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Diversity and Relative Abundance of Pollinator Fauna of Canola (*Brassica napus* L. Var Chakwal Sarsoon) with Managed *Apis mellifera* L. in Pothwar Region, Gujar Khan, Pakistan

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Authors' Contribution
TA and AA conceived and designed the study, and performed experimental work. IB and MN identified the bees. MS helped in data analysis.

Key words
***Brassica napus*, Insect pollinators, Relative abundance, *Apis mellifera*, Foraging activity.**

ABSTRACT

Insect pollinators are essential for reproduction and survival of several plant species. Agricultural productivity depends on population interactions of these pollinators. A field experiment was conducted at PMAS-Arid Agriculture University Research Farm, Koont, Gujar Khan during 2015 to compare the diversity and abundance of different insect pollinators on canola (*Brassica napus* L. Var. Chakwal Sarsoon) crops along with managed *Apis mellifera*. Thirty five insect species belonging to twenty families under five orders were recorded on canola. Among the hymenopterans, abundance of managed *A. mellifera* was maximum (87.76%) followed by *Apis florea* (1.11%) and *Apis dorsata* (0.98%). Peak activity of the insect visitors was observed at the mid of the day *i.e.,* 12:00 pm. The activity of managed *A. mellifera* started to increase from the third week of blooming (20th Jan, 2015) and reached to maximum in the sixth week (10thFeb, 2015). Among the abiotic environmental factors, temperature had a strong, significant and positive correlation with the foraging activity of *A. mellifera* on *B. napus* (r= 0.766; P= 0.0037 **), whereas relative humidity and rainfall had significantly negative correlation (r= -0.759; P= 0.0041 **, r= -0.715; P= 0.0089 **).

INTRODUCTION

Canola (*Brassica napus* L.) has been recognized as one of the most important oilseed crops and is the third most important source of the edible vegetable oil worldwide (Carvalho, 2011). Pakistan is the third largest importer of edible oil in the world. Although it is cultivated in all five provinces of Pakistan, Punjab has the largest share of 53% in the total area. During the last few years, it was reported that the area under canola cultivation in Punjab (153000 ha) has increased, but that productivity (148000 m tons) has not increased accordingly (MINFAL, 2014). This decline in yield can be attributed to pests and diseases damage, poor soil fertility, water stress or insufficient crop pollination (Free, 1993).

Pollination is the most important ecosystem service provided by insects resulting in sustainability of the majority of food plants. Approximately, 75% of the main crop species of the world rely on pollinators for fruit and seed set (Klein *et al*., 2007). Insufficient numbers of suitable pollinators causes severe decline in fruit and seed production (Partap, 2001). Production deficit due to the absence of pollination ranges between 3- 5% in the developed world and up to 8% in the developing world (Aizen *et al*., 2009). In Pakistan, this deficit in edible oilseed crops costs $55 billion, out of which 47% is only in the oilseed crops toria and sarsoon (Stephan and Irshad, 2012), which may be attributed to low and insufficient density of pollinator's population per unit area (Munawar *et al*., 2009).

Honeybees contribute nearly 80% of the total insect pollination community and therefore, are considered as the best pollinators (Robinson and Morse, 1989). Among honeybees, *Apis mellifera* are the main pollinators of *B. napus*, accounting for 46 to 95% of all insect pollinators of this crop (Pierre *et al*., 2003). *B. napus* is not only a good oilseed and fodder crop but also a major source of nectar and pollen that supports population buildup of pollinators (Klein *et al*., 2007). Previous studies have shown that insect pollinators especially managed *A. mellifera* populations, can increase canola seed productivity (Delaplane and Mayer, 2000; Westcott and Nelson, 2001). Other rape pollinators, such as solitary bees can account for about 4% or sometimes 9% of all insect pollinators (Koltowaski, 2001).

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Although a large number of beekeepers migrate towards the Gujar Khan and Chakwal area of Punjab province during Brassica season, but farmers normally show reluctance to cooperate with the beekeepers. The reason is that farmers' perception that bees deprive flowers of valuable products (nectar and pollen) which is based on local myth that bees harm the crop. Keeping in view the above situation, there was a need to conduct a systematic study in Pothwar region (Gujar Khan) of Punjab, Pakistan to explore the diversity of local pollinators attracted by canola and compare their abundance with managed *A. mellifera* and to provide scientific information regarding the contribution of *A. mellifera* along with other insects towards crop pollination. The findings of our research will help to orientate the farmer's attitude to cooperate with beekeepers; which is beneficial for both communities.

MATERIALS AND METHODS

Study site

The investigations were carried out at University Research Farm, Koont, Gujar Khan located at latitude 233º06'N and 73º00'E at an elevation 518.76 meter under arid conditions. Canola crop was sown with 45cm R×R and 15cm P×P on 15 October, 2014 over an area of 1 acre. From this area, four plots at the corners of field measuring 9×2m² were selected for recording data of pollinators diversity and abundance. Five strong colonies of *Apis mellifera* L. were kept near the experimental field and data were started from 6th January, 2015 when about 10% of the flowers were blooming. All recommended agronomic practices were applied to the crop. Data regarding temperature, relative humidity and rainfall of twelve weeks were also taken from Meteorological Station, Department of Environmental Sciences at University Research Farm, Koont to correlate them with foraging activity of *A. mellifera*.

Pollinator's diversity

Plants from four plots were observed three times a day (10:00, 12:00 and 2:00 pm) on weekly basis, during the whole flowering period for collection and identification of insect pollinators (Roy *et al*., 2014). Observation time was ten minutes for each period in each plot. The collected insects were killed in a poison bottle (potassium cyanide) and transferred into the laboratory, where they were pinned, labeled and preserved in the collection boxes. All the pollinators were identified to genus level and some of them were possible to be identified up to species level with the use of published systematic keys and direct comparison with museum specimens housed at Biosystematics Laboratory, Department of PMAS-Arid Agriculture University, Rawalpindi.

Pollinator's abundance

Pollinators abundance was calculated by randomly observing 15 plants for 60 seconds/plant from each plot and counting the number of visiting individuals of the different pollinator species with the help of a stop watch. Observations were made at two hourly intervals from 10:00 am to 2:00 pm on a weekly basis throughout the flowering season. We intentionally delayed the observation period until 10:00 am because of heavy fog incidence in January in this area.

Data analysis

The data of pollinators abundance at different timings were subjected to statistical analysis using analysis of variance (ANOVA), followed by means comparison with Least Significant Difference (LSD) at P = 0.05. Coefficient correlation was used to determine the relationship between pollinator abundance (*A. mellifera* L.) and abiotic factors (temperature, relative humidity and rainfall). Statistical analysis was performed using XLSTAT (available at: http://xlstat.com/en/download).

RESULTS

Pollinator's diversity

The canola crop was found to be visited by 35 insect species belonging to five orders. Twenty-seven out of thirty five species were frequent visitors of canola flowers. This pollinator community comprised six bees, two wasps, twelve flies, seven butterflies, two moths, three beetles and three bugs. Bees were among the most abundant floral visitors with total abundance of 4555 individuals (89.79%), followed by 260 Diptera (5.12%) and 148 butterflies (3.24%). Moths and wasps were the rarest floral visitors with 18 and 2 individuals, respectively (Table I).

Among the eight Hymenoptera visitors, four families were recorded; five species from Apidae, (*A. mellifera, A. florea, A. dorsata, Amegilla cingulata* and *Xylocopa* sp.), one species each from Halictidae (*Halictus* sp.), Ichneumonidae (*Ichneumon* sp.) and Sphecidae (*Sphex* sp.) were found on *B. napus* during flowering season. From Diptera, six species from Syrphidae (*Eristalis tenax, Eupeodes corollae, Melanostoma* sp., *Ischniodon scutellaris, Episyrphus balteatus, Eristalis smilis*), two from Calliphoridae (*Stomorpgina discolor*, *Chrysomya megacephala*) one species each from Sarcophagidae (*Sarcophaga* sp.), Muscidae (*Musca domestica*), Tabanidae (*Tabanus suleifrons*) and Tachnidae (*Prosena siberita*) were recorded. From Lepidoptera six species (*Pieris brassicae, Anaphaeis aurota, Eurema nicippe, Eurema smilax, Catopsilia pomona* and *Pieris canidia*) belonging to family Pieridae and one species each from Nymphalidae (*Vanessa cardui*), Erebidae (*Callimorpha*

sp.) and Sphingidae (*Macroglossum nycteris*) were found to visit *B. napus* flowers. The remaining species belonging to the orders Coleoptera and Hemiptera were found as casual visitors of the flowers and are not reported to participate in nectar or pollen collection (Bhowmik *et al*., 2014; Roy *et al*., 2014) (Table I).

Table I.- Insect species in canola flowers, with their total abundance and foraging behavior.

Order	Family	Name of the species	Total abundance	Percentage	Foraging behavior		
					Pollen foragers (PF)	Nectar foragers (NF)	Casual visitors (CV)
Hymenoptera	Apidae	*Apis mellifera*	4447	87.66	PF	NF	
		Apis dorsata	43	0.85	PF	NF	
		Apis florea	56	1.10	PF	NF	
		Amegilla cingulata	2	0.04		NF	
		Xylocopa sp.	3	0.06	PF	NF	
	Halictidae	*Halictus* sp.	4	0.08	PF	NF	
	Ichneumonidae	*Ichneumon* sp.	1	0.02			CV
	Sphecidae	*Sphex* sp.	1	0.02			CV
Diptera	Syrphidae	*Eristalis tenax*	24	0.47		NF	
		Eupeodes corollae	32	0.63	PF	NF	
		Melanostoma sp.	31	0.61	PF	NF	
		Ischniodon scutellaris	39	0.77	PF	NF	
		Episyrphus balteatus	25	0.49		NF	
		Eristalis smilis	32	0.63		NF	
	Sarcophagidae	*Sarcophaga* sp.	19	0.37		NF	
	Muscidae	*Musca domestica*	25	0.49	PF	NF	
	Calliphoridae	*Stomorhina discolor*	9	0.17		NF	
		Chrysomya megacephala	8	0.16		NF	
	Tabanidae	*Tabanus suleifrons*	4	0.08		NF	
		Prosena siberita	12	0.24		NF	
Lepidoptera	Pieridae	*Pieris brassicae*	44	0.87		NF	
		Anaphaeis aurota	23	0.45		NF	
		Eurema nicippe	8	0.16		NF	
		Eurema smilax	22	0.43		NF	
		Catopsilia pomona	13	0.26		NF	
		Pieris canidia	16	0.31		NF	
	Nymphalidae	*Vanessa cardui*	22	0.43		NF	
	Erebidae	*Callimorpha* sp.	10	0.02		NF	
	Sphingidae	*Macroglossum nycteris*	8	0.16		NF	
Coleoptera	Tenebrionidae	*Entomoscelis americana*	36	0.71			CV
	Meloidae	*Lytta* sp.	42	0.83			CV
	Chrysomelidae	*Aulacophora foveicollis*	9	0.17			CV
Hemiptera	Lygaeidae	*Oncopeltus fasciatus*	1	0.02			CV
	Cydnidae	*Sehirus luctuosus*	1	0.02			CV
	Pentatominae	*Bagrada hilaris*	1	0.02			CV

Table II.- Activity of certain insect pollinators on canola plants at the day time during 2015 season under the environmental conditions of Pothwar region (Gujar Khan).

Day time	Average number of pollinators/15 plants and (%)						
	Apis mellifera	***Apis florea***	***Apis dorsata***	**Beetles**	**Diptrous flies**	**Lepidoptrans**	**Grand total**
10 A.M.	32.083 (86.63)	0.42 (1.13)	0.33 (0.89)	0.92 (2.48)	1.78 (4.81)	1.5 (4.06)	37.033
12 P.M.	52.58 (88.79)	0.75 (1.26)	0.56 (0.95)	0.69 (1.16)	2.97(5.02)	1.67 (2.82)	59.22
2 P.M.	38.86 (87.38)	0.39 (0.88)	0.5 (1.12)	0.81 (1.82)	2.47 (5.55)	1.44 (3.25)	44.47
Mean	41.17	0.52	0.46	0.81	2.41	1.54	46.91
±SE	±6.03 A	±0.12 B	±0.068 B	±0.07 B	±0.34 B	±0.07 B	
%	87.76	1.11	0.98	1.73	5.14	3.28	100

Pollinator abundance

Data in Table II shows the abundance of insect pollinators during different day time, *A. mellifera* was noticed as pre dominant pollinator with 87.76% abundance among all insect pollinators; whereas *A. florea* and *A. dorsata* comprised of 1.11% and 0.98% abundance, respectively. All the three honeybee species, Dipteran flies and Lepidopteran insects foraged throughout the day. The maximum activity of *A. mellifera* (52.58%), *A. florea* (0.75%), *A. dorsata* (0.56%), Diptera (2.97%) and Lepidoptera (1.67%) was noticed at 12:00 pm. The least activity of *A. mellifera* was observed at 10:00 am.

Table III.- Correlation between honey bees visitation on *Brassica napus* L. inflorescences with weather variables (n=12).

Weather variables	r	P value
Air temperature (°C)	0.766	0.0037**
Relative humidity (%)	-0.759	0.0041**
Rainfall (mm)	-0.715	0.0089**

Relationship of foraging activity of Apis mellifera *with environmental factors*

Total daily visits of the *A. mellifera* were dependent on weather conditions. The correlation of foraging activity of *A. mellifera* comprising of twelve weeks was calculated against weather conditions. The results revealed that temperature, relative humidity and rainfall played a significant role in the foraging activity of *A. mellifera* on *B. napus* flowers. Temperature had significantly strong and positive correlation with the visitation rate of *A. mellifera* (r= 0.766). Rainfall had significantly negative correlation (r= -0.715) with inflorescence visits of *A. mellifera* on *B. napus* (Table III). Relative humidity curve showed steep peaks in the weeks of rain fall with no foraging activity (Fig. 1). Co-efficient of correlation between relative humidity and *A. mellifera* foragers revealed significantly negative correlation (r=-0.759) (Table III).

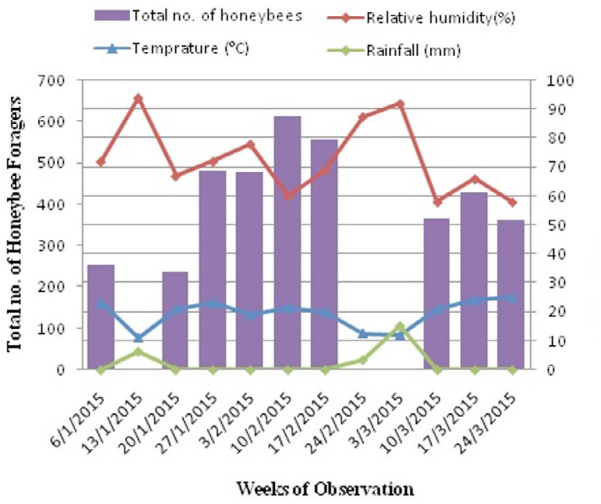

Fig. 1. Influence of air temperature, relative humidity and rainfall on honey bees visit of *Brassica napus* L. inflorescences during flowering time.

DISCUSSION

The general structure of the canola flower welcomes a large range of foragers, *e.g.,* bees, wasps, flies, butterflies, and beetles (Pierre, 2001), which help in cross pollination, early seed set and higher yield (Free, 1993). In the present study, Thirty five insect species belonging to five orders: Hymenoptera, Diptera, Lepidoptera, Coleoptera and Hemiptera were recorded on *B. napus* (Table I). Pollinator's abundance and composition varies with geographical area, latitude and time (Ollerton and Louise, 2002). Roy *et al.* (2014) documented 24 insect species belonging to 13 families under six orders (Hymenoptera, Lepidoptera, Coleoptera, Diptera, Odonata and Hemiptera) on *B. juncea* crop. Kunjwal (2014) observed 30 species visiting *B.*

juncea flowers under three orders, 23 from Hymenoptera, 5 from Diptera and one from Lepidoptera. In the same year, Goswami and Khan (2014) reported 19 insect visitors belonging to two orders, 15 from Hymenoptera and 4 from Diptera during mustard blossom period. Atmowidi *et al*. (2007) also found 19 species of insect visitors on mustard crop. From Kingdom of Saudia Arabia, Ahmed (2005) reported 22 Hymenopterans and 16 Dipterans species as visitors of mustard flowers in Diriyah region and 7 Hymenopterans and 5 Dipterans species in Derab.

In the present investigation, eight species belonged to Hymenoptera (*A. mellifera, A. dorsata, A. florea, Amegilla cingulata, Xylocopa* sp*., Halictus* sp*., Ichneumon* sp. and *Sphex* sp.). Among Hymenopteran species, five species (*A. mellifera, A. dorsata, A. florea, Xylocopa* sp*., Halictus* sp.) were found as both pollen and nectar foragers and one (*Amegilla cingulata*) as only nectar forager, while two species (*Ichneumon* sp and *Sphex* sp.) as casual visitors. Shakeel *et al*. (2015) recorded five species of Hymenopterans on *B. napus*, among which *A. mellifera* was the major pollinator. Mahindru *et al*. (1995) found that *A. mellifera*, *A. florea*, *A. dorsata* and *Andrena* sp. are the dominant visitors of brown sarsoon at Ludhiana, Punjab India. Chakravarty (2000) reported different pollinators' diversity on *B. napus i.e., A. mellifera*, *A. ceranaindica*, *Eristalis*, *Syrphus* sp., *A. dorsata*, *Bumbus* sp., *Mellipona* sp., *Haliothis armigera*, *Pieris brassicacae* and *Plusia orichalcea* at Pantnagar, Uttarakhand. From our studies, it is evident that Gujar Khan area of Punjab is rich in insect pollinator fauna and thus has better potential for pollination of *B. napus* to enhance crop yield with the help of these pollinators. In present results, all recorded families of order Diptera are reported as nectar feeders except Syrphidae; in Syrphidae out of six recorded species, three (*Eupeodes corolla, Melanostoma* sp*., Ischniodons cutellaris*) are reported as both pollen and nectar foragers (Ali *et al*., 2011). Lepidopterans are reported as nectar feeders only; they visit flowers to satisfy their own needs and accidently transfer pollens, so may help in the process of pollination of canola (Jauker and Wolters, 2008; Jauker *et al*., 2012). Therefore nine species of Lepidoptera recorded in present study may be regarded as secondary pollinators of this crop.

Among all families of Hymenoptera, Apidea was the most abundant (89.71%) (Table I). Goswami and Khan (2014) also recorded the maximum abundance of *Apis* bees (57.55%) followed by the non *Apis* bees (21.06%) on *B. juncea* in experiments without taking support from managed *A. mellifera* pollination. In Hymenopterans, honeybee workers are the predominant group of pollinating insects of rapeseed and mustard, their total numbers on flowers can account up to 95% (Koltowski, 2007). In another study, Bhowmik *et al*. (2014) observed maximum abundance of *A. mellifera* (18%) followed by *A. dorsata* (16%) and *A. ceranaindica* (14%) on *B. juncea* along with no record of *A. florea* in their experiments. In contrary, *A. florae* ranked 2nd (1.11%) followed by *A. dorsata* (0.98%) in our study, although *A. mellifera* was the most abundant visitor (87.76%) of *B. napus* (Table II). The inconsistency regarding abundance of *A. mellifera* (87.66%) may be due to the fact that we used managed pollination as compared to the non- managed pollination of *A. mellifera* used by Bhowmik *et al*. (2014). Abundance of *A. florae* more than *A. dorsata* reflects that our research area has also better potential to support a large population of *A. florea*.

Ali *et al*. (2011) and Roy *et al*. (2014) reported *A. dorsata* and *A. cerana* more abundant as compared to *A. mellifera* and *A. florea*; this may be due to the difference in distribution of *A. dorsata* and *A. cerana* and experimental conditions in that area *A. cerana* is commonly found in hilly areas of Pakistan, while the area under study is in rainfed region. *A. mellifera* was the most efficient pollinator in our study, because of higher foraging rate, highest abundance and adaptation to adherence of loose pollen grains. Present results are similar to Kumar and Singh (2005), who declared *A. mellifera* as the most dominant species followed by other insect visitors on canola crop.

The visitation of *A. mellifera* on *B. napus* crop may be affected by the time of day. In present study, the highest numbers of *A. mellifera* were recorded at 12:00 to 2:00 pm, while the least number of *A. mellifera* was found at 10:00 am (Table II). Similarly, Ali *et al*. (2011) and Goswami and Khan (2014) also found that foraging activity of *A. mellifera* was higher at 12.00 pm. Semida and Elbanna (2006) documented that the abundance of pollinators differed across the time of the day and increased gradually up to maximum around the midday (10:00-12:00 pm). Some researchers reported slight differences regarding peak activity of *A. mellifera* like between 9:00 and 1:00 pm in Brazil (Nascimento and Nascimento, 2012), at 2:00 pm (Kunjwal *et al*., 2014) and at about 3:00 pm (Williams, 1985). These differences may be due to variable geographical conditions and weather patterns in those areas.

In our study, the activity of *A. mellifera* started to increase from 3rd week of blooming (20th Jan, 2015) and reached to maximum at 6th week (10thFeb, 2015) (Fig. 1). This may be due to peak flowering season and favorable weather conditions. During this period, available floral food resources are very limited because of winter season, so the pollinators are attracted more towards this crop. The attractiveness of *B. napus* flowers to *A. mellifera* is highly related with the availability of food resources in the form of pollen or nectar (Free, 1993; Delaplane and Mayer,

2000; Mussury*et al*., 2003).

Among weather variables, temperature was only positively related factor with the visitation rate of *A. mellifera* during twelve weeks (r= 0.766; P= .0037 **) whereas relative humidity and rainfall had significantly negative correlation (r=-0.759; P= .0041 **; r= -0.715; P= .0089 **) with inflorescence visits of bees on *B. napus* (Table III). Our results are in accordance with Kasper *et al*. (2008) who stated that temperature positively influenced the insect pollinators' activity on foraged flowers. Omoloye and Akinsola (2006) also declared that bee activity was found to be significantly positive correlated with the temperature and significantly negative with the relative humidity in all the three honeybee species on different cultivars of oilseed crops. Knowledge of pollinator relationship with abiotic variables is very helpful for making future conservation strategies regarding efficient pollinators like *A. mellifera* (Lenzi *et al*., 2005).

In conclusion, our study provides insight of 35 local insect pollinators belonged to Hymenoptera out of which managed *A. mellifera* was the most efficient pollinator. Foraging activities of *A. mellifera* were directly related to blooming progression (20th Jan to 10th Feb, 2015) with the most favorable temperature 20±1°C at 12:00 pm. Pollinators of this crop may be protected and well utilized by intelligent pest management tactics to get higher seed yield through better crop pollination.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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