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Yearlong association of *Apis dorsata* and *Apis florea* with flowering plants: planted forest vs. agricultural landscape

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Abstract

The yearlong association of two native honey bee species (*Apis dorsata* and *A. florea*) with 49 plant species was recorded in a planted forest and adjacent agricultural landscape at Multan, Pakistan. The study resulted in 588 interactions of *A. dorsata* with 40 plant species and 454 interactions of *A. florea* on 38 plant species. The most visited plants species by *A. dorsata* included *Helianthus annuus*, *Citrus reticulata*, *Trifolium alexandrinum*, *Moringa oleifera* and *Calotropis procera*, while the most visited plant species by *A. florea* included *C. procera*, *Mangifera indica*, *T. alexandrinum*, *Coriandrum sativum* and *H. annuus*. The peak abundance of bees and floral resources (i.e. number of plant species in flowering and abundance of floral units) was recorded during early March to late May followed by a gradual decline until December. Monthly abundance of both bee species was positively related to the floral resources, negatively related to relative humidity while it was not significantly related to temperature. The current study may serve as a baseline to track the degradation in ecosystem service of cross pollination and making new conservation strategies at local scale while future research should focus on tempo-spatial variations in foraging preferences, floral constancy and effect of foraging competition on crop pollination in different ecological regions of Pakistan.

Introduction

Honey bees play a vital role in crop production and in preserving the wild habitats. They have coevolved with native flora and sometimes this coevolution results in specialized relationships. Because of their diverse nesting strategies and specific host plant biochemical relationships, honey bees are regarded as the most suitable social insect for the monitoring of environmental processes (Nathan et al., 1999).

There are three native honey bee species in Pakistan i.e. the wild bee or rock bee, *Apis dorsata* (Fabricius, 1793); the little bee or dwarf bee *A. florea* (Fabricius, 1787) and domesticated bee, *A. cerana* (Fabricius, 1793) whereas, the exotic Italian bee, *Apis mellifera* (Linnaeus, 1758) has been successfully domesticated since 1977. *Apis cerana* occurs in Northern and Western hills and foot-hills in some parts of KPK, Punjab, Baluchistan and Kashmir, while the *A. dorsata* and *A. florea* occur in the foot hills and plains of Pakistan. Although Asia is not rich in bee diversity compared with some other biogeographical regions e.g. Neotropics (Michener, 1979) however, social bees (Apis sp.) – in terms of their abundance – play a critical role in maintaining the vital ecological process of cross pollination (Momose et al., 1998; Roubik et al., 2005; Devy & Davidar, 2006).

Both, *A. dorsta* and *A. florea* tend to migrate locally in response to the availability of floral resources and have the ability to increase rapidly in number in response to flowering events (Itioka et al., 2001). Honey bees require carbohydrates, proteins, fats, minerals, vitamins, and water for the maintenance, growth and development of their colonies (Loper & Berdel, 1980). These elements are sourced by flowering plants in shape of pollen and nectar. However, the foraging activity of honey bees is greatly influenced by the weather conditions and availability of nectar and floral resources (Neupane & Thapa, 2005).

Both *Apis* bees have already been reported as the most abundant and efficient pollinators of various economically important crops in Pakistan e.g. canola; *Brassica napus* (Ali et al., 2011), onion; *Allium cepa* (Saeed et al., 2008; Sajjad et al., 2008), fodder; *Sesbania sesban* (Sajjad et al. 2009), fodder alfalfa; *Medicago sativa* (Ahmad 1976) and Bitter gourd; *Momordica charantia* (Saeed et al., 2012). Using bio-economical methods Irshad and Stephen (2013) quantified economic value of pollination in Pakistan. They estimated production value of pollination dependent crops at US$1.59 billion i.e. 62% fruits, 20% vegetables, 9% nuts, 8% oilseed and 0.25% spices.

A sharp decline in number of honey bee combs has been reported during last two decades in neighboring India (Sihag, 2014) however, for Pakistan, there is no study in hand based on systematic observations. The decline is probably due to the increase in pesticide usage and destruction of natural habitats (Kremen et al., 2002). Khan et al. (2002) reported the direct annual loss of 9.91 million rupees on account of a loss of 5661 MT of honey while net annual loss of 6.55 million rupees in sunflower production in the selected nine districts of the cotton zone of Punjab, Pakistan.

The associations between plant diversity, social bee abundance, honey production and local livelihoods have poorly been documented for Pakistan. The flora visited by honeybees remains scarce in most parts of the beekeeping areas for a fairly long period during the year (Ahmad, 1984; Aziz, 2015). Moreover, the strong turnover in quality and quantity of floral resources are known to influence the behavior and foraging strategy of pollinator species (Subbareddi & Reddi, 1994; Murugan et al., 1997).

Site list of floral host plants, their temporal dynamics in providing floral resources and ways they interact with honey bees temporally, serve as baseline studies without which it is difficult to track the degradation in ecosystem service of cross pollination and making new conservation strategies (Mark et al., 2002). Several preferred plant species for managed honey bees (*A. mellifera*) have been documented from Himalayan foothills and northern Pakistan (Noor et al., 2009; Partap, 1997) however no such data is available for the plain areas of Southern Punjab. The purpose of this study was to enlist the floral host plants and identify the *Apis* most loving plant species for the first time from southern part of Punjab, Pakistan. Moreover, we hypothesized that seasonal population fluctuations of *Apis* is influenced by abiotic (relative humidity and temperature) and biotic (number of plant species in flowering and total floral abundance) factors.

Materials and Methods

The study was conducted in a planted forest of 20 hectares and an adjacent agricultural farm at Bahauddin Zakariya University campus Multan (30.255°N; 71.513°E; 114±6 meter above sea level), Pakistan from January to December, 2008. The planted forest is comprised of trees/ shrubs of the species *Prosopis juliflora, Moringa oleifera, Eucalyptus camaldulensis, Tamarix aphylla, Albizia procera, Ziziphus jujuba, Dalbergia sissoo, Capparis decidua, Leucaena leucocephala* and *Acacia nilotica.* Besides planted trees, there also grows a variety of natural vegetation including annual wild plants and perennial shrubs (Table 1). Some seasonal crops and fruit trees are grown in adjacent agricultural landscape including *Helianthus annuus, Trifolium alexandrinum, Coriandrum sativum, Momordica charantia, Brassica campestris, Daucus carota, Allium cepa, Citrus reticulata*, *Grewia sub inaequalis* and *mangifera indica*. Climate of the area is sub-tropical with a long hot summer and short cold winter. The mean daily maximum and minimum temperatures are in the range of 30 to 35°C and 15 to 20°C, respectively with the mean monthly summer rainfall of *ca*. 18mm. The highest temperature (45 to 51°C) is recorded in May and June while the lowest (3 to 0°C) is recorded in January (Khan et al., 2010).

A variety of annual wild plants and perennial shrubs naturally grow in the forest. There were eight hives of *A. dorsata* while the number of hives of *A. florea* was uncertain as they are smaller in size and mostly build combs in thick vegetation. As different plant species had different type of inflorescences, we defined the floral units for each plant species separately and each time recorded observations on those floral units. Floral abundance was estimated by randomly tagging 15 plants of each plant species and counting total floral units at every two weeks interval.

During each census, we performed random walks in forest and selected15 plants of each plant species at their flowering stage. For agricultural crops, 15 plants were selected randomly from the margins of the field. Each plant was observed for 60 seconds in its floral units for any visit of honey bees. In this way there was a total of 15 minutes of observation per plant species in one census. Only those plant species were selected which were in the phase of flowering. For each plant we counted the number of visiting individuals of both *Apis* species by visual observation. Visiting bees other than *Apis* species were also recorded during the census. The census of each flowering plant species was carried out at two weeks interval throughout the flowering period. The observations were taken on clear sunny days, while rainy or cloudy days were avoided.

To avoid the phenomenon of floral constancy i.e. bees tend to visit single plant species even in the presence of many other flowering plant species in that particular area (Gruter et al., 2011), we selected wild plants of a particular species at a considerable distance from each other (>50m).

We used rank-abundance plot with fitted curve as a way to visualize the overall visitation pattern of bees on 36 plant species (Magurran, 2004). Frequency distribution test was applied to identify various classes of plant species based on abundance of *Apis* bees. We used linear regression

Table 1. Monthly abundance of bees, on 49 plant species at Bahauddin Zakariya University Campus, Multan Pakistan during January to December, 2008.

Plant species	Family	Flowering period	Flower color	*A. dorsata*	*A. florea*	Total	Class boundary Lower-Upper (contribution to χ^2)
Calotropis procera	Asclepiadaceae	Jul-Sep	White+purple	36	93	129	103-129 (13.82)
Helianthus annuus	Asteraceae	Feb-Mar	Yellow	59	24	83	77-103 (1.63)
Trifolium alexandrinum	Fabaceae	Apr-May	Pale	42	39	81	
Citrus reticulata	Rutaceae	Feb-Mar	White	56	6	62	52-77 (1.66)
Coriandrum sativum	Apiaceae	Feb-Mar	White	27	30	57	
Prosopis juliflora	Fabaceae	Mar-June	Pale	21	22	43	
Melilotus indica	Fabaceae	Apr-Aug	Yellow	-	41	41	
Moringa oleifera	Moringaceae	Apr-Jun	White	41	-	41	
Grewia subinaequalis	Malvaceae	Mar	Yellow	34	2	36	
Eucalyptus camaldulensis	Myrtaceae	Apr-May	Pale	34	1	35	26-52 (3.15)
Launaea nudicaulis	Asteraceae	Feb-Sep	Yellow	15	17	32	
Raphanus sativus	Brassicaceae	Feb-Mar	White+pink	12	18	30	
Momordica charantia	Cucurbitaceae	Apr-Jul	Yellow	6	24	30	
Cirsium arvense	Asteraceae	Jan-Mar	Purple	19	10	29	
Brassica campestris	Brassicaceae	Nov-Jan	Yellow	8	19	27	
Tamarix aphylla	Tamaricaceae	Jun-Oct	Pink	11	15	26	
Albizia procera	Fabaceae	Jun-Aug	White	21	1	22	
Ageratum conyzoides	Asteraceae	Mar-May	Purple	13	7	20	
Tribulus terrestris	Zygophyllaceae	Jun-Aug	Yellow	9	7	16	
Haloxylon recurvum	Amaranthaceae	Sep-Oct	Green	1	14	15	
Ziziphus jujuba	Rhamnaceae	Jun-Jul	Green	14	1	15	
Daucus carota	Apiaceae	Feb-Mar	White	1	13	14	
Salsola baryosma	Amaranthaceae	Aug-Oct	Green	13	1	14	
Launaea procumbens	Asteraceae	Feb-Sep	Yellow	12	-	12	
Pulicaria crispa	Asteraceae	Mar-April	Yellow	11	1	12	
Dalbergia sissoo	Fabaceae	Mar-April	Pale	9	3	12	
Trianthema portulacastrum	Aizoaceae	Sep-Oct	White+pnik	3	9	12	
Malvastrum coromendelianum	Malvaceae	Round the year	Yellow	8	3	11	
Capparis decidua	Capparaceae	Mar-Apr	Pink	11	-	11	
Convolvulus arvensis	Convolvulaceae	Round the year	White	-	8	8	
Parkinsonia aculeata	Fabaceae	Mar-May	Yellow	7	1	8	
Leucaena leucocephala	Fabaceae	Jun-Nov	Pale	7	-	7	
Achyranthes aspera	Amaranthaceae	Oct-Nov	Purple	6	1	7	
Sonchus oleraceus	Asteraceae	Mar-Apr	Yellow	-	7	7	
Heliotropium europaeum	Verbenaceae	Apr-Jun	White	4	2	6	
Carthamus oxycantha	Asteraceae	Mar-May	Yellow	4	2	6	
Spergula arvensis	Caryophyllaceae	Mar-Apr	White	-	5	5	1-26 (31.59)
Acacia nilotica	Mimosaceae	Jun-Nov	Yellow	4	-	4	
Sonchus asper	Asteraceae	Feb-Sep	Yellow	-	2	2	
Lantana camara	Verbenaceae	Round the year	Yellow+pink	-	2	2	
Physalis peruviana	Solanaceae	July-Oct	Yellow+brown	2	-	2	
Oxystelma esculentum	Asclepiadaceae	Dec	White+purple	2	-	2	
Cleome viscose	Cleomaceae	July-Sep	Yellow	2	-	2	
Euphorbia helioscopia	Euphorbiaceae	Mar-Apr	Green	-	1	1	
Oxalis corniculata	Oxalidaceae	Mar-Dec	Yellow	-	1	1	
Allium cepa	Liliaceae	Mar-Apr	White	1	-	1	
Suaeda fruticosa	Amaranthaceae	Dec-Jan	Green	-	1	1	
Chenopodium album	Amaranthaceae	Mar-Apr	Green	1	-	1	
Portulaca oleracea	Portulacaeae	Aug-Dec	Yellow	1	-	1	

analysis to explore the relationship between abundance of bees and abiotic (relative humidity and temperature) and biotic (number of plant species in flowering and total floral abundance) factors.

Results

A total of 72 flowering plant species in 30 families were observed for recording the visitation of *A. dorsata* and *A. florea*. The total sampling efforts of 121 hours yielded 1049 interactions of both bee species (constituting 44.36% of total native bee visits) on 49 plant species in 26 families (Table1). Twenty three plant species in 18 families were not visited by any of the two *Apis* species (Table 2). A total of 588 individuals of *A. dorsata* were recorded on 40 plant species in 21 families while 454 individuals of *A. florea* were recorded on 38 plant species in 19 families. Eleven plant species were exclusively visited by *A. dorsata* while 13 by *A. florea*. Twenty five plant species were visited by both the bees (Table 1).

Table 2. Plant species which were not visited by *A. dorsata* and *A. florea* during January to December, 2008.

Plant species	Family	Flower color	Flowering period
Sesuvium sesuvioides	Aizoaceae	Purple	Dec
Mangifera indica	Anacardiaceae	Green+ white	Mar-Apr
Asphodelus tenuifolius	Asphodelaceae	White	Nov-Apr
Conyza canadensis	Asteraceae	Green+ white	May-Jun
Sisymbrium irio	Brassicaceae	Yellow	Mar-May
Stellaria media	Caryophyllaceae	White	Apr-Aug
Chenopodium murale	Chenopodiaceae	Green	Jan-July
Convolvulus sp.	Convolvulaceae	White	Mar-Jun
Cucumis prophetarum	Cucurbitaceae	Yellow	Jun-July
Phyllanthus niruri	Euphorbiaceae	Green	Aug-Sep
Cajanus cajan	Fabaceae	Yellow	Jul-Aug
Medicago sativa	Fabaceae	Purple	May-Sep
Lathyrus sativus	Fabaceae	Pruple+ White	Feb-Mar
Vicia sativa	Fabaceae	Purple	Jul-Aug
Alhagi graecorum	Fabaceae	Pink	Mar
Fumaria indica	Fumariaceae	Pink	Mar- Jun
Centaurium pulchellum	Gentianaceae	Pink	May-Jun
Abutilon indicum	Malvaceae	Yellow	Aug-Dec
Abelmoschus esculentus	Malvaceae	Yellow	Jun-Aug
Melia azedarach	Meliaceae	White+ Purple	Mar-Apr
Ranunculus muricatus	Ranunculaceae	Yellow	Mar-Apr
Solanum surattense	Solanaceae	Purple	Apr-Aug
Torilis japonica	Apiaceae	White	Feb-Mar

The rank abundance curve shows only a few plant species with higher bee abundance while many plant species showed lower bee abundance (Fig 1). The frequency distribution test identified five classes of plant species based on visitation frequency of both bee species. *Calotropis procera* (class 1) was visited by the maximum number (129) of bees followed by *Helianthus annuus*, *Trifolium alexandrinum* (class 2) and *Citrus reticulata* and *C. sativum* (class 3). Class 4 included ten plant species while class 5 included 34 plant species (Table 1). The plant species most visited by *A. dorsata* included *H. annuus*, *C. reticulata*, *T. alexandrium*, *Moringa oleifera* and *C. procera*, while the most visited plant species by *A. florea* included *C. procera*, *M. indica*, *T. alexandrium*, *Coriandrum sativum* and *H. annuus* (Table 1).

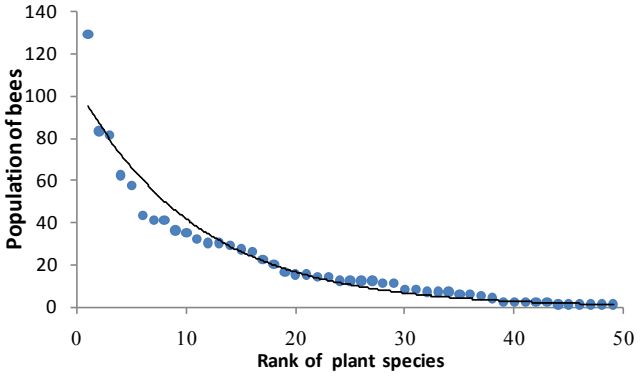

Fig 1. Rank abundance curve based on population of *A. dorsata* and *A. florea* on 49 plant species at Bahauddin Zakariya University Campus, Multan, Pakistan during January to December, 2008.

The seasonal dynamics revealed the peak activity of both *Apis* bees during early March to late May. Almost no activity was recorded in the months of November, December and January (Fig 2). Similarly, the maximum availability of floral resources (abundance of floral units and number of plant species in flowering) was also recorded in the spring season (March to May) followed by a gradual decline until December (Fig 3). Average monthly temperature and relative humidity is shown in Fig 4.

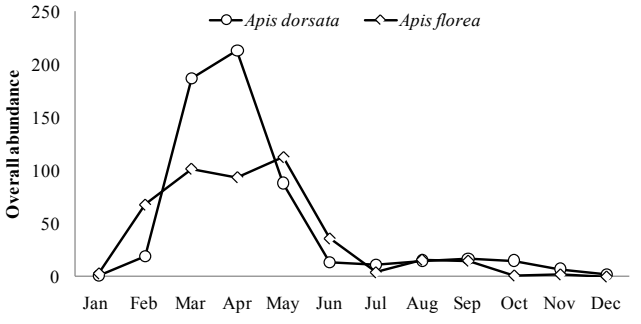

Fig 2. Monthly abundance of *A. dorsata* and *A. florea* at Bahauddin Zakariya University Campus, Multan, Pakistan during January to December, 2008.

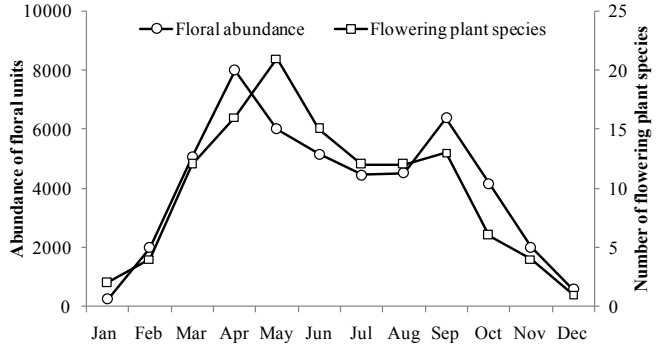

Fig 3. Availability of floral resources (number of flowering plant species and floral abundance) at Bahauddin Zakariya University Campus, Multan, Pakistan during January to December, 2008.

Monthly abundance of both bee species was positively related to the floral resources i.e. number of plant species in flowering ($y = 10.43x-15.81$, $R^2 = 0.338$, $P = 0.047$) and abundance of floral units ($y = 0.029x - 31.76$, $R^2 = 0.382$, $P = 0.032$). The monthly abundance of bees had a significant negative relationship ($y = -5.674x + 474.9$, $R^2 = 0.409$ $P = 0.025$) with relative humidity while no relationship was verified with temperature.

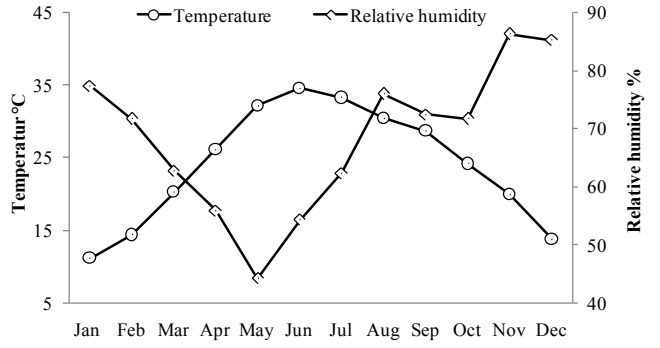

Fig 4. Average monthly temperature and relative humidity at Bahauddin Zakariya University Campus, Multan, Pakistan during January to December, 2008.

Discussion

In this study, we recorded 1049 visits of both *Apis* species which constituted 44.36% of the total pollinating bee visits. This high abundance of honey bees signifies the importance of semi-natural habitats (i.e. forest in our case) in maintaining the viable population of pollinators (Johannsmeier & Mostert, 2001). Moreover, the size and quality of semi-natural habitats have been reported to impact significantly on the bee abundance in adjacent agricultural landscapes (Liow et al., 2001). Since insect pollination contributes 75% of total global crop pollination while honey bees constitute 70-80% of insect pollination (Klein et al., 2007), loss of honey bees therefore can significantly impact the biodiversity and availability of food in general while livelihoods of local communities in specific (Devy & Davidar, 2006).

Therefore, in order to maintain honeybee populations, it is important to ensure the conservation and management of sufficient forage resources within agricultural and surrounding natural landscapes (Zhang et al., 2007). These resources include both suitable nesting habitat and sufficient floral resources i.e. nectar and pollen (Kremenet al., 2007). In various parts of India, *A. dorsata*, *A. cerana*, *A. florea* and *A. mellifera* are the most effective pollinators (Chandel et al., 2004). Apid bees probably play an important role in pollinating crop plants in India, although quantitative data are lacking (Potts et al., 2003).

In our study, both honey bee species visited 49 species across the year. Honey bees are generalist feeders and visit several plant species for getting nectar and pollen (Michener, 1990) yet they prefer the flowers with exposed nectar, abundant pollen production, zygomorphic nature and compact umbels (Free & Ferguson, 1983; Koul et al.,1989; Diederichsenn, 1996). That is why in our study, their higher abundance was recorded mostly on open shaped flowers (*C. procera, H.annus*) along with zygomorphic (*M. indica*, *T. alexandrium*) and umbelliferous flowers (*C. sativum*). Previous studies have also reported honey bees as the frequent visitors of *Calotropis* sp. (Betz et al., 1994; Fishbein & Venable, 1996), *H. annus* (Jadhavet al., 2011) and *C. sativum* (Abou-Shaara, 2015).

Twenty five plant species were visited by both *Apis* species. There was evidence of ecological niche overlapping among them i.e. value of Pianka's index was 0.66 (simulated indices mean = 0.66, simulated indices variance = 0.000, P (observed ≥expected) = 0.122) at alpha 0.05. Different floral nectar compositions are preferred by different species (Abrol, 2011). For example, *A. florea* prefers flowers having low caloric rewards (Sihag & Rathi, 1992) whereas *A.dorsata* prefers flowers with high caloric rewards. Similarly, in our studies, *A. dorsata* did not visit two of the most preferred host plants of *A. florea* i.e. *M. indica; C. sativum* which contain low nectar rewards.

Twenty three plant species were not visited by both of the *Apis* species. However, some of these plant species have already been reported as good source of pollen and nectar for *A. mellifera* i.e. *Sisymbrium irio*, *Cucumis prophetarum*, *Medicago sativa* (Taha, 2015). Besides this, there seems no specific reason of avoiding some other very good sources of nectar and pollenin this study like *Mangifera indica*, *Conyzacan adensis*, *Cajanus cajan*, *Abutilon indicum* and *Torilis japonica* etc. This might be due to the marked behavior of floral constancy among bees (Amaya-Marquez, 2009). Floral constancy is the behavior of restricting visits largely to a single floral type (Waser, 1986) yet little is known about the reasons and thereby no any general theory is in place that can explain all kinds of floral constancy (Amaya-Marquez, 2009). This constraint suggests taking into account rather a large geographical area when attempting quantification of floral host plants of bees in order to avoid this behavior.

The seasonal dynamics revealed the peak activity of both *Apis* bees during early March to late May (summer months) while almost no activity was recorded from November to January (winter months). This could be due to the availability of high foraging resources in summer months compared to the winter months as it is clear from the significant positive relationship of monthly bee abundance with the floral resources (number of plant species in flowering and availability of floral units) in our data. Hussein et al. (1992) also reported the maximum honeybee foraging activities during the summer months (July to September) and minimum in winter months (November to January). Whereas, it has also been studied that *A. dorsata* migrates locally in response to the availability of floral resources and its abundance showed positive relationship with the floral resources (Itioka et al., 2001; Sihag, 2014).

The monthly bee abundance was also found to have significant negative relationship with relative humidity while it had no relationship with the temperature. The previous studies have also reported a decreased daily activity of honey bees on the days characterized by maximum temperature and highest humidity (Nargis et al., 2001; Kumar et al., 2002; Mordago et al., 2002). High humidity, heavy rain fall, wind and low temperature had negative influence on sunflower inflorescences visits.

Knowledge of the honeybee flora of an area is a basic tool for the development and sustainability of apiculture as well as commercial agricultural crop pollination (Dimou et al., 2006). The availability of adequate forage resources positively impact the honeybee colony health and beekeeping profitability leading to improved crop pollination (van Engelsdorp & Meixner, 2010). The current study may serve as a baseline to track the degradation in ecosystem service of cross pollination and making new conservation strategies at local scale while future research should focus on tempo-spatial variations in foraging preferences, floral constancy and effect of foraging competition on crop pollination in different ecological regions of Pakistan.

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