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Studies on Visitation Pattern of Honeybee (Hymenoptera: Apidae) and its Impact on the Yield and Oil Contents of Sunflower (*Helianthus annuus* L.) Seed in Peshawar Valley, Pakistan

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ABSTRACT

Field studies were carried out at New Developmental Farm (NDF), The University of Agriculture-Peshawar, (34.01° N, 71.53° E) Khyber Pakhtunkhwa-Pakistan during spring and fall 2012 and 2013. Objectives of current trial were to assess visitation pattern of *Apis mellifera* and *A. florea* and to examine their influence on yield increase as well as on oil contents of sunflower seed. Frequent visitations of *A. mellifera* observed at 1200 noon, where 29 individuals of bee visitors were found on sunflower blossoms. The next high (25 honeybee/3 m^2) visitation rate of bee was recorded at 1400 h. Individuals of *A. florea* showed two peaks of visitation between 1400 to 1600 h. First peak (15 honeybee/3 m^2) was recorded at 1600 h, whereas second peak (12 bees) was recorded at 1400 h. Lowest visitation rate of both species was noticed during evening after 1800 h. High frequency of both species found engaged in foraging during 20th and 25th day after initiation of blooms on sunflower due to more number of pollen and nectar. Minimum number of honeybees was recorded during initial and very last days of flowering due to less number of plants with blooms and less availability of pollens and nectar on flowers. Maximum seed production as well as oil contents was obtained from sunflower plots kept under natural conditions, where bee visitors had access to sunflower blossoms. In contrast, sunflower plots covered with insect-proof bags gave minimum seed production and oil contents, which most probably was because of bee visitors denied to forage on flowers of the crop. Results revealed that sunflower in blooming stage attracted large number of bee visitors particularly *Apis mellifera* and *A. florea*. Visitation/foraging of honeybee contributed significantly towards increase in the yield and quality of sunflower seed.

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Authors' Contributions
FS and MI designed the study, performed the experiments, statistical analysis and initial writing. HA analysed the data statistically, graphs, writing, editing and submission.

Key words
***Apis mellifera, Apis florea*, Visitation pattern, Oil contents, Yield and sunflower.**

INTRODUCTION

Sunflower (*Helianthus annuus* L.), which belongs to the family Compositae (Khan and Atta, 2007), is one of the most important oilseed crops worldwide (Cantamutto and Poverene, 2007; Skoric *et al*., 2007). Being a potential oilseed crop, cultivation of sunflower growing in both developed and developing countries due to which this crop has emerged as an economically important crop across the globe (Skoric, 1992). Sunflower is a good and major source of high quality edible oil (40-47%) and its seeds contain 20-25% protein (Saleem *et al*., 2003). It is renowned as poor man's meat in several developing countries (Potter and Hotchkiss, 1997). In Pakistan, sunflower cultivation started in 1960 to connect the gap between production and utilization of edible oil across the country (Burney *et al*., 1990). As per annual report of Agricultural Statistics of Pakistan (2013), the area under cultivation of various oilseed crops in Pakistan is 3,704,940 (ha) and its production is 3,947,000 (tons), while area under cultivation of sunflower and its production is 155.30 thousand hectares and 189.73 thousand tons, respectively. In general, sunflower is considered self-fertile and a self-pollinated crop, but lack of self-compatibility considerably declines its yield (Neff and Simpson, 1990; Free, 1993). The degree to which a plant is able to self-pollinate, depends on the position and the time of release of anthers in relation to the stigma (Free, 1993). In some cases, the tip of the stigma of sunflower expands above the anthers to get pollens; thus enhancing the probability of self-pollination. Self-incompatible and cross-pollinated crops need pollinating agents for efficient pollination and seed set (Thapa, 2006).

Sunflower visited by many insects/pollinator but among them *Apis* species are very important (Ali *et al*., 2015). *Apis mellifera* L. is an efficient and successful pollinating agent of a large number of crops (Free,

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1993; Johannsmeier and Mostert, 2001) that contributes significantly to the economic growth and sustainability of humanity. Forging and pollination by honeybee varies in different crops (Yucel and Duman, 2005). Earlier investigation conducted by Moeller and Koval (1973) have confirmed that honeybee pollination can increase fruit set by 10 to 25 percent and yield by 18 to 100 percent. Nderitu *et al*. (2008) and Kumar and Singh (2005) also reported that among *Apis* bees, *A. mellifera* is thought to be the most efficient and well-organized species. It increases the yield of sunflower compared to other insect visitors. Oz *et al*. (2009) pointed out that because of honey bees sunflower hybrid seed production improved percentage of seed set, 100-seed weight and number of filled/healthy seed per capitulum and seed yield. Shrestha (2004) concluded that the contribution of honeybee pollination to crop production and quality has been anticipated to be more than the overall value of honey and wax production. In addition to oil content, seed yield, is the most important component of sunflower (Mijić *et al*., 2006). Some studies were also done by Ali *et al*. (2015) on visitation patterns of *Apis* species, though they did not study the effect of pollinator on yield.

Keeping in view the importance of honeybees, the current investigation was undertaken with an objective to record observation on the commencement, cessation and peak timings of visitation/foraging of *A. mellifera* and *A. florea* and to study their influence on seed yield and oil content of sunflower in Peshawar Valley of Khyber Pakhtunkhwa (KP) in Pakistan.

MATERIALS AND METHODS

Visitation pattern of Apis mellifera *and* Apis florea

The current experiments were carried out at the New Developmental Farm (NDF) of The University of Agriculture, Peshawar (34.01° N, 71.53° E). The trial was conducted during spring and fall 2012 and were repeated in 2013. The experiments were laid out in a completely randomized block design with four replications of each treatment. Plot size was 3 x 3 m with 5 rows that were 60 cm apart from each other and 3 m long with plant-to-plant distance of 30 cm, respectively. The sunflower hybrid Hysun-33 was planted in February 2012-2013 as a test crop at the rate of 2.5 kg per hectare manually on ridges by dibbling three seeds per hull up to a depth of 3 cm to maintain optimum population per plot. Following seedling emergence, plants were hand-thinned. Weekly irrigation and other recommended agronomic and cultural practices adapted uniformly to all the treatments. The experimental area was kept free from any insecticidal application during the blooming period of the crop.

The observations were recorded from commencement of 5% flowers until the end of flowering session of the crop. Observation on visitation pattern of *A. mellifera* and *A. florea* was recorded regardless of pollen collection and time spent on each inflorescence. There were four such randomly selected points in the field for recording observations on visitation of bee visitors. The experiment comprised of following six treatments: T1, 0800 h; T2, 1000 h; T3, 1200 h; T4, 1400 h; T5, 1600 h; T6, 1800 h.

Determination of effects of bee visitors on seed yield and oil contents of sunflower

In order to determine the effects of bee visitors on seed yield and its oil contents, the sets of experimental plots (T_1) were kept covered with a muslin cloth bag until the florets opened. The purpose was to prevent bees to visit the flowers (Free, 1993). In the other plots (T_2) sunflower heads were kept uncovered throughout the flowering period so that honeybees had an easy access to the flowers so that natural pollination may take place.

To determine total seed yield (kg ha^{-1}), sunflower heads from all plots were collected, dried and threshed. Seeds were weighted by using electric balance and each net plot yield was later converted into kg ha^{-1}.

For determination of percent oil contents, samples of 100 g of sun-dried and clean grains were taken from each plot and dried in an oven for six hours. Oven-dried samples (20 g) were taken in a glass tube and the oil contents were determined with the help of Newport 4000 NMR (Nuclear Magnetic Resonance) analyzer in Laboratory at Department of Nutrition, The University of Agriculture, Peshawar.

Assessment of differentiation in percent oil content and yield of sunflower

Total seed yield was recorded from the plots where the honeybees were denied access to the flowers and from the plots where they had visited the flowers. The monetary net gain by farmers was calculated. Percent increment in both oil content and yield of sunflower differentiated by the calculation followed by (Morse and Calderone, 2000).

$$\text{Percent Increase} = \frac{\text{Yield from opened heads} - \text{Yield from covered heads}}{\text{Average yield obtained from opened heads}} \times 100$$

Statistical analysis

Means of spring and fall for both years were calculated. Then the data were analyzed by using statistical software, Gen-Stat discovery edition 3rd and F-test was used in order to signify the difference between the variables.

RESULTS

Visitation of Apis mellifera *on sunflower Hysun-33*

Table I shows visitation pattern of *Apis mellifera* and

Apis florea on sunflower inflorescence at different time intervals and days. The population density recorded for both species of honeybee varied significantly. Average counts on bee foragers normally increased in the morning hours, which peaked at 1200 noon and subsequently declined toward the end of the day. After 5 days of initial flowers appearance on the crop, the most frequent visitants (19) of *A. mellifera* was observed at 1200 noon that happened to be the highest degree of foraging of bee visitors, whereas second peak frequency of bee visitants was noticed at 1400 h with average count of 15 individuals. On 10th flowering day, the peak (24) foraging of bee visitors was remarkable at 1200 noon and minimum mean incidence of only 11 number of *A. mellifera* estimated at 1800 h. Intensity of bees foraging recorded on 15th day after inflorescence of the crop revealed that the highest (29 and 23) density of foragers was calculated at 1200 h and 1400 h, respectively, whereas, minimum (14) foraging activities of visitors were observed at 1800 h of the day. Total number of visitations made by *A. mellifera* foragers typically increased between 20th and 25th flowering days of the crop. Maximum (36 and 30) strength of bee pollinators noticed at 1200 and 1400 h on 20th day, whereas, it was recorded maximum (33 and 27) at 1200 and 1400 h on 25th day. Similarly, least counts of bee foragers with 22 and 20 individuals recorded at 1800 h in the evening. Visitations by bee visitors gradually declined towards the end of flowering session of sunflower (Table I).

Overall duration of foraging movement of both species comprised of 10 h that was recorded from 0800 h during early morning and continued until 1800 h in the evening hours of the day. Significant variation was found in visitant's frequency, different hours of the day and time intervals after inflorescence. It is evident that throughout the spring season in 2012 and 2013 the peak abundance (29 bees/3 m^2) of *A. mellifera* was estimated at 1200 noon and was further followed by a mean numbers of 25 and 23 visitants at 1400 and 1000 h, respectively. At 0800 and 1600 h, the counts of individuals of *A. mellifera* had no significant differences and were recorded parallel with 19 insect visitors. The lowest (14) population of *A. mellifera* individuals was recorded at 1800 h. During autumn season, the highest (25) number of honeybees was recorded at 1200 noon, whereas, the lowest (11) mean density of bees visitants was recorded at 1800 h (Fig. 1).

Visitation of Apis florea *on sunflower Hysun-33*

After 5th and 10th flowering day during spring season, first and second peak of visitation observed at 1600 and 1400 h, which comprised of 11 and 09 and 13 and 11 bee visitors, respectively. Number of foragers estimated on 5th day at 0800 and 1800 h recorded with similar (06) numbers

Table I.- Visitation pattern of *Apis mellifera* and *Apis florea* on sunflower during spring 2012-13.

Time of observation (Hours)	Interval x Treatment						
	5DAF	10DAF	15DAF	20DAF	25DAF	30DAF	35DAF
Apis mellifera							
0800	10s-u	13p-r	17k-m	23e-g	22f-l	17k-n	11q-t
1000	13o-r	16l-n	21g-j	27cd	25de	20h-j	16m-o
1200	19j-l	24ef	29bc	36a	33a	28b-d	22f-h
1400	15m-p	19i-k	23e-g	30b	27de	23e-h	17k-n
1600	12q-t	15m-q	18j-l	22f-i	20h-j	17k-n	12q-t
1800	8u	11r-u	14n-p	17k-m	15m-o	13p-s	9tu
Apis florea							
0800	6qr	8n-q	10j-o	12f-i	11g-k	9l-p	6qr
1000	7p-r	91-p	11g-k	13e-h	12f-i	9l-p	7p-r
1200	8n-q	10j-o	12fi	14ef	13e-h	10j-o	7p-r
1400	9l-p	11g-k	13e-h	17c	17c	12f-i	8n-q
1600	11g-k	13e-h	16cd	20ab	20ab	14ef	10j-o
1800	6qr	7p-r	8n-q	10j-o	9l-p	7p-r	5i-m

Mean within the coloumns followed by different letters are significantly different from one another at ($P \leq 0.05$) level of propability. $LSD_{0.05}$ for SxT=0.71 (*Apis mellifera*) and $LSD_{0.05}$ for SxT=0.55 (*Apis florea*). DAF, days after flowering.

of honeybees that constituted the lowest level of bees foraging. Similarly, lowest visitors (07 and 08) found on 10th flowering day at 1800 and 0800 h, respectively. Mean number of visits made of individuals of *A. florea* from 15th to 25th flowering day represented highest level of foraging. Highest foraging rate of bee visitors estimated on 15th day with a total of 16 individuals at 1600 h followed by 13 bees at 1400 h. Minimum (08) number of bee foragers made at 1800 h of the day. Visitation frequency of *A. florea* individuals observed on 20th and 25th flowering day revealed that first peak (20) of foraging recorded at 1600 h followed by second peak of 17 visitants at 1400 h. least visitation rate was calculated at 1800 h with a total of 10 and 09 visitors during 20th and 25th day. Data recorded on 30th flowering day showed highest (14 and 12) density at 1600 and 1400 h, whereas, lowest foraging recorded at 1800 h with a total of 07 bee visitors. Maximum (10) number of visitants on 35th flowering day noticed at 1400 h followed by 08 foragers at 1400 h. Least flowers foraged by a mean number of only 05 bee visitors (Table I).

Visitation frequency of small honeybees, *A. florea* slightly increased from morning, reached to its highest level in the evening hours at 1600, and then again attained its lowest incidence at 1800 h of the day. During spring, 2012-2013 peak visitations with a total 17 individuals of *A. florea* recorded at 1600 h followed by an average incidence of 15 foragers at 1400 h. Bee visitants made minimum visits at 1800 h in the evening and observed only 09 individuals. In case of autumn-sown crop, maximum (13) number of bees found engaged in foraging of sunflower capitula at 1600 h. Second peak of visitants recorded at 1400 h with 10 honeybees. Subsequently, Bee foragers observed at 0800 and 1800 h were more or less similar that showed mean population of 07 and 06 visitors, respectively (Fig. 1).

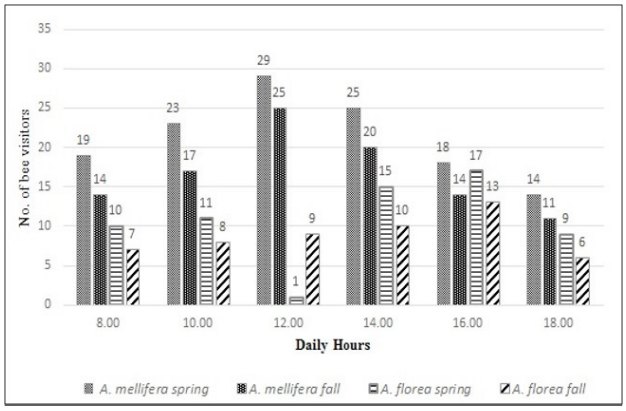

Fig. 1. Comparison of visitation pattern of *Apis mellifera* and *Apis florea* during spring and fall 2012-13.

Sunflower seed yield per hectare (kg)

Interaction of seasons and treatments found significant effect on seed yield. During spring season, significantly more seed yield ha^{-1} (2388 kg) recorded in open- pollinated plots than close-pollinated plots (1924 kg). In case of fall season, highest (2129 kg) seed yield ha^{-1} obtained from un-bagged plots, while 1794 kg yield ha^{-1} recorded on bagged plots. In case of both treatments, significant (2258 kg) yield per hectare recorded in open-pollinated plots, whereas 1859 kg yield obtained from close-pollinated plots (Fig. 2A).

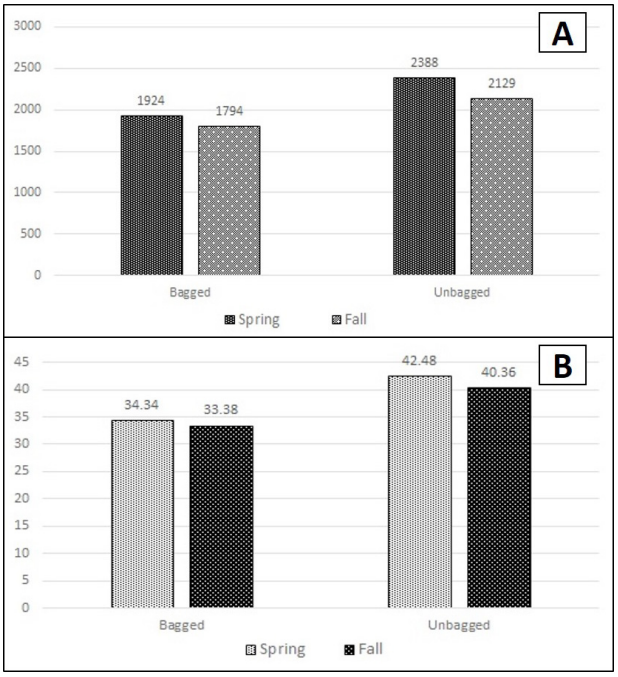

Fig. 2. Impact of honybees on **(A)** yield/ha and oil contents **(B)** of sunflower during 2012-13.

Percent oil contents

Interaction of season and treatments recorded significant effect on percent oil contents of sunflower. Highest (42.48%) oil contents recorded on un-bagged plots during spring season. On the other hand, significantly lower (34.34%) oil contents recovered from bagged plots in the same season. During fall season, again highest (40.36%) oil contents recorded on open pollinated plots as compare to close-pollinated plots (33.38%). When both treatments compared individually, highest (41.42%) oil contents observed on open-pollinated plots as compare to close-pollinated plots, which recorded 33.86% oil contents (Fig. 2B).

DISCUSSION

Visitation pattern of Apis mellifera *and* Apis florea *on sunflower Hysun-33*

It is known that the foraging activity of insect visitors in common and honeybee species in particular are commenced in morning period and completely cease during evening hours of the day. In some earlier studies, workers of honeybee initiated foraging at 6.17 am (Joshi and Joshi, 2010) but this commencement phase may be significantly varied region to region. Current investigation validates that visitation rate as well as foraging behavior of honeybees on sunflower greatly fluctuates during different hours of the day. Incidence of bee visitors normally increases with days after initial flowering on the crop. Generally, the visitation of bee visitors was found throughout the day with varying level of fluctuations but the most frequent visitation and foraging activities of *A. mellifera* individuals were noticed at 1200 noon and afterward at 1400 h. our findings are in line with Nderitu *et al*. (2008) who recorded peak foraging of honeybee at 1200 noon. Ali *et al*. (2015) also obtained similar results and reported that peak foraging of *A. mellifera* happened between 1200 to 1400 h. Furthermore, Pernal and Currie (2010) confirmed a greater foraging degree (36.02 foragers/min) during the afternoon than during the morning hours of the day (17.66 foragers/min). Yucel and Duman (2005) stated that honey bee visitants mostly forage onion blossoms from 0815 to 1630 h and the highest foraging was recorded between 1100 to 1200 h of the day, respectively. The reason could be high nectar secretion of the plant in the period, as some plants nectar secretion is greatly related to high temperature, low humidity and dry conditions. Naik *et al*. (2011) also presented similar results that glands secreting nectars are much active during mid-day hours, which accordingly attracted more bee visitors to forage on sunflower at 1200 h. Results obtained by Fell (1986) in Virginia further supported our findings that mean number of bee visitants generally increased from morning hours and reached to peak level from 1300 to 1400 hr of the day and after that little by little decreased towards end of the day. Lowest visitation of bee visitors noticed at 1800 h, which is in agreement with Adjare (1990) who reported that honeybee visitation greatly varied with time of the day, and no foraging activity recorded by 1830 h.

Yield per hectare (kg)

Significantly, high yield per hectare (kg) recorded on open-pollinated plots during spring season as compared to close-pollinated plots. Similarly, during fall season, again significantly high yield obtained in those plots, which kept under natural conditions, while minimum seed yield recorded on those plots where access of honeybee prevented by covering of sunflower heads with bags. Reduction in grain yield mainly accompanied by decrease in flower size, total number of seeds per flower head and weight of seeds. According to a study conducted by Furgala *et al*. (1979) and Jyoti and Brewer (1999) sunflower benefits from insect visitors that visit its capitulum for pollen and nectar collection. Cross pollination carried out by honeybees enhances the grain yield by 30% and oil contents by more than 6% in hybrid varieties. Our findings are also in compliance with the previous reports made by Chambo *et al*. (2011), who recorded similar results and stated that significantly higher seed yield obtained from those sunflower plants which were open to different pollinator agents as compare to those plants where insect visitors were excluded.

Oil contents

The finding that sunflower seeds which, remained exposed to honeybee workers had higher oil contents strongly agrees with other previous studies conducted by Krause and Wilson (1981), El-Sarrag *et al*. (1993), Kamler (1997) and Nderitu *et al*. (2008). The exclusion of honeybees showed an unpleasant impact on percent increase of oil contents in sunflower. Oil contents are extremely important finding of the current study to consider for imploring sunflower growers to conserve and protect pollinator species.

Percent increase/difference in seed yield (kg ha^{-1}) and oil contents due to honeybee

There was significant impact of bee visitors on percent increment of total yield (kg ha^{-1}). Plots kept opened throughout flowering session of the crop produced 17.67 % more net grain yield (kg ha^{-1}) whereas bagged plots gave significantly less amount of seed yield (kg ha^{-1}). Similarly, the outcome of the estimation as per method indicated that honeybee visitation/ foraging led to a total increment of 18.25% in oil in open-pollinated plots as compared to bagged sunflower plots.

When the percent increase/difference in sunflower seed yield (kg ha^{-1}) determined, it revealed that there was visible increase in the yield obtained from open-pollinated plots, whereas the yield achieved from controlled plots was obviously poor. Studies previously conducted in the developed countries by Moeller and Koval (1973) and Nye and Anderson (1974) revealed that pollination services provided by different honeybee species, increased seed setting by 10 to 25 percent and total seed yield by18 to 100 percent depending upon cultivar of the crop.

When the percent increase/difference in oil contents was determined, it was found that because of frequent

visitation made by bee visitors, percent oil contents of open-pollinated plots considerably enhanced as compared to control-pollinated plots, where less amount of oil contents was recorded. Rajasri *et al*. (2012) made similar observation and concluded that different insect visitors obtained higher oil contents due to good pollination services, whereas oil contents achieved through self-fertility was not encouraging.

CONCLUSION

A. mellifera and *A. florea* were the major pollinators of sunflower in the region during the current studies. Foraging is positively related and significantly influenced by daily hours during inflorescences period in sunflower. Pollinator also greatly affected the quality and quantity of grains which has significant effect production.

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Conflict of interest statement

We declare that we have no conflict of interest.

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