small arthropod pests especially for the capturing of whiteflies during both cropping seasons i.e. 2018 and 2019 and at both districts. Sampling was done by following standard protocols outlined by Ahmed et al. (2011). Capturing of smaller flying arthropods for evaluating their abundance was not an easy task so suction devices became a genuine need of the researchers as such insects were hard to collect even by beat-sheet method (Whitehouse et al., 2007).

2.2.7. Pitfall trapping method

Twenty-five glass jars (Barber, 1931) were installed in three-layer pattern (outer, middle and center) in the selected fields to achieve maximum sampling targets of different pest species residing near the margins, in the middle and in the central parts of the cotton fields plots. Each pitfall jar had a three-inch-wide opening (mouth) and seven inch deep, buried into the soil with its mouth lies parallel to the soil surface i.e. at the level of the ground (Tahir and Butt, 2008). A mixture of alcohol and glycerol with few drops of liquid detergent was poured up to one-third portion of each jar. Weekly visits were made for the collection of already fallen insects and for refilling of pitfall jars.

2.3. Sample preservation

Collected samples were placed in 20 ml glass vials filled with 95 % alcohol. Each bottle was carefully labeled and assigned specific field collection numbers along with related details. All samples were shifted carefully to the Applied Entomology and Toxicology Laboratory, Department of Zoology, Government College University for further study. In laboratory, they were washed with seventy percent alcohol to remove different soil particles, or other debris attached with their bodies and preserved in 95 % alcohol.

2.4. Morphological identification of insect pests

All pests were identified upto lowest possible rank by carefully examining different morphological characters examined under stereo dissecting microscope (BCVS 121 & BIOCUM UK) with the help of available keys and catalogues, such as Vreden and Ahmadzabidi (1986), Pathak and Khan (1994), Chanthay et al. (2010), Gupta and Singh (2013), Murthy et al. (2015), Whiting (2017) and data available on BOLD. Identified specimens were also photographed with the help of dissecting microscope and digital camera (Model: DS126431 Canon Inc. Japan).

2.5. Statistical analysis

The data regarding collection date, time, site and name of collector was also noted before leaving the field. The whole data of different pests was arranged and percentages or means were calculated for each record. The recorded data was then used for further comparison and statistical analysis. Normality of data was assessed using Shapiro-Wilk test. As there was non-significant difference in data of two years, therefore, it was pooled together for further analyses. Species accumulation curves were prepared using SPDI-VERS.BAS program of Ludwig et al. (1988). The richness of the insect pests of cotton ecosystems was computed using the non-parametric estimator Chao1 and Margalef richness index. To check the completeness of inventories, ratio between Chao1 and observed richness was calculated. Shannon-wiener index was used for comparing the diversity. However, evenness Hill's ratio (E5) was used to compute evenness. Repeated measure ANOVA was used to compare the richness, diversity and evenness among trapping sites and trapping session. All the statistical analyses including the cluster analysis were done by using SPSS® version 16.

3. Results

A total of 94,343 insect pests representing 43 species, 40 genera, 28 families and 6 orders were recorded from district Layyah (n = 46276) and district Vehari (n = 48067) during sampling period of two years. Out of total catch 32,496 insects were immature and identified up to genus level due to the unavailability of keys to juvenile identification while remaining 61,847 insects were mature. List of insect pest's species identified on the basis of morphological characters is given in Table 1. Family Aleyrodidae (39.68 %) was found to be the most abundant of all families. It was followed by family Lygaeidae (11.18 %), Cicadellidae (10.21 %), Aphididae (7.21 %), Gelechiidae (5.95 %), Thripidae (5.63 %), Pyrrhocoridae (4.68 %), Pseudococcidae (4.42 %), Noctuidae (4.36 %), Nolidae (3.93 %), Curculionidae (1.39 %), while all the remaining families had a collective share of <1.5 % of the total catch. *Bemisia tabaci* (Gennadius 1889) of family Aleyrodidae was the most dominant species that represented 39.68 % of the total catch followed by *Oxycarenus laetus* (Kirby 1891) of family Lygaeidae with 10.78 % representation of the total catch (Table 1). Table 2 is showing percent relative abundance of ten most common families of insect pests recorded from cotton fields.

Fig. 1 is showing the pooled species accumulation curves (combined for two years) for insect pests of both districts under study. The number of trappable insect pest species increased continuously with the increase of sample size. Initially the number of species increased rapidly but after sample count of 36,000 individuals, the increase in the species became slow. The curve did not reach asymptote. Estimated species richness was 42 and 43 for district Layyah and Vehari respectively on the bases of Chao 2 estimates. The ratio of observed to estimated number of species was 94 % (combined for two districts) which suggests at least 6 percent

Table 1
Relative abundance of different insect pest species associated with cotton ecosystems of two major cotton producing districts of Punjab, Pakistan.

Order Family Species	Layyah	Vehari	Total	R.A.
Coleoptera				
Chrysomelidae				
<i>Chrysomelidae</i> sp.	17	6	23	0.024
<i>Calligrapha bidenticola</i> (Brown, 1945)	0	6	6	0.006
Curculionidae				
<i>Mylocerus undecimpustulatus</i> (Faust, J. 1891)	507	674	1181	1.252
<i>Anthonomus grandis</i> (Boheman, 1843)	75	59	134	0.142
Scarabaeidae				
<i>Heteronychus arator</i> (Fabricius, 1775)	6	7	13	0.014
<i>Oxygryllus ruginasus</i> (LeConte, 1856)	7	11	18	0.019
Tenebrionidae				
<i>Tenebrionidae</i> sp.	7	0	7	0.007
Meloidae				
<i>Meloidae</i> sp.	4	9	13	0.014
Diptera				
Ceratopogonidae				
<i>Culicoides</i> sp.	0	8	8	0.008
Hemiptera				
Aleyrodidae				
<i>Bemisia tabaci</i> (Gennadius, 1889)	18,093	19,338	37,431	39.68
Aphididae				
<i>Aphis gossypii</i> (Glover, 1877)	3117	3646	6763	7.169
<i>Cinara</i> sp.	27	14	41	0.043
Cicadellidae				
<i>Amrasca biguttula</i> (Ishida, 1912)	4670	4927	9597	10.17
<i>Nephotettix parvus</i> (Ishihara & Kawase, 1968)	15	21	36	0.038
Fulgoridae				
<i>Fulgoridae</i> sp.	0	9	9	0.01
Lygaeidae				
<i>Oxycarenus laetus</i> (Kirby, 1891)	5117	5055	10,172	10.78
<i>Spilostethus saxatilis</i> (Scopoli, 1763)	98	80	178	0.189
<i>Lygaeus</i> sp.	103	94	197	0.209
Coreidae				
<i>Cletus pugnator</i> (Fabricius, 1787)	78	95	173	0.183
Pentatomidae				
<i>Dolycoris baccarum</i> (Linnaeus, 1758)	89	36	125	0.132
<i>Eysarcoris ventralis</i> (Westwood, 1837)	81	52	133	0.141
<i>Nezara viridula</i> (Linnaeus, 1758)	46	28	74	0.078
Order				
Family	Layyah	Vehari	Total	R.A.
Species				
Pseudococcidae				
<i>Phenacoccus solenopsis</i> (Tinsley, 1898)	1995	2174	4169	4.419
Pyrrhocoridae				
<i>Dysdercus cingulatus</i> (Fabricius, 1775)	2349	2063	4412	4.677
Lepidoptera				
Erebidae				
<i>Laelia suffusa</i> (Hampson, 1893)	11	21	32	0.034
Gelechiidae				
<i>Pectinophora gossypiella</i> (Saunders, 1844)	2679	2938	5617	5.954
Geometridae				
<i>Geometridae</i> sp.	12	17	29	0.031
Hesperiidae				
<i>Pelopidas</i> sp.	7	15	22	0.023
Noctuidae				
<i>Agrotis</i> sp.	147	265	412	0.437
<i>Spodoptera litura</i> (Fabricius, 1775)	985	930	1915	2.03
<i>Spodoptera frugiperda</i> (J.E. Smith, 1797)	982	805	1787	1.894
Nolidae				
<i>Earias insulana</i> (Boisduval, 1833)	1634	1268	2902	3.076
<i>Earias vittella</i> (Fabricius, 1794)	335	471	806	0.854
Pieridae				
<i>Pieris brassicae</i> (Linnaeus, 1758)	21	43	64	0.068
<i>Pieris</i> sp.	7	13	20	0.021
Crambidae				
<i>Cnaphalocrosis medinalis</i> (Guenee, 1854)	6	15	21	0.022
Orthoptera				
Pyrgomorphidae				
<i>Chrotogonus trachypterus</i> (Blanchard, 1836)	66	87	153	0.162
<i>Atractomorpha</i> sp.	35	48	83	0.088
Tettigoniidae				

Table 1 (continued)

Order	Layyah	Vehari	Total	R.A.
Family				
Species				
<i>Oxyiachinensis</i> sp.	18	31	49	0.052
<i>Tettigoniidae</i> sp.	19	29	48	0.051
Acrididae				
<i>Acrida willemsei</i> (Dirsh, 1954)	48	29	77	0.082
Gryllidae				
<i>Gryllidae</i> sp.	34	41	75	0.079
Thysanoptera				
Thripidae				
<i>Thrips tabaci</i> (Lindeman, 1889)	2729	2589	5318	5.637
Total	46,276	48,067	94,343	100

Table 2

Percent relative abundance (on yearly basis) of ten most common families of insect pests recorded from cotton fields of both districts.

Family	LAYYAH		VEHARI	
	2018	2019	2018	2019
Aleyrodidae	9352 (40.66)	8741 (39.60)	9088 (41.71)	10,250 (41.19)
Lygaeidae	2616 (11.37)	2702 (12.24)	2473 (11.35)	2756 (11.07)
Cicadellidae	2461 (10.70)	2224 (10.08)	2405 (11.04)	2543 (10.22)
Aphididae	1637 (7.12)	1507 (6.83)	1689 (7.75)	1971 (7.92)
Gelechiidae	1208 (5.25)	1471 (6.66)	1307 (5.99)	1631 (6.55)
Thripidae	1446 (6.29)	1283 (5.81)	1217 (5.58)	1372 (5.51)
Pyrrhocoridae	1210 (5.26)	1139 (5.16)	892 (4.10)	1171 (4.70)
Pseudococcidae	897 (3.90)	1098 (4.97)	1009 (4.63)	1165 (4.68)
Noctuidae	1139 (4.95)	975 (4.42)	894 (4.10)	1106 (4.44)
Nolidae	1037 (4.51)	932 (4.22)	817 (3.75)	922 (3.70)
TOTAL	23,003 (100)	22,072 (100)	21,791 (100)	24,887 (100)

Note: In above table numbers are representing total count of insect pests belonging to each family and values in brackets are their percentage relative abundance.

more species are present in the area than were actually collected (Table 3).

Figs. 2 and 3 are showing seasonal dynamics of sucking and chewing insects collected from cotton ecosystems of district Layyah and Vehari, respectively. The sucking pests' active density peaks were observed in the district Layyah during the third week of July in both years while they peaked in district Vehari in the third week of August during both years i.e., 2018 and 2019. The chewing pests' active densities peaked in the districts Layyah during the third week of September in both years while they showed their active density peaks in district Vehari during the third week of September in 2018 and first week of October in 2019.

Table 4 is showing the total abundance, richness, diversity, and evenness indices calculated for both sampling years combined for the pests collected from district Layyah and district Vehari. Total catch of pests from district Vehari (48067) was higher than the total pests collected from district Layyah (46276). For calculating richness Menhinick and Margalef indices were used. It is clear from the Table 4 that both indices of richness were slightly higher for district Vehari as compared to district Layyah. Simpson index and Shannon-Weiner index values for both districts were nearly same. Likewise, the value of evenness index was a little higher in Vehari (0.71) district as compared to Layyah (0.67) district (Table 4).

Results of cluster analysis (Fig. 4) show 95 percent faunal similarity on all six locations of the study sites belonging to two different districts. Results of repeated measure ANOVA showed non-significant differences in the richness ($F_{1,7} = 1.55$; $P = 0.17$), diversity ($F_{1,7} = 1.46$; $P = 0.19$ and evenness ($F_{1,7} = 1.91$; $P = 0.09$) of insects among areas. However, there was significant different in richness ($F_{1,7} = 4.99$; $P < 0.01$), diversity ($F_{1,7} = 7.32$; $P < 0.001$) and evenness ($F_{1,7} = 2.99$; $P < 0.01$) among trapping sessions.

There was a strong negative correlation between population densities of Jassids, army worm and pink bollworm with temperature whereas all bollworms population densities show negative correlation with different selected weather parameters (Table 5).

4. Discussion

During the present study 94,343 arthropod pests belonging to 43 species were recorded from the two cotton growing districts of Punjab i.e. district Layyah and district Vehari during 2018 and 2019 cotton cropping seasons (Table 1). Family Aleyrodidae was the most dominant family in both districts during both years of this study and it all alone showed more than 39 percent family share among all families of different pests recorded while possessed 39.60 to 41.71 percent share among the top ten pest families (Table 2). Silverleaf whitefly *Bemisia tabaci* (Gennadius), Thrips *Thrips tabaci* (Lindeman), Jassids *Amrasca biguttula* (Ishida) and Aphid *Aphis gossypii* (Glover) are collectively known as sucking pest complex and are considered as the major sucking pests of cotton while Pink bollworm *Pectinophora gossypiella* (Saunders), American bollworm *Helicoverpa armigera* (Hubner), Spotted bollworm *Earias insulana* (Boisduval), Spiny bollworm *Earias vittella* (Fabricius), Army worm *Spodoptera litura* (Fabricius) collectively made the chewing pest complex of cotton crop. These pest complexes cause serious damage to the cotton crop either by sucking its sap or by chewing its different tender parts or by transmitting serious diseases to the plant (Abou-Elhagag 1998, Sarode et al., 2020).

The sucking insect pests attack cotton plants at its early phenological stage. As per expectation the first invaders of the sibling cotton were thrips. They invaded the cotton crop at an early stage as reported by Layton and Reed (2002). Thrips are highly polypha-

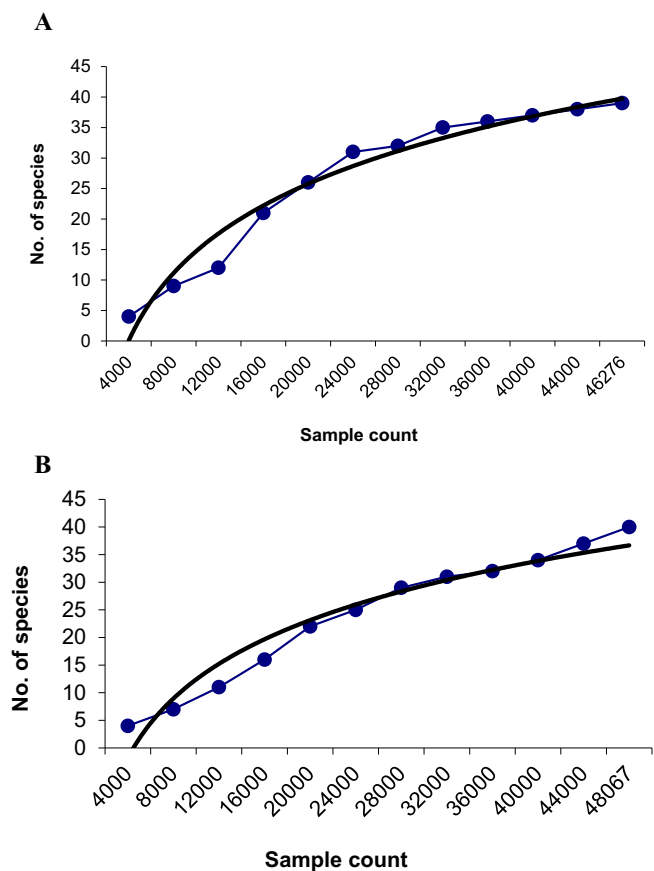


Fig. 1. Species accumulation curve for insect pests recorded from cotton fields of district Layyah (A) and district Vehari (B). **Note:** Each curved line in Fig. 1 is showing species accumulation curve and is obtained by using SPDIVERS.BAS program while each circular dot is showing actual number of species recovered from the collected specimen.

Table 3

Species diversity and inventory completeness for insect pests collected from district Layyah and Vehari.

Insect pests	Layyah	Vehari
No of specimens	46,276	48,067
Observed richness	40	42
No of singletons	4	4
No of Doubletons	3	3
Chao 2	42	43
% completeness	94	94

gous in nature (Cook et al., 2011). Along with cotton they are known for their feeding on more than 300 cultivated crops and other uncultivated plants belonging to at least 25 different families (Lewis, 1997; Shelton et al., 2008; Shera et al., 2020). It is observed that their number increased dramatically during the period of last two weeks of June and first two weeks of July during both cropping seasons (i.e. 2018 and 2019) suggesting a positive relationship with the high temperature. This rapid increase in their number and population size favored the idea of reproduction through parthenogenesis as mentioned by Layton and Reed (2002). In the experimental fields it was observed that all those plants which were severely infected by thrips showed stunted growth and also showed a delay in their maturity and fruiting behavior. They showed compromised growth as compared to other plants of the field. Such observations have also been reported by other researchers (Stewart et al., 2013; Allen et al., 2018). Aphids attack plant's

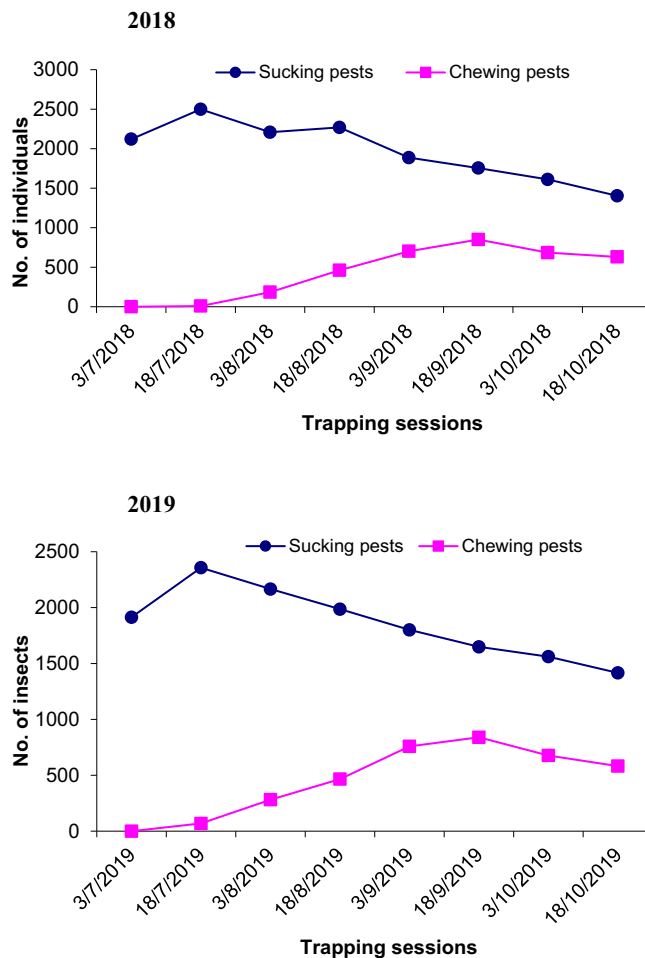


Fig. 2. Seasonal dynamics of sucking and chewing insects collected from cotton fields of district Layyah of Punjab, Pakistan.

soft tissue like leaves and growing points which are easy to puncture with the help of their piercing mouth-parts (Yang et al., 2020). They are also having polyphagous nature and damage various crops belonging to different families like Malvaceae, Rutaceae, Cucurbitaceae along with some ornamental plants like chrysanthemum (Ebert and Cartwright, 1997). Aphids like other sap suckers also secrete honeydews which pave the way for the development of sooty mold on cotton leaves and reduces its photosynthetic activity along with transmitting viral diseases into the plants they attack (Yang et al., 2020). Jassids were also found as serious sucking pests of cotton which contributed towards the overall yield loss. It caused 19 % yield reduction single handedly (Ali et al., 1993) along with the transmission of mosaic viral disease (Samal and Patnaik, 2008). Such yield loss was also reported by Bhat et al. (1984) and Ali (1992). Its nymphs and adults, both inject their poisonous saliva into the plant's tissue (Siraj et al., 2019).

Silverleaf whitefly, *B. tabaci* was recorded as the most dominating pest species among different pests of the cotton crop. Due to the Silverleaf whitefly family Aleyrodidae was the most dominant family in both districts during the study period with more than 39 % family share among all families of different pests recorded (Table 1). This pest species also appeared at an early phenological stage of cotton crop and kept its numbers near or above economic threshold levels during both cotton cropping seasons of this study. Whitefly not only inflicted damage to the cotton crop by sucking its sap from plant's lower leaf surfaces but also acted as a vector of viral disease (Shukla et al., 2016). Moreover, due to their sap suck-

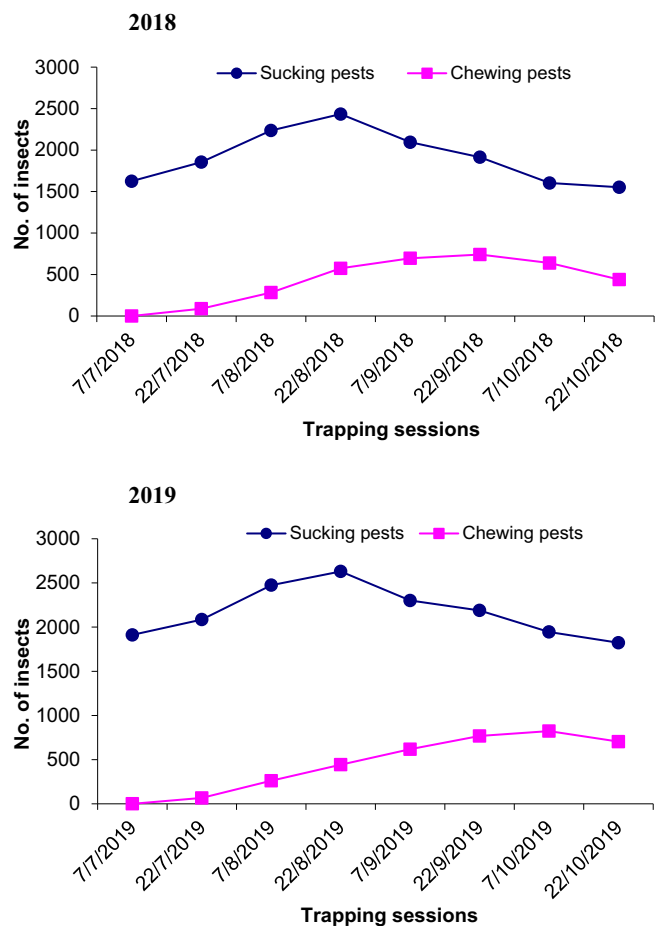


Fig. 3. Seasonal dynamics of sucking and chewing insects collected from cotton fields of district Vehari of Punjab, Pakistan.

Table 4

Total abundance, richness, diversity and evenness indices for the pests collected from district Layyah and district Vehari during 2018 and 2019.

Parameters	Study areas	
	Layyah	Vehari
Total abundance	46,276	48,067
Richness indices		
Margalef Index	3.537	3.618
Menhinick Index	0.1813	0.1824
Diversity indices		
Shannon-Wiener Index	3.078	3.056
Simpson's Index	0.1943	0.202
Evenness index		
Evenness (E5)	0.67	0.71

ing nature they reduced the plant's boll formation ability and its vigor. These findings were in agreement with the findings of Umaharan et al. (1998) and Ahmad et al. (2002). It was further noted that different insecticidal applications had not shown any promising effect against whitefly infestation and this was also in agreement with the findings of Shukla et al. (2016). The findings of positive correlation of whitefly with high temperature, is in accordance with the results of Bala et al. (2019). It is due to the fact that whiteflies' developmental activities are enhanced in slightly high temperatures (Zeshan et al., 2015).

Mealy bug is another polyphagous pest and have been reported to cause severe damages to its host plants including members of family Fabaceae, Malvaceae, Solanaceae, Verbenaceae, Compositae, Amaranthaceae, Asteraceae, Cucurbitaceae and Zygophyllaceae

(Nagrare et al., 2011). It was also recorded from the cotton fields during this study and it was found that its attack caused stunted plant growth, reduced boll formation and due to their excretion of honeydews, plants got sooty mold growth which reduced surface area for plants photosynthetic activity. These findings were in alignment with the findings of Saeed et al. (2007). Fuchs et al. (1991) was the first one who reported it as a pest of cotton in Texas, U.S.A in 1989. After that, it was reported from various countries in subsequent years like Chile (Larrain, 2002), Argentina (Granara, 2003), Nigeria (Akintola and Ande, 2008), Pakistan (Hodgson et al., 2008) and China (Wang et al., 2009; Wu and Zhang, 2009). Mealy-bug all alone reduced 40–50 % yield in the severely attacked cotton fields (Nagrare et al., 2009). Red cotton bug or cotton strainer was also observed to affect cotton seeds, its overall weight and its oil producing ability along with deteriorating lint quality by staining it. These findings were parallel with the findings of Shahid et al. (2014) and Khan et al. (2019). According to Jaleel et al. (2014) red cotton bugs caused 16–25 % yield reduction in cotton fields and are also responsible for deteriorating its lint quality. It was also reported to injure other plants of families Bombacaceae and Malvaceae (Kohn and Thi, 2004). Dusky cotton bugs were also observed causing injury, not only to cotton seeds but also to its stems and leaves as well. These observations are in agreement with the findings of Sarmad et al. (2020). The cotton plant is a preferred choice of this pest (Sarmad et al., 2020) and it attacks about 40 different host plants (Schaefer and Panizzi, 2000). It causes economic loss in almost all cotton growing areas of the world (Sarmad et al., 2020).

Among bollworms' complex, spotted bollworms were the first to be observed in the cotton fields. Two species i.e. *Earias insulana* and *Earias vittella* were recovered from the selected cotton fields. Severe damage to cotton plants was observed due to their phytophagous nature which is in accordance with the findings of Akhtar and Farooq (2019). Some of them were collected from newly growing but ruptured floral buds and even recovered from stems of the plants which caused severe damage to crop and impart a severe economic setback to its growers as reported by Sarate et al. (2012) and Sisterson et al. (2004). Pink bollworm was recovered from young bolls which were felt softened on examination. On opening of a few of them, they were found feeding inside the infected bolls. Moreover, due to their attack, premature opening of bolls was also observed which deteriorates the cotton fiber quality which was in alignment with the findings of Singh et al. (1988) and Fand et al. (2019). Pink bollworm was also reported as a native of Indo-Pakistan area of South East Asia (Saunders, 1843; CABI, 2018). It showed quite remarkable resistance towards *Bt* cotton as it was found in good numbers. Their resistance was also reported by many other researchers (Henneberry and Naranjo 1998; Tabashnik et al., 2013, 2014). Now it is ranked as a global, important chewing pest of cotton crops (CABI, 2018). Two species of the genus *Spodoptera* i.e. *Spodoptera frugiperda* and *Spodoptera litura* were recovered from the selected cotton fields. They were observed as one of the most destructive and notorious chewing pests of cotton affecting their different parts including leaves, stems and fruiting points adversely. Such damage was also reported by Saleem et al. (2016) and Ahmad et al. (2018). It was also observed as a polyphagous and cosmopolitan pest which was commonly reported from South Asia, Europe and Africa (El-Helaly et al., 2013). The bollworm infestation showed negative significant correlation with minimum temperature (Akhtar and Farooq, 2019). The black cutworms of genus *Agrotis* were also recovered from cotton fields at an early phenological stage and they also proved themselves as a severe and destructive pest of the crop which caused great loss to the overall crop yield. Similar observations were reported by Atwa et al. (2016).

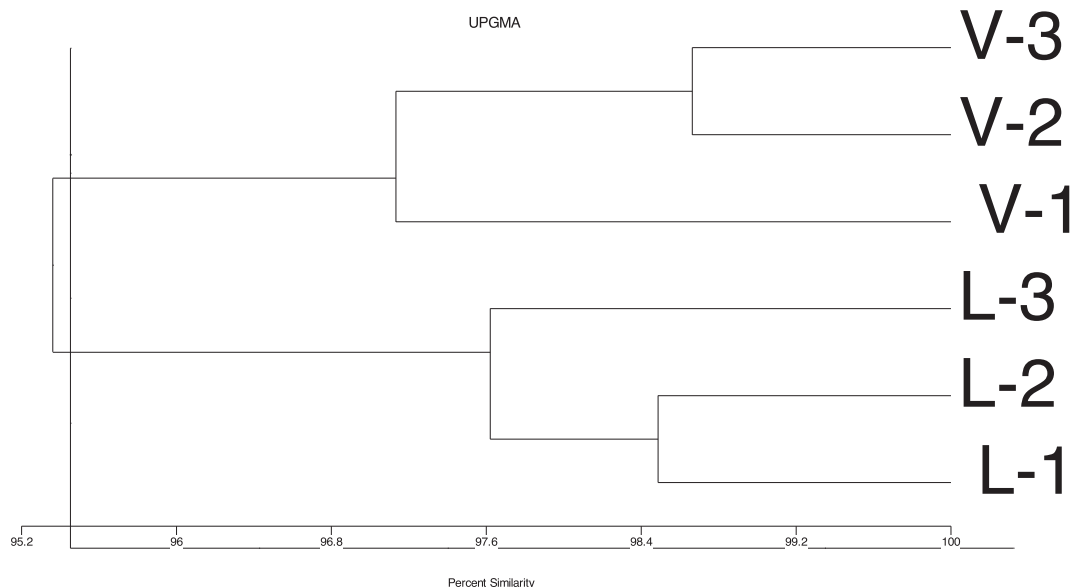


Fig. 4. Cluster analysis of different trapping sites showing percent similarity on the basis of abundance data of different Pests of district Layyah and Vehari. **Note:** (L-1, L-2 and L-3 were three selected sites of district Layyah while V-1, V-2 and V-3 were three selected sites of district Vehari).

Table 5
Correlation of different environmental parameters with the population densities of major pests of cotton.

Sr. No.	Pest	Temperature	Humidity	Rainfall
1	Jassid	r = -0.814* p = 0.014	r = -0.162 p = 0.701	r = -0.277 p = 0.507
2	Whitefly	r = -0.084 p = 0.843	r = -0.017 p = 0.967	r = 0.100 p = 0.815
3	Thrips	r = 0.585 p = 0.128	r = 0.398 p = 0.329	r = 0.363 p = 0.377
4	Army worm	r = -0.756* p = 0.030	r = -0.184 p = 0.663	r = -0.246 p = 0.557
5	Spotted bollworm	r = -0.433 p = 0.284	r = -0.108 p = 0.799	r = -0.287 p = 0.491
6	Pink bollworm	r = -0.790* p = 0.020	r = -0.368 p = 0.370	r = -0.360 p = 0.381

* Correlation is significant at the 0.05 level.

Many beetle species are ground dwelling arthropods and pitfall traps were mentioned for the collection of such arthropods more than a century ago (Dahl, 1896). It is considered as one of the best methods for the study of ground-dwelling arthropods (Sabu and Shiju, 2010). Mostly these traps were used to capture insects like beetles, ants and other ground dwelling arthropods (Philips and Cobb, 2005). Individuals belonging to the family Meloidae, Tenebrionidae, Scarabaeidae, Curculionidae and Chrysomelidae were captured during the sampling period with pitfall traps (Table 1). In addition to these, some other ground dwelling arthropods were also collected by these installed pitfall traps during the course of the sampling period of this study.

Cotton grey weevil (Sri Lankan weevil) was collected from the cotton fields at maturation stage of the crop (George et al., 2015). Typical leaf notching was observed as they moved inward from leave margins during its feeding. It was reported as another pest of cotton crop in the past few years from Pakistan and India (O'Brien et al., 2006). Their adults showed a peculiar behavior of feigned death in order to escape from dangers of different nature like spray of insecticides. This behavior was also reported by Josephraj Kumar et al. (2011). Another weevil *Anthonomus grandis* was also collected from the cotton fields which was found damaging the bolls and other parts of the cotton plants. It is an economically important pest of cotton agroecosystems especially in tropical and sub-tropical countries (Franco et al., 2004). It was

observed that their females cause more damage to the cotton bolls as compared to males for ovipositional punctures or for feeding. Similar findings were reported by Lloyd et al. (1961) and Santos et al. (2003). They also reported that boll weevils preferred feeding on ovules and anthers and due to this preference, they perforate through sepal and petal tissues which result in mechanical damage to the bolls.

Some other beetles of the family Scarabaeidae and Chrysomelidae were also observed in small numbers from the cotton fields of both districts during the cropping seasons of both years of this study (Table 1). Many species of Orthopteran pests were also observed in small numbers (i.e. about 1–2 % of the total pest count) during the field visits, including members of different families like Pyrgomorphidae, Tettigoniidae, Acrididae and Gryllidae from all selected cotton fields (Table 1).

In this study 94 percent species were successfully captured from the study areas of both districts during the study period (i.e. 2018 and 2019) (Table 3). The remaining 6 percent pests of the crop may consist of some rare pest species or our sampling timing and their activity time might have some difference due to which they were not captured due to which pests' accumulations curves didn't reach asymptote (Fig. 1). Similar findings were also reported by Schmidt and Balakrishnan (2015), and they also reported that different insect species used different activity time in order to avoid competition. It might also be possible that the dif-

ferent techniques used for capturing these pests were not enough to get 100 % complete inventory of the pest species of cotton agroecosystem of study area (Table 3). Some pest species may appear for a very short time during the cropping season and due to this they might have skipped themselves from getting captured during sampling. Different other researchers who worked on biodiversity of different arthropods or even some particular species estimation projects in a particular area reported almost similar experimental findings as of our observations. For example, Borges and Brown (2003) reported 90 % inventory completeness in their study of estimation of different arthropod species while Sherawat (2012) observed 96 % species of spiders recovered from wheat agroecosystem. Similarly, Mokam et al. (2014) reported more than 92 % insect species recovered from their area of study while Pineda et al. (2005) reported capturing of 94 % beetles from their study area.

At district Layyah, sucking insect pests showed their maximum numbers during the third week of July in both years (Fig. 2) and these observations were in complete alignment with the observations of Shera et al. (2013) who recorded peak populations of jassid during these weeks. The chewing pests had peaked during the third week of September in both years of sampling i.e. 2018 and 2019 (Fig. 2) and it was in total agreement with the findings of Akhtar and Farooq (2019). At district Vehari sucking insect pests showed their population peaks during the third week of August in both cropping seasons (Fig. 3) and again these observations were in accordance with the observations of Shera et al. (2013) who reported peak populations of whitefly during these weeks, but contradictory to those of Rashida et al. (2010), which was due to unusually rainier days in August during their observation period. The chewing insect pests had shown their maximum numbers in the third week of September during 2018 and in the first week of October during 2019 cotton cropping season (Fig. 3) and the present recorded data is in total agreement with the observation made by Akhtar and Farooq (2019).

There were minor differences in diversity indices of both districts (Table 4). This was expected as there were minor differences in environmental conditions of both districts. The maximum number of species estimated by using Chao quantitative estimator showed some deviation between observed and estimated number of species at both districts. This difference shows that the sampling efforts used were not adequate enough. So, there is a need of conducting more intensive studies with some modifications of used sampling techniques or even by adding some more methods of sampling. Moreover, enhancing sampling time i.e. sampling during different times of a day may be considered as an option as well. From the dendrogram of the cluster analysis (Fig. 4), it is evident that localities of each district formed one group as they share the same environmental and geographical range. It is also confirmed by the data obtained from the cotton fields as it shows minimal differences among the data obtained from different sites of each district. Similar findings were reported by Inayat et al. (2010), who worked on four different crops from the same geographical zone.

In the present study, the jassid population showed strong negative correlation with the temperature which is affirmative with the results of Sarode et al. (2020). Similarly, the whitefly population showed weak negative correlation with temperature during the period of study (Table 5), which was completely in agreement with the findings of Dhaka and Pareek (2008), Khan et al. (2010), Bhute et al. (2012), and Kedar et al. (2016). Thrips population showed moderate positive correlation with the temperature (Table 5). Similar results were observed by Bhute et al. (2012) and Majeed et al. (2016), who reported that thrips multiplied their number during hot and dry days. Venilla et al. (2007a, b) also reported population peaks of thrips during the days with high temperature and low humidity which is in confirmation with the present findings. All Bollworms' population densities showed negative

correlation with the temperature (Table 5). By the end of July or mid-August the temperature started to decline and at this time the crop was mostly at boll formation stage. So, the population densities of boll worms increased many folds during this crucial stage. These findings were completely in agreement with the findings of Akhtar and Farooq (2019). Bollworms' population densities also showed negative correlation with humidity and rainfall during this study which was in-line with the findings of Akhtar and Farooq (2019).

The non-significant association between rainfall and humidity with the total number of all major pests showed the minimal role of these climatic factors on their population densities. Similarly, the population densities of some major pests of the crop also showed negative significant correlation with temperature. All this shows a minimal role of these climatic factors in controlling the overall population densities of these major pests (Table 5).

5. Conclusion

It is concluded that population densities of various pest species fluctuate during the cropping season. Different pests show their peaks at different standard meteorological weeks but their combined effect show that at first sucking pests dominate the fields during the early phenological stage of the crop i.e. growth stage of the crop as more and more fresh, green and soft tissue is added at this stage to the plant and it supports sucking pest population to grow rapidly by sucking sap from these newly grown soft parts of the cotton plants. Later, as the plants enter next phenological stages, chewing pests dominate the fields as more and more bolls are available for the attack of different bollworms attacking species and other chewing pests. The cotton growers have to remain vigilant throughout cropping season as all these pests attack the crop in consecutive standard meteorological weeks. In agricultural fields, different insects respond differently to the weather parameters. Even different life stages of each insect also show varied response to same environmental factor. So, to mention any single factor as the sole cause of increase or decrease in the population of any given pest is quite difficult to claim with certainty. In future the current finding may be extended to explore the arthropods diversity by considering different habitats and in multiple cropping systems.

Author contribution statement

Amir Nadeem, Hafiz M. Tahir, Azhar A. Khan, Zeshan Hassan and Arif M. Khan contributed to the design, data collection, analysis, writing and review of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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