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Observing Pollinator Attraction to Winter Annual Flowering Plants in an Urban Ecosystem

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MA and GA conceived and designed the experiments, and contributed reagents/materials/analysis tools. MTA performed the experiments. MA and FZAK analysed the data, reviewed and edited the manuscript. MMA and MA wrote the manuscript. All the authors read and approved the manuscript.

Key words
Biodiversity, Pollinators, Foraging needs, Conservation

ABSTRACT

Pollination is an important ecosystem service as 87% of angiosperm plants are dependent on insect pollinators, particularly bees, for their fruit and seed production. However, the pollinators are experiencing a global decline, mainly due to a shortage of food and nectar resources. Therefore, it is imperative to adapt various pollinator conservation practices, such as growing seasonally attractive flowering plants that can serve as foraging resources for these insect pollinators. Therefore, the current study was planned to screen the variation in the attraction of annual winter flowers for bees and other floral visitors. Different annual flowering plants were grown locally in the winter season. The plants were grown in polythene bags and shifted to the field just before flowering. The group of each specific plant was arranged in a circular planting pattern. The attraction of bees and other available insect floral visitors to these flowering plants was assessed through their arrangement in a randomized complete block design (RCBD). Syrphid flies were the most abundant pollinators of seasonal winter ornamental flowers, followed by bees (*Apis mellifera* L. and *Nomia* sp.). The flower of the genus *Petunia* attracted diverse pollinators, followed by pansy (Violaceae) and antirrhinum (Plantaginaceae). Insect pollinators were highly attracted towards *Dahlia* spp. (Asteraceae) observed by *Petunia* spp. (Solanaceae) and African daisy *Dimorphotheca ecklonis* (Asteraceae), while the least attractive flower was *Gazania* spp. (Asteraceae). Butterflies and moths were most abundant in Phlox (Polemoniaceae), followed by pansy (Violaceae). This study helped identify the most attractive winter annual flowers for bees and other floral visitors. Also, the floral patterns will be recommended for the future flowering scheme of this region as a step towards urban pollinator conservation.

INTRODUCTION

Biodiversity of the pollinators is decreasing globally due to various anthropogenic activities, i.e., increased habitat loss, increased pesticide use, and low availability of flora (Goulson *et al*., 2015; Ceballos *et al*., 2017; Rhodes, 2018). Habitat loss is one of the most critical factors causing the decline of pollinators (Winfree *et al*., 2009; Roulston and Goodell, 2011; Hinkel *et al*., 2014) and the key drivers are expanding urban settlements and intensive agricultural practices, including monoculture and high use of agricultural inputs like pesticides and fertilizers (Maxwell *et al*., 2016).

Pollination is vital for the production of many crops i.e., oilseed, fodder, fruits and vegetables. Bees are the essential pollinators of different crops including berries, melons, squashes, mango, apple, citrus (Liow *et al*., 2001; Fontaine *et al*., 2006) and other flowering plants (Rollings and Goulson, 2019; Marquard *et al*., 2020; Erickson *et al*., 2020). Similarly, syrphid flies are also important pollinators and biological control agents (Sajjad and Saeed, 2010). Additionally, dipteran pollinators are the major pollinators of the angiosperm (Endress, 2001). Much research has been done on pollination services provided by the bees (Jauker and Wolters, 2008). However, very few studies have been conducted to understand pollination services offered by syrphid flies compared to bees (Symmank *et al*., 2008). Syrphid flies are most attracted to ornamental flowering plants (Salisbury *et al*., 2015).

Ornamental flowering plants play a vital role in human life due to their economic and aesthetic values (Lawson, 1996). Ornamental flowering plants are the main component of gardening, landscaping, and cut flowers marketing. About 90% of the flowering plants provide foraging resources for various insect pollinators (Ollerton *et al*., 2011). These are the primary sources of pollen

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and nectar (Nicolson, 2011; Sampson and Cane, 1999; Konzmann and Lunau, 2014). These flowering plants attract different pollinators (Jachuła *et al*., 2019: Rollings and Goulson, 2019: Oyewole *et al*., 2019: Fukase and Simons, 2016: Salisbury *et al*., 2015). Pollen and nectar concentration attract different pollinators due to their taste, quantity, and quality (Gardener and Gillman, 2002; Galetto and Bernardello, 2004). Moreover, ornamental plants vary in their attraction to insect pollinators (including bees, syrphid flies, moths, and butterflies) (Garbuzov and Ratnieks, 2014) due to flowering abundance, color, shelter, and food (Colley and Luna, 2000; Miller *et al*., 2011; Wood *et al*., 2017; Jachuła *et al*., 2018; Mach and Potter, 2018).

The temporal stability of floral resources is important for the reproductive success of the pollinators (Bosch, 2008; Zurbuchen *et al*., 2010). Pollinators need fats, protein, carbohydrates, and minerals in pollen and nectar for optimum growth (Loper and Berdel, 1980; Neupane and Thapa, 2005). Flowering plants are the primary source of pollen and nectar (Konzmann and Lunau, 2014; Hicks *et al*., 2016). Pollinators face variable floral resources landscapes due to seasonal pollen and nectar availability changes, which might have ecological impacts on their fitness (Donkersley *et al*., 2014; Requier *et al*., 2020). For instance, if the availability of pollen and nectar is limited during critical periods of a pollinator's life cycle, such as during breeding or hibernation, it can negatively impact their fitness by reducing their survival rates and reproductive success (de Manincor *et al*., 2023). Additionally, a lack of floral resources can result in reduced population sizes of pollinators, which can further impact the ecological interactions and dynamics within the ecosystem (Theodorou *et al*., 2021). In seasons with low bloom density, pollinators prefer to fly greater distances to obtain pollen or nectar loads (Bosch, 2008; Peterson and Roitberg, 2006; Zurbuchen *et al*., 2010). Flowering plants benefit syrphid flies by providing food for adults, ultimately enhancing biological control in crops (Heimpel and Jervis, 2005).

Studies have been reported insect pollinators associated with flowering plants in Pakistan (Sajjad *et al*., 2017; Sajjad and Saeed, 2010). The year-long association of *Apis florea* F. and *A. dorsata* L. (Hymenoptera: Apidae) has been recorded in 49 plant species planted in forests and farmland. *Calotropis procera* (Aiton), *Helianthus annuus* L., *Moringa oleifera* Lamark, *Citrus reticulata* Blanco, and *Trifolium alexandrinum* Juslen were among the plant species which were highly visited by *A. dorsata*. On the contrary, *H. annuus, C. procera, Coriandrum sativum,* and *Mangifera indica* were highly visited plants by the *A. florea*. The abundance of the pollinators was positively correlated with the floral resources while it was negatively related to the humidity, and the maximum abundance of pollinators was observed from March to May (Sajjad *et al*., 2017). Different host plants for the syrphid flies were assessed under natural climate in another study, where syrphid flies were frequent visitors of wild carrot (*Daucus carota*; Apiaceae), juliflora (*Prosopis juliflora*; Legumes) coriander (*Coriandrum sativum;* Apiaceae), onion (*Allium cepa;* Amaryllidaceae) and dandelion creeping launaea (*Launaea procumbens;* Asteraceae) (Sajjad and Saeed, 2010). The temporal dynamics of the plants present in an area, pollen resources that these host plants provided, and their interaction with the pollinators gave us the base for the study of depletion of cross-pollination ecosystem services and the development of conservation strategies (Michener, 2002).

Previously, few studies have reported the diversity of insect pollinators on wild flowering plants globally (Garibaldi *et al*., 2013; Pardo and Borges, 2020). However, no research was conducted in the studied area regarding the attraction of annual winter flowers for bees and other pollinators. Therefore, the objective of the current study was: (1) to evaluate pollinator abundance on the ornamental flowers, and (2) to document the most attractive winter annual flowers for the pollinations in the southern Punjab Pakistan. This study will provide new information on pollinator abundance and attractive winter annual flowers for pollination in southern Punjab, Pakistan, thus contributing to the scientific literature on the ecology of pollinators in ornamental flower gardens and their preferences for specific plant species. Furthermore, the study may have broader implications for conservation efforts aimed at understanding the factors that affect pollinator abundance and diversity in urban and suburban environments.

MATERIALS AND METHODS

Experimental area

The research was conducted during the winter growing season 2021 at the experimental farm of MNS University of Agriculture Multan Pakistan. An area of 505.857m^2 was selected where different winter annual flowering plants were grown by using the RCBD layout. The experimental field was surrounded by buckwheat (*Fagopyrum esculentum*; Polygonaceae), wild radish (*Raphanus raphanistrum;* Brassicaceae, gum arabic tree (*Acacia nilotica*; Fabaceae), Indian jujube (*Ziziphus mauritiana*; Rhamnaceae) and mulberry (*Morus alba*; Moraceae). Pesticides and fertilizers were not used in the experimental plots. Surface irrigation was done at two days intervals, and it depended on the condition of the plants and environment. The average annual mean value of wind speed for the area is 0.514m/s. The mean temperature was 25-30°C, with a maximum of 35-40°C (during June-July) and the lowest temperatures of 10-20°C (during December-January). The warmest ever measured highest temperature was between 45 and 51°C in May and June (the warmer months), while the lowest ever measured lowest temperature is between 0 and -5°C in January (coldest). The area's average yearly rainfall is 175 mm (Hussain *et al*., 2020).

Flowering plants

Twelve annual winter plant genotypes were selected, which are primarily grown in this area. These included: *Tagetes erecta* L. (Asteracea), *Viola tricolor* L. (Violaceae), *Osteospermum fruticosum* L. (Asteraceae), *Dianthus chinensis* L. (Caryophyllaceae), *Gazania rigens* L. (Asteraceae), *Petunia* spp. (Solanaceae), *Matthiola incana* (L.) W.T.Aiton (Brassicaceae), *Antirrhinum majus* L. (Asteraceae), *Salvia splendens* Sellow ex JA Schultes (Lamiaceae), *Phlox drummondii* Hook (Polemoniaceae*)*, *Dahlia pinnata* Cav. (Asteraceae) and *Pericallis* spp. (Asteraceae).

Nursery preparation

The nursery was grown in polythene bags, with slit and sewage sludge 70% and 30%, respectively in last week of January 2021. Holes were made in the polythene bag for aeration. Single seedling flowering plants were then shifted into a single polythene bag. These bags were placed under the open field conditions for 40 days till the ten percent flowers started. Daily irrigation and weekly weed removal were performed.

Transplanting plants

Flowering plots were prepared at the start of March. There were twelve treatments with three replications for each treatment. Plots were made of 1.219×1.219m with a 3.048m distance from one replication to another replication and 1.524m from one plot to another plot in a treatment. Plants were placed randomly in the experimental field. Twenty plants from each variety were placed in the plot in a circular shape (Garbuzov and Ratnieks, 2014). This layout was designed to avoid any edge effects that may have influenced insect visits.

Recording abundance and diversity of floral visitors

Data were recorded in the morning hours during the active period of insect pollinators (09:00 am to 11:00 am). The number of flowers per plant was counted during each data observation. The abundance of insect visitors was assessed by observing and counting their visits on each floral patch for three minutes. Observations were taken across the entire flowering season from first week of March to 3rd week of April 2021. Data were recorded daily on clear sunny days. Sweep nets were used to trap insects, which were then identified for the study purpose. Pollinators were collected and sent to the experts for identification (see acknowledgements). Syrphid flies and bees were identified up to lower taxonomic levels using taxonomic keys (Michener, 2000).

Statistical analysis

The variation in abundance in different varieties of annual winter flowers was assessed by using analyses of variance (ANOVA). Mean will be compared by LSD test at $P < 0.05$. All analyses were done by using statistical software XLStat 8.1 (XLSTAT Product, Addinsoft).

RESULTS

Insects pollinator groups visiting ornamental flowering plants

There was significant difference among the four pollinator groups among the 12 varieties of flowering plants. Four groups of the main insect pollinators which include honey bees, solitary bees, syrphid flies, moth and butterflies were the most abundant. Our results showed that the pollinator abundance on marigold (*Tagets erecta*; Asteracea), dahlia *(Dahlia pinnata;* Asteraceae), petunia (*Petunia hybrid*; Solanaceae) and antirrhinum *(Antirrhinum majus;* Asteraceae) was higher as compared to other flowering plants with the least abundance on salvia (*Salvia splendens;* Lamiaceae) (Table I).

Attractiveness of insect pollinators on different ornamental flowers

There was significant difference among the pollinators among the 12 varieties of flowering plants (F= 26.6, df = 11, $P < 0.001$). We selected 12 local verities of the winter annual plants that are mostly grown in this area. Our results showed that abundance of the insect pollinators was significantly higher in *Petunia hybrid* followed by *Phlox drummondii* and *Vida tricolor* while it was lowest in *Dahlia pinnata.* Data regarding to the total abundance of honeybees on different ornamental flowering plants revealed higher abundance in *Dahlia pinnata*was followed by *Petunia hybrid* and *Osteospermum fruticosum* flowers. Abundance of syrphid flies was also varied significantly across the 12 varieties of flowering plants (F= 26.6, df= 11, P < 0.001). Syrphid flies mostly preffered *Petunia hybrid* followed by the *Phlox drummondii* and *Vida tricolor* while the *Dahlia pinnata* is the least attractive. Moths and butterflies abundance was higher on the phlox followed by the pansy and dianthus (Fig. 1).

Abundance and diversity of insect pollinators

In terms of the abundance of pollinators, it was observed that a variety of pollinators visited the selected ornamental flowering plants throughout the winter flowering season (Fig. 3). Different bees (honey bees and solitary bees), syrphid flies (*Eupeodes corollae* male, *Episyrphus balteatus* female, *Spharophoria bengensis* male, *Spharophoria bengalensis* female and *Eristalinus aeneus*), moths and butterflies visited the selected plants. These pollinators belong to insect order Hymenoptera, Diptera and Lepidoptera. Syrphid flies were the most abundant pollinator of the flowering plants followed by the solitary bees and butterflies (F= 22.3, df = 2, P < 0.001). The honey bees were the least abundant pollinators of the selected ornamental flowers (Fig. 2).

Table I. Insect pollinator groups (honey bees, solitary bees, syrphid flies, moth and butterflies) visiting ornamental flowering plants.

Flowering plant	Flowering period	No. of insect pollinator visitor groups
Family: Asteraceae		
Marigold	March-April	4
Dahlia	April-May	4
African daisy	March-April	2
Gazania	March-April	2
Cineraria	March-April	2
Family: Solanaceae		
Petunia	March-April	4
Family: Brassicaceae		
Stock	March-April	3
Family: Plantaginaceae		
Antirrhinum	March-April	4
Family: Lamiaceae		
Salvia	March-April	1
Family: Violaceae		
Pansy	March-April	3
Family: Polemoniaceae		
Phlox	March-April	2
Family: Caryophyllaceae		
Dianthus	March-April	3

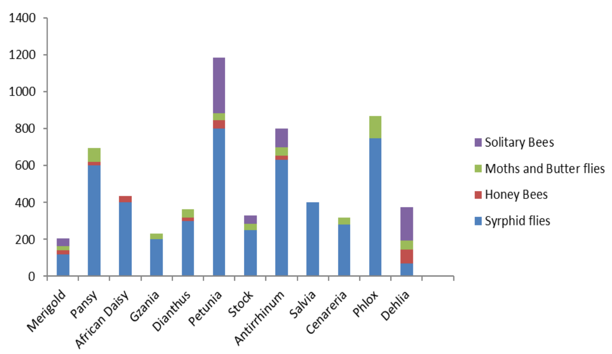

Fig. 1. Attractiveness of insect pollinators on different ornamental flowers.

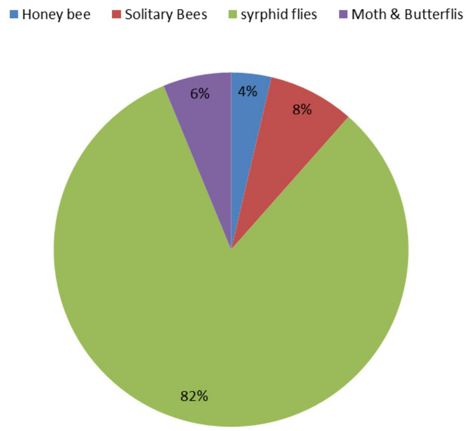

Fig. 2. Abundance and diversity of Insect pollinators visiting ornamental flowers.

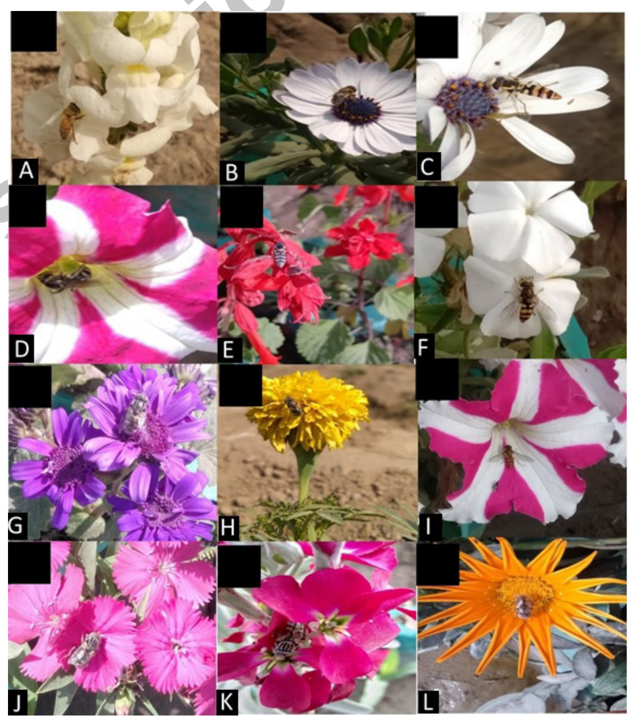

Fig. 3. Floral visitors. (A) *Apis mellifera* visiting *Antirrhinum majus,* (B) *Eristalinus aeneus* visiting *Osteospermum fruticosum*, (C) *Spharophoria bengalensis* male visiting *Osteospermum fruticosum,* (D) *Nomia* sp. visiting *Petunia hybrid,* (E) *Spharophoria bengalensis* female visitting *Salvia splendens,* (F) *Eupeodes corollae* male visiting *Phlox drummondii,* (G) *Eristalinus aeneus* visiting *Pericallis senetti,* (H) *Eristalinus aeneus* visiting *Tagets erecta,* (I) *Episyrphus balteatus* female visiting *Petunia hybrid,* (J) *Eristalinus aeneus* visiting *Dianthus chinensis,* (K) *Eristalinus aeneus* visiting *Matthiola incana,* (L) *Eristalinus aeneus* visiting *Gazania rigens.*

DISCUSSION

A wide diversity of pollinators, including different species of bees, syrphid flies, moths, and butterflies, were observed on different ornamental flowering plants. During the trial, almost 6220 pollinators visited the ornamental flowering plants. Most flowering plants were visited due to pollen and nectar, which serve as food sources for the pollinators (Marquardt *et al*., 2020). Based on previous findings, pollinators such as bumblebees, honeybees, flies, hoverflies, moths, and butterflies were attracted to the flowering plants. This was consistent with our findings (Garbuzov *et al*., 2015; Salisbury *et al*., 2015).

Based on total abundance of insect pollinators, petunia was the most preferred flower followed by phlox and antirrhinum. The highest attraction on petunia might be due to the morphological and physiological characters, especially the floral odor, which may drive the attraction of insect pollinators (Beltrame *et al*., 2022). Previously studies have also reported varying attraction of insect pollinators towards the flowering plants, i.e., purple loosestrife (*Lythrum salicaria*; Lythraceae) was the highly attractive flowering plant for the hoverflies and butterflies (Corbet *et al*., 2001). White laceflower (*Orlaya grandiflora;* Apiaceae) was most visited by syrphid flies (Basteri and Benvenuti, 2009). Indian chrysanthemum (*Chrysanthemum indicum;* Asteraceae) was received with more *Apis mellifera* (Gupta *et al*., 2020), Mexican sunflower *Tithonia rotundifolia*; Asteraceae) and dahlia (*Dahlia pinnata* were most attractive for the bumble bees (Wróblewska *et al*., 2016). In contrast, a study shows that petunias (Petunia) are prominent urban landscaping plants that have been proven to give little nourishment to bees (Tommasi *et al*., 2012).

The attractiveness of the flowering plants varied among different pollinators. Our findings revealed that Syrphid flies were the most abundant pollinators among all the others. Among syrphid flies, *Eristalinus aeneus* (S.), *Spharophoria bengalensis* female (C.), *S. bengensis* male (C.) and *Episyrphus balteatus* (G.) female visited the flowering plants, and among these species, *E. aeneus* was the most abundant pollinator followed by *S. bengensis* male and *E. balteatus* female. Similar results showed that in another study, *Hibiscus syriacus*, *O. grandiflora* and *L. salicaria* were highly visited by the syrphid flies (Basteri and Benvenuti 2009; Corbet *et al*., 2001).

Honey bees only attracted to the Dahlia flower similarly, another study showed that dahlia is most attract the honey bees (Garbuzov *et al*., 2015). The higher attraction of honey bees on the dahlia flowers might be due to the better nectar availability (Tew *et al*., 2021). In our study, about five plant species from the total species of selected flowering plants were not visited by any honey bees. Different studies also reported that garden plants do not attract honey bees (Garbuzov *et al*., 2017). While in contrast, some studies showed the honey bee is highly attracted to the flowering plants i.e., *Chrysanthemum indicum* and *Nepeta* x *faassenii* (Jachuła *et al*., 2018; Majewska *et al*., 2018; Daniels *et al*., 2020). Similarly, lepidopterans (moths and butterflies) were highly attractive pollinators (Wróblewska *et al*., 2016).

Ornamental flowering plants provide pollen and nectar to the pollinators (Nicolson, 2011; Sampson and Cane, 1999; Konzmann and Lunau, 2014) which is necessary for the development and the growth of pollinators. About 90 percent of the flowering plants provide food to pollinators (Ollerton*et al*., 2011). Since pollinators are declining globally due to habitat loss and other factors, therefore, by giving these floral resources, we can conserve the pollinators and increase the production of many important crops. The temporal stability of floral resources is important for the reproduction of the pollinators (Bosch, 2008; Zurbuchen *et al*., 2010). Therefore, we suggest that pollinators attracting flowering plants should be planted on a massive scale in the urban ecosystems. There is some sucking and chewing insect pests associated with ornamental flowers that degrade their aesthetic value, however, some insect predators have also been reported to attack these insect pests on the same plants (Lanjar *et al*., 2014).

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Statement of conflict of interest

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