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Comparison of wild honeybees in the pollination of strawberries in Bahawalpur, Pakistan

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Comparación de abejas silvestres en la polinización de frutilla en Bahawalpur, Pakistán

RESUMEN. Las abejas silvestres, *Apis dorsata* Fabricius, 1793 y *Apis florea* Fabricius, 1787 constituyen una gran parte de la abundancia de polinizadores en la mayoría de las especies de cultivos en Punjab, Pakistán. En el presente estudio, se evaluó el comportamiento de forrajeo de ambas especies de abejas melíferas y se determinó su rol en el rendimiento reproductivo de la fresa en la granja de The Islamia University of Bahawalpur. Ambas especies aportan el 53% de la abundancia total de polinizadores. *Apis florea* presentó una alta frecuencia de visitas (0,449±0,03 individuos por 120 segundos) en comparación con *A. dorasta* (0,211±0,02). La actividad de *A. florea* fue la máxima a las 12:00 pm, mientras que *A. dorsata* se mantuvo activa en baja abundancia durante todo el día. *Apis florea* exhibió un mayor tiempo de estadía pero una tasa de visitas más baja que *A. dorsata*. *Apis dorsata* demostró ser un polinizador más eficaz de la fresa en lugar de *A. florea* en términos de longitud, peso y diámetro de la fruta. Existe la necesidad de conservar las abejas silvestres bajo el escenario actual de cambios antropogénicos en el uso de la tierra e intensificación en la agricultura.

PALABRAS CLAVE. Abejas melíferas. Abejas nativas. Efectividad de visita individual. Fresa. Polinización.

ABSTRACT. The wild honeybees, *Apis dorsata* Fabricius, 1793 and *Apis florea* Fabricius, 1787 constitute a large proportion of pollinators' abundance on most crop species in Punjab, Pakistan. In the present study, we studied the foraging behavior of both the honeybee species and their comparative role in the reproductive performance of the strawberry at the research farm of The Islamia University of Bahawalpur. Both the species comprised 53% of the total pollinators' abundance. *Apis florea* had high visitation frequency (0.449±0.03 individuals per 120 seconds) than *A. dorsata* (0.211±0.02). The activity of *A. florea* was the maximum at 12:00 pm while *A. dorsata* remained active in low abundance throughout the day. *Apis florea* exhibited a higher stay time but a lower visitation rate than *A. dorsata*. *Apis dorsata* proved to be an effective pollinator of the strawberry rather than *A. florea* in terms of fruit length, weight and diameter. There is a need to conserve wild honeybees under the current scenario of anthropogenic land use changes and intensification in agriculture.

KEYWORDS. Honey bees. Native bees. Pollination. Strawberry. Single visit effectiveness.

INTRODUCTION

Pollination is an essential ecosystem service responsible for reproducing flowering plants and producing one-third of the human diet (Klein et al., 2007; Partap et al., 2012). Bees are considered as potential pollinators because they can visit the plants during peak hours with the maximum abundance and the highest stay time (Willmer et al., 2017). Thanks to their fine body hairs, bee pollinators can transfer the pollen grains more efficiently than other

pollinators (Karunaratne & Edirisinghe, 2006). Thus, bee pollination improves the commercial value of most fruits, including strawberry, in terms of their post-harvest features (e.g. shelf life, weight, firmness, color, sugar contents etc.), size, shape and weight (Garibaldi et al. 2014; Klatt, 2013).

Bees worldwide are declining due to degradation of the natural habitats and the intensification of agriculture (Orford et al., 2015). Contrary to this general perception, Aizen et al. (2022) proposed in their recent findings that the agricultural intensification has led to the increased global food supply during last six years. However, they still warned that the global pollination capacity could be drastically reduced in coming decades, as the environmental costs of agricultural intensification outweigh its production benefits. The intensification in agriculture is inevitable due to ever increasing human populations. However, the recent concept of ecological intensification suggests that there are many farm management practices that can contribute to mitigating the drivers of pollinator decline e.g., using compost or manure, agro-forestry, reduced or no-till and crop rotation etc. (Kovács-Hostyánszki et al. 2017).

Both the wild and managed honeybees are essential pollinators in any agro-ecosystem and protecting them is important for the sustainable ecological services (Hristov et al., 2020). It is assumed that more *Apis mellifera* Linnaeus, 1758 as an introduced species is always better for crop yield world-wide; however, Rollin & Garibaldi (2019) suggested that beyond an optimum visitation rate they can even be detrimental for crop productivity. Therefore, crop specific recommendation of *A. mellifera* should be followed keeping in view the ecological structure of the surroundings. In the strawberry crop, to have the optimum fruit production, pollen transported by bees is necessary (Chagnon et al., 1989). Wild bees can be more effective pollinators of many agriculture crops and wild plants than managed honeybees in terms of fruit and seed sets (Garibaldi et al., 2014). Many fruit growers rely on solitary bees like *Osmia lignaria* Say, 1837 and *Megachile rotundata* (Fabricius, 1787) for pollination in different countries (Joshi et al., 2011, Park et al., 2018). Presence of solitary bees usually improves the pollination efficiency of honeybees (Greenleaf & Kremen, 2006).

The behavioral attributes of pollinators such as visitation rate, visitation frequency, pollen deposition and nectar theft, affect the key function in crop pollination (Ivey et al., 2003; Fenster et al., 2004). For instance, the number and length of floral visits can raise the amount of pollen deposition on stigma (Kudo, 2003). The visitation rate of bees is an important factor for pollination; more visits, the better the pollination will be (Proctor et al., 1996). However, exceeding the optimum visitation level can even be detrimental for crop productivity (Rollin & Garibaldi, 2019). On the other hand, the visitation rate of pollinators is affected by temporal variation in the production of the nectar and pollen (Thomson & Thomson, 1989). The single visit efficiency –in terms of pollen deposition and fruit set- is the most important tool to know the relative pollination effectiveness of different available pollinators (Inouye et al., 1994). Flowers of all existing strawberry cultivars are self-compatible and hermaphrodite (McGregor, 1976). Poor pollination is one of the main causes of deformity in strawberry. Carew et al. (2003) suggested that at least 70-80% of carpels must be pollinated to develop fruit normally. MacInnis & Forrest (2019) reported that strawberry fruits pollinated by native bees were denser in weight than those pollinated by managed honeybees.

The arid climate of Bahawalpur, Punjab, Pakistan does not favor *A. mellifera,* therefore, beekeeping is not a trend (Ali et al. 2020). Some recent studies have shown that native *Apis dorsata* Fabricius, 1793 and *Apis florea* Fabricius, 1787 constitute between 30 to 80 % of the total pollinators' abundance in various crops and play a vital role in pollination of many agricultural crops i.e., canola, radish, falsa, bitter gourd and pumpkin etc. (Ali et al. 2011; Saeed et al. 2012; Ali et al. 2014; Zameer et al. 2017; Akram et al. 2022). It seems to be an excellent choice to conserve wild native pollinators and basic studies must be conducted regarding the biology of these species including foraging and nesting behaviors (Sajjad et al., 2008). The present study aimed to analyze the role of the most abundant native honeybees (*A. dorsata* and *A. florea*) in strawberry pollination in terms of their behavior and single visit effectiveness. The comparison between different pollination methods such as open-pollination and self-pollination, was also performed.

MATERIAL AND METHODS

Study area and plant material. The present research was carried out at the research farm of The Islamia University of Bahawalpur (IUB), Punjab, Pakistan (29°22'16.3"N 71°45'52.9"E; 181 meters above sea level). Strawberry plants, *Fragaria* × *ananassa, Duch.* (Rosaceae) were sown in mid-October. The study lasted from mid-December to mid-March (from onset of flowering to harvest). District Bahawalpur is situated in southern Punjab which is featured by cold winters and hot summers. The area's climate is arid with average annual rainfall of 83-218 mm and average daily minimum and maximum temperatures of 18.8˚C and 33.5˚C, respectively (Ahmad et al., 2019).

Foraging behavior of pollinators. Visitation frequencies (number of bees visiting a specific plant in 120 seconds), visitation rate (number of flowers visited by an individual bee in 120 seconds) and stay time (an individual's time spent on a flower during its single visit) of both the honeybee species were recorded throughout the flowering period with three-day intervals. On each observation day, we recorded data from 8.00 am to 4:00 pm with two hours of breaks (five censuses in an

observation day). For recording visitation frequencies, during each census, ten plants were randomly selected and each plant was observed for 120 seconds and counted the number of floral visitors on wings. For recording visitation rate, during each census, ten individuals of each species were chased and recorded the number of flowers visited in 120 seconds. Similarly, for recording stay time, during each census, ten individuals of each species were observed for the time spent on a flower in a single visit. During 12 observation days, a total of 180 such censuses were performed i.e., 60 censuses each for visitation frequency, visitation rate and stay time. Three observers recorded that data with net observation time of 60 hours i.e., 20 hours each for behavior. For recording the foraging behaviors, a stopwatch was used (Akram et al., 2019) and visits were considered only when the bees made contact with the reproductive parts of the flowers.

Pollination effectiveness. We randomly caged 30 unopened flower buds on 30 plants with nylon mesh bags to measure the single-visit efficacy of wild honeybees. They were un-caged during their peak activity hours of pollinators and then re-caged after a single visit had been made. Fifteen such observations were recorded each for *A. dorsata* and *A. florea*. The percent fruit set was recorded and upon the maturity, the resultant fruit length, fruit weight and fruit diameter was measured as a proxy of their pollination effectiveness in a single visit. Strawberry fruit length and diameter in centimeters were recorded using a digital Vernier caliper, and fruit weight was measured in grams using electronic weight balance. For the comparison, fifteen un-opened flowers were caged with nylon mesh bags (self-pollination) and other 15 flower buds were tagged only and kept exposed to unrestricted insect visitation (open-pollination).

Statistical analysis. A rank abundance curve was drawn (using log series) to visualize pollinator community structure based on their relative abundance. The diurnal dynamic patterns of foraging behaviors were visualized through simple line graphs. Both the honeybee species' visitation frequency, visitation rate and stay time were compared using Generalized Linear Model (GLM). The dependent variable included visitation frequency, visitation rate and stay time. Plants were the random variables since they were selected randomly in each census and honeybees were the fixed factors. We used Poisson distributions with linear-log link functions. Kruskal-Wallis test was applied to see the significant difference among four pollination treatments (open-pollination, self-pollination and single visits of *A. florea* and *A. dorsata*) in fruit weight, fruit length and fruit diameter. The means of ranks were compared among treatments using Dunn's post hoc test at alpha 0.05. Statistical analysis was performed with SPSS Version 25.0 statistic software package.

RESULTS

Visitors' profile. The rank abundance curve showed that *A. dorsata* was a more abundant floral visitor (271 individuals) than *A. florea* (127). Both comprised 53.50 % of the total floral visitors (743 individuals) (Fig. 1). Other floral visitors included two more bee species (Hymenoptera) i.e. *Lasioglossum* sp. Curtis, 1833, *Sphecodes* sp. Latreille, 1804 and five true fly species (Diptera) i.e., *Eupeodes corollae* (Fabricius, 1794), *Episyrphus balteatus* (De Geer, 1776), *Ischiodon scutellaris* (Fabricius, 1805), *Syrphus ribesii* Linnaeus, 1758 and *Eristalinus aeneus* (Scopoli, 1763).

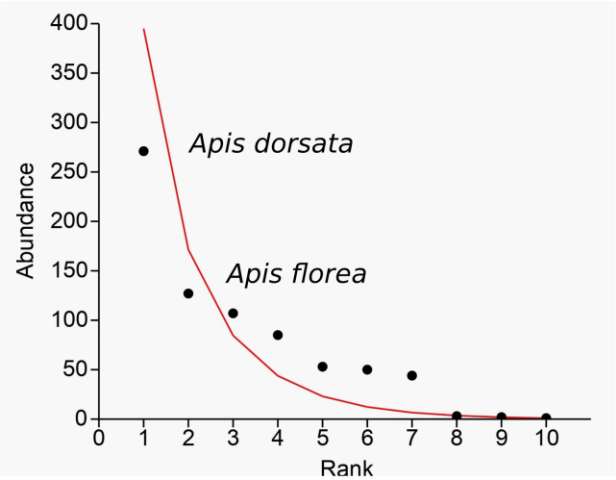

Fig. 1. Rank abundance curve of floral visitors of the strawberry crop at the research farm of IUB, Bahawalpur (Punjab), Pakistan. Names of most abundant visitors are mentioned.

Foraging behavior. The activity of both the bees started somewhere around 8:00 am. The visitation frequency of *A. florea* gradually increased until 12:00 pm followed by a sharp decline until 4:00 pm. *Apis dorsata*, on the other hand, remained active in a low abundance throughout the observation hours. The stay time of *A. dorsata* never exceeded that of *A. florea* throughout the observation hours (Fig. 2). The stay time of *A. florea* reached the maximum (32.40±4.81 seconds) at 12:00 pm, while for *A. dorsata* the maximum (14.89±1.83 seconds) was obtained at 2:00 pm. Similarly, the visitation rate of *A. florea* never exceeded that of *A. dorsata*. The visitation rate of *A. dorsata* reached a peak (12.73±2.20 flowers per 120 seconds) at 10:00 am, while *A. florea* did it at 4:00 pm (5.74±0.58 flowers per 120 seconds, Fig. 2). Results of the GLM showed that *A. florea* exhibited significantly higher stay time (26.44±2.46 seconds per flower; $P< 0.000$) and visitation frequency (0.45±0.029 flowers per 120 seconds; $P< 0.000$) but lower visitation rate (4.67±0.24 flowers per 120 seconds; $P< 0.000$) than *A. dorsata* (Fig. 3).

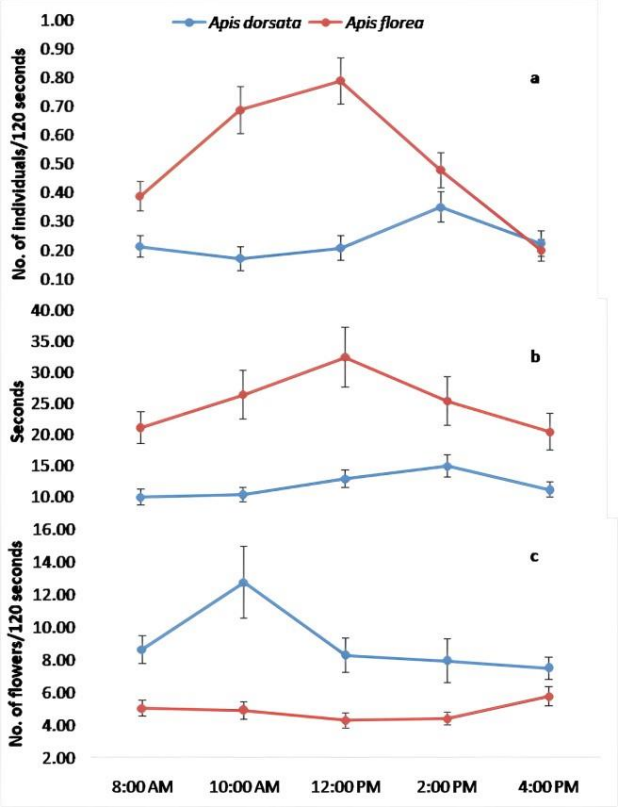

Fig. 2. Diurnal dynamic pattern of (a) visitation frequency, (b) stay time and (c) visitation rate of *A. dorsata* and *A. florea* at the research farm of IUB, Bahawalpur (Punjab), Pakistan.

Pollination effectiveness. The single visits of *A. dorsata* and *A. florea* resulted in 80% and 60% fruit sets, respectively as compared to 100% fruit set in open pollinated flowers. Kruskal-Wallis test showed a significant difference in fruit weigh (K obs.= 31.9, P< 0.0001), length (K obs.= 19.87, P< 0.0001) and diameter (K obs.= 25.52, P< 0.0001) among four pollination treatments i.e., single visit of *A. dorsata*, single visit of *A. florea*, open-pollination and self-pollination. Box-and-whisker plots of fruit weight, length and diameter showed that although the data was highly scattered and skewed, single visit of *A. dorsata* produced significantly heavier, longer and wider fruits (10.93±0.89 g, 2.60±0.11 cm and 2.36±0.14 cm, respectively) than those pollinated by *A. florea* (5.47±0.27 g, 2.49±0.09 cm and 1.74±0.06 cm, respectively). The minimum fruit weight, length and width were recorded in self-pollination treatment (2.13±0.87 g, 0.90±0.34 cm and 0.67±0.26 cm, respectively) while the maximum in open-pollination treatment (13.47±1.91 g, 2.79±0.38 cm and 2.56±0.35 cm, respectively) (Fig. 4). There was a significant positive relationship between fruit weight, width and diameter (Table I).

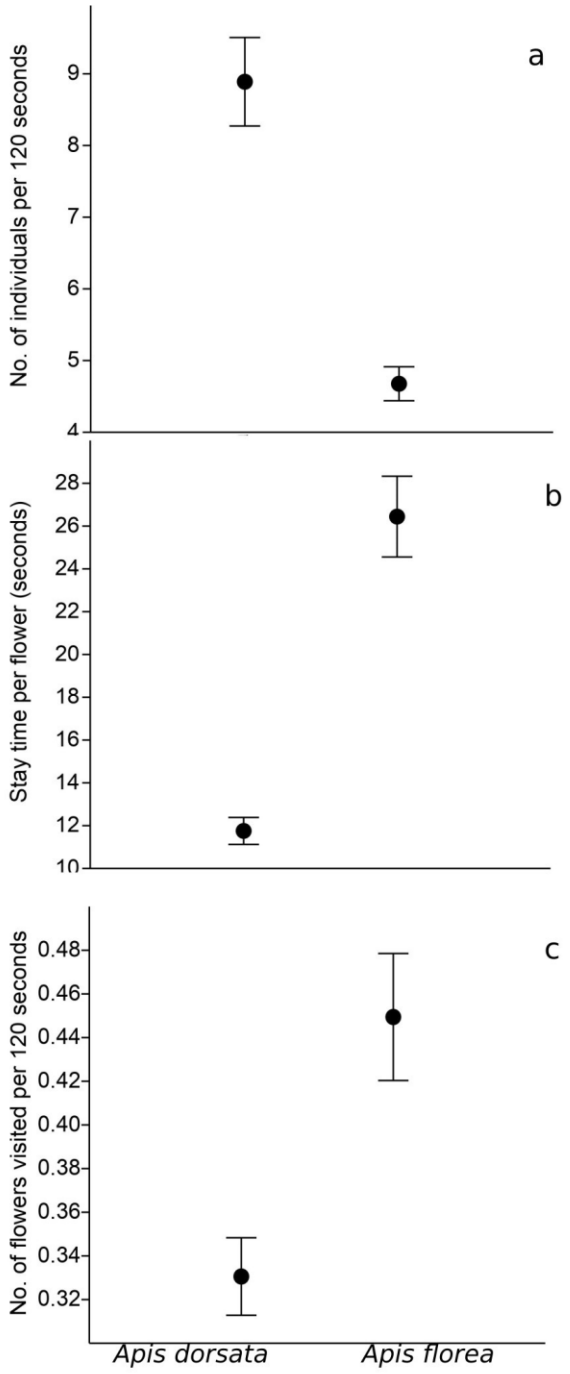

Fig. 3. Means of foraging behavior of *Apis dorsata* and *Apis florea* in terms of (a) visitation frequency, (b) stay time and (c) visitation rate. Error bars show standard errors.

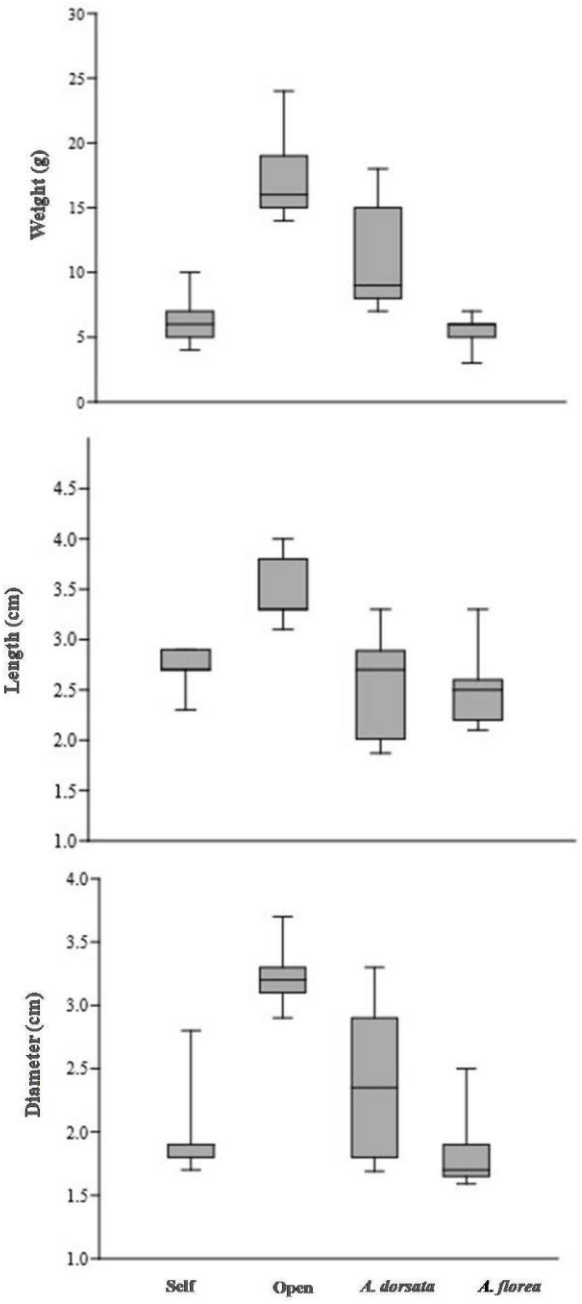

Fig. 4. Box-and-whisker plot of strawberry (a) fruit weight, (b) length and (c) diameter obtained in different pollination treatments.

Table I. Pearson's correlation coefficients among yield attributing components of strawberry fruits.

	Weight (g)	Length (cm)
Length	0.843*	
Diameter	0.898*	0.882*

*Correlation significant at the 0.05 level.

DISCUSION

In the present study, *A. florea* and *A. dorsata* comprised 53.50 % of the total floral visitors. Other floral visitors included two more bee species (Hymenoptera) and five true fly species (Diptera). The actinomorphic open flowers of strawberry offer pollen and nectar along with a landing platform of white petals. This generalized structure of the strawberry blossom welcomes a wide array of pollinator groups, e.g., bees, flies and beetles (Nye & Anderson, 1974; Woo et al., 1986; Abrol, 1989; Abrol et al., 2019; Widhiono, 2020). Sajjad et al. (2017) conducted a year-around survey of *A. dorsata* and *A. florea* in the study area. They found that both the species constituted 44.36 % of pollinating bee visits. They noticed eight hives of *A. dorsata* within 25 acres in the study area while they needed clarification about the number of *A. florea* hives, as they were smaller and mostly built combs within thick vegetation. The size and quality of semi-natural habitats significantly affect the bee abundance in adjacent agricultural landscapes (Maurer et al., 2022).

In Pakistan, both, *A. dorsata* and *A. florea* have been reported as efficient and the most abundant pollinators of canola, onion, fodders and bitter gourd (Sajjad et al., 2008; Sajjad et al., 2009; Saeed et al., 2012; Ali et al., 2011). Since honeybees contribute 70-80 % of insect pollination, the loss of honeybees therefore can significantly affect the overall pollination process (Carreck & Williams, 1998). Several investigators have reported that managed honeybees (*A. mellifera*) contribute for about 63 to 80 % of pollination in strawberry crop (Nye & Anderson, 1974; Pion et al., 1980; Abrol, 1989). However, strawberry blossoms may face competition from pollinators with other nearby crops or natural vegetation (Currie, 1997; McGregor, 1976). A sharp decline in natural honeybee colonies has been observed during the last two decades in subcontinent, probably due to increased pesticide usage and the destruction of natural habitats (Sihag, 2014). In a recent continent level analysis, Zattara & Aizen (2021) showed a clear signal of bee decline in Europe and North America, affirming the existing local, national, and sub-continental levels reports on pollinators decline.

The foraging activity of bees started around 8:00 am. The visitation frequency of *A. florea* gradually increased until 12:00 pm while *A. dorsata* gradually increased until 2:00 pm followed by a sharp decline until 4:00 pm. The already published literature from the study area suggests that the activity of *A. dorsata* and *A. florea* starts at 10:00 h, 6:00 h, 7:00 h during winter, spring and summer seasons, respectively, whereas their peak activity is attained at 12:00 to 14:00 h, 10:00 to 12:00 h and 8:00 to 09:00, respectively (Sajjad et al., 2008; Ali et al., 2011; Ali et al., 2016). Seasonal population fluctuations of *Apis* are influenced by biotic (number of flowering plant species and total floral abundance) and abiotic (relative temperature and humidity) factors (Farooqi et al. 2021).

We observed a significant difference in the stay time and visitation rate between *A. dorsata* and *A. florea*. The stay time of *A. florea* was higher than *A. dorsata* and vice versa in the visitation rate. Villalobos & Shelly (1996) found that *A. florea* had a lower visitation rate but more extended

stay time than *A. dorsata* at Chinese violets. Some previous studies confirm significantly higher visitation rate of *A. dorsata* as compared to *A. florea* in onion (Sajjad et al., 2008), canola (Ali et al., 2011) and pumpkin (Ali et al., 2014). Zameer et al. (2017) reported a lower visitation rate of *A. dorsata* than *A. florea* in radish. In contrast, Saeed et al. (2012) did not find any significant difference between visitation rates of two honeybees in bitter gourd. Bee species that are intensive foragers (having high foraging rates) usually work at a rapid pace but stay for a shorter period on each flower than species with less foraging rates (Sajjad et al., 2008; Ali et al., 2011; Ali et al., 2014; Ali et al., 2016; Zameer et al., 2017; Farooqi et al., 2021). Longer stay time on flower is not always beneficial as it leads to low visitation rate and poor pollen dispersal, while shorter stay time leads to high visitation rates and more out-crossing (Ali et al., 2011). However, not always a high visitation rate guarantees more pollen deposition. Many species with high visitation rates could be poor pollen depositors due to other foraging behaviors and morphology (Engel & Irwin, 2003).

Apis dorsata prefers large-sized sucrose-based flowers, while *A. florea* prefers flowers with glucose and fructose (Abrol, 2016) however, energy costs and rewards show that the larger *A. dorsata* forage flowers provide a higher energy reward than *A. florea* (Abrol, 2016). Pollen load and harvest are crucial for good pollination (Canto-Aguilar & ParraTabla, 2000). Taxa of different body types carry different pollen loads and behave differently on inflorescence heads (Chagnon et al., 1993; Hoehn et al., 2008). According to reports, wild bees of the family Apidae have the vitality to collect pollen and nectar (Widhiono & Sudiana, 2017). Wietzke et al., (2018) pointed out that the uniform distribution of pollen on strawberry stigmas is essential for fruit development, and the minimum threshold of pollen required for each stigma is also important. Therefore, under the lack of these conditions, deformities may occur.

In reference to pollination effectiveness, flowers pollinated in single visits of *A. dorsata* produced significantly heavier, longer and wider fruits than that of *A. florea*. Some previous studies have also reported that pollination affects the yield and is responsible for various physiological processes that increase fruit quality and the commercial value of many pollinator-dependent crops (Wietzke et al., 2018). For better and higher quality fruit set in the strawberry crop, the success of pollination should be 90% which has been confirmed in the previous studies (Özbek, 2008). Adhikari & Miyanaga (2016) reported higher quality large fruits from cross-pollinated strawberry plants than fruits from self-pollinated plants. Sharma et al. (2014) observed significant changes in fruit length, fruit width (2.40-2.80 cm) under bee pollination in enclosure and open pollination (1.96-2.43 cm) in strawberry cultivars. Tuohimetsä et al. (2014) observed that in cross-pollinated varieties Ria and Malling Opal, the length and width of the berries were larger.

We conclude that native honeybees play a significant role in pollination of strawberry owing to their foraging behavior and single the visit efficiency in terms of plant reproductive performance. In order to maintain sustainable strawberry cultivation, there is a need to conserve and enhance wild honeybees in southern Punjab, as weather does not support managed honeybees in this part of the country. Future studies should focus on determining other efficient native bee species and their conservation strategies for sustainable strawberry production in Pakistan.

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