Pakistan J. Zool., vol. 46(2), pp. 531-539, 2014.

Exploring the Best Native Pollinators for Pumpkin (*Cucurbita pepo*) Production in Punjab, Pakistan

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Abstract.- Pollination in pumpkin (*Cucrubita pepo*) largely depends on activity of native insect pollinators, especially the bees. In order to explore the pollinator diversity and the best native pollinators for pumpkin production, an experiment was performed at the research farm of the Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan. The visitation frequency of the bees across staminate and pistillate flowers, pattern of diurnal and seasonal visitation, efficiency in harvesting and depositing pollen and resultant fruit set per single visit were evaluated. The insect pollinator community was composed of 18 species in 3 orders and 6 families. *Nomia* sp., *Apis dorsata* and *Halictus* sp. were among the most abundant (189, 399, 117 respectively) and efficient pollinators in terms of number of pollen grains harvested (3121.50±199.54, 2090.40±139.03, 798.45±109.39, respectively) and deposited (376.60±23.01, 204.15±20.63, 177.85±16.31, respectively). The single visit fruit set percentage also revealed *Nomia* sp. (36.66) as the best pollinator followed by the *A. dorsata* (23.33) and *Halictus* sp. (20.0). Conserving and enhancing these bee species may boost pumpkin production in Pakistan.

Key words: Pumpkin, staminate and pistillate flowers, effective pollinators, single visit efficacy.

INTRODUCTION

Pollination is one of the essential ecosystem service provided by insects resulting in the sustainability of both natural and agro-ecosystems (Buchmann and Nabhan, 1996). The importance of pollinators to agro-ecosystems is high as 75% of the important crop species of the world depend on them for fruit or seed set (Klein *et al*., 2007). Pollinators contribute 35% to global food volume and provide essential nutrients for human subsistence (Gallai *et al*., 2009). Different crops vary in their pollination requirements and thus their dependence on insect pollinators (Morse and Calderone, 2000).

Pumpkin (*C. pepo*) attracts a wide array of insect visitors due to its large, monoecious and showy flowers that open before sunrise and wilt or close by early afternoon (Hurd, 1964; McGregor, 1976). The staminate flowers are more numerous than pistillate flowers, and produce both nectar and pollen, whereas pistillate flowers produce nectar only (Tepedino, 1981). The pollen grains in *C. pepo* are too large and sticky to be carried by the wind and highly favour insect pollination (Hodges and Baxendale, 1995; Eischen, 2000).

Honey bees are important pollinators of pumpkin (Nicodemo *et al*., 2009; Vidal *et al*., 2010) and their importance is more in areas where wild bees are absent or their number is unpredictable (Jaycox *et al*., 1975). Walters and Taylor (2006) have reported increased fruit set, size, weight and number of seeds per fruit in the presence of managed honey bee (*A. mellifera*) pollination. *Apis dorsata* and *A. florea* along with some solitary bees (Anthophoridae, Xylocopidae, Megachilidae and Halictidae) have been documented visiting *C. pepo* flowers in India (Grewal and Sidhu, 1979; Girish, 1981).

However, a few studies have suggested that honeybees are less effective pollinators in pumpkin due to their inefficacy in harvesting pollen from the anthers (Linsley, 1961; Michelbacher *et al*., 1964) and uncertain visitation patterns in the presence of nearby competing crops (Westerkamp, 1991). The non *Apis* bees, particularly the squash bees, have been documented as more effective pollinators than the honey bees in a number of squash crops in Europe (Canto-Aguilar and Parra-Tabla, 2000; Sampson *et al*., 2007). These bees out compete honey bees in terms of visiting flowers and

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0030-9923/2014/0002-0531 $ 8.00/0

depositing pollen to the conspecific stigmas (Ordway *et al*., 1987). Besides pollinating a number of other economically important crops in India and Pakistan (Kumar and Rao, 1991; Ali *et al*., 2011), bees from the family Halictidae have also been recognized as the most frequent and important pollinators of cucurbit crops (Melendez-Ramirez *et al*., 2002; Krug *et al*., 2010; Saeed *et al*., 2012).

Determination of the pollination efficiency is the first step for the management and conservation of wild bee species (Primack, 1993). The efficiency of pollinators is measured in terms of different parameters such as visitation frequency, time spent per visit, pollen harvest and deposition and fruit and seed set in their single or multiple visits (Ne'Eman *et al*., 2010). The computation of these diverse parameters, for instance, single-visit pollen deposition, floral preference (visits to pistillate and staminate flowers) and time spent per flower helps in measuring the overall performance of a pollinator to the reproductive success of a plant (Inouye *et al*., 1994; Harder and Wilson, 1998; Ne'Eman *et al*., 2010).

The bee fauna of cucurbit crops of Pakistan is still unexplored and consequently their role in crop pollination is poorly understood. Honey bees (*A. mellifera*) are considered as the important pollinators of cucurbit crops (Vidal *et al*., 2010; Saeed *et al*., 2012) and are used to provide the managed pollination services in many countries. In Pakistan, however, managed honey bees are not the successful pollinators in vegetable growing areas of Southern Punjab due to intensive use of pesticides and high temperature (Sajjad *et al*., 2008). Moreover, the vegetable growers lack sufficient resources to rent honey bees, therefore the services of wild pollinators may be of key importance (Kremen *et al.*, 2007). The crop pollination efficiency of these wild native pollinators (in terms of fruit or seed set) should be evaluated (Primack, 1993; Torchio, 1994) so that these may be protected and managed within the agricultural context (Kevan *et al.*, 1990).

The objectives of this study were to determine the diversity of native floral visitors of *C. pepo*, their diurnal and seasonal dynamic pattern, and their single visit efficacy from the perspective of finding the best pollinators for future conservation at Multan, Pakistan.

MATERIALS AND METHODS

Study area

The studies were conducted between August to November, 2011 at the research farm of Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan (30.255º N and 071.513º E) Pakistan. Pumpkin (*Cucurbita pepo*) was grown in an area of one hectare located at a distance of 1 km north of a 20 ha planted forest, dominated by perennial trees *i.e., Acacia nilotica, Dalbergia sissoo* etc. and different flowering weed species. The forest was a source of *A. dorsata* since there were many hives of this species located in it.

The area has a sub-tropical climate with hot long moist summer and short warm dry winter; the mean daily maximum and minimum temperatures range from 30 to 35ºC and 15 to 20ºC, respectively (Khan *et al*., 2010). The average annual rainfall of the area is 26 mm (Salma *et al*., 2012).

Floral visitor census

We conducted 15 minutes observations each for staminate and pistillate flowers. Observations were made on the hour at 07:00, 8:00, 9:00, 10:00 and 11:00 in seven-day intervals throughout the flowering season. During each observation, pollinator abundance (total visits) and the visitation frequency (number of visits per staminate or pistillate flowers per 15 minutes) were observed. A visit was only counted if an insect made a contact with either the anther or the stigma. The syrphid fly species were identified by a taxonomist (Acknowledgements) while the bee genera were identified to the lowest taxonomic level following Michener (2000). Most of the bees were not identified to species level due to lack of local taxonomic literature. Therefore, we maintained morphospecies based on their strong interactive morphological features. Voucher specimens were submitted to the Agricultural Museum of the Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan.

Foraging behavior

Foraging behavior of the abundant pollinators was recorded in terms of visitation rate (number of flowers visited per min), time spent on pistillate and staminate flowers and their thrust for nectar and/or pollen. Since different insects had different diurnal and seasonal dynamics, observations were made with one hour interval from 07:00-11:00h on weekly basis.

Pollen harvest

Pollen harvest by a particular pollinator species was estimated by caging buds 24 hours before they opened and uncaging them after they had opened fully. One pollinator species was allowed to visit which was subsequently captured and identified. To avoid overestimating the pollen harvest, this procedure was executed early in the morning, when individuals arriving at flowers were beginning foraging activity. Pollen harvested by 50 individuals per pollinator species was calculated by using the Canto-Aguilar and Parra-Tabla (2000) method. Thirty flowers were caged for estimating total number of pollen grains produced.

Pollen deposition

For estimating the pollen deposition on the floral stigma, floral buds were caged with muslin cloth bags 24 h before their opening. The flowers were un-caged during the peak activity period of pollinators (8:00 to 9:00) (Fig. 1) and stigmas were excised once the flowers had been visited by a particular pollinator species. The stigmas were then fixed in alcohol-acetic acid solution and stained with safranine, aniline blue and acetic acid (Dafni, 1992). Pollen grains were counted by using the stereoscopic microscope at 40x magnification.

Single visit fruit set

To confirm the pollen deposition potential of different pollinators during a single visit, their effectiveness in terms of fruit set percentage was also calculated. For this purpose, we caged 30 other buds before they opened and re-caged them after a single visit was made by a particular pollinator species. Fruit set was evaluated 5-6 days after pollination when the difference in ovary swelling or abscission was obvious (Tepedino, 1981; Vidal *et al*., 2010). Fifty floral buds for both open-pollinated (unrestricted insect visitation) and caged (no insect visitation) were also maintained for the comparison.

Nectar production and sugar concentration

Nectar produced by each flower type (staminate and pistillate) was measured at two hours interval during the anthesis period (07:00-11:00). Over two days, each of ten pistillate and staminate flowers was caged 24 hours before their opening. The nectar from these fresh flowers was extracted by using the graduated micropipette (10 µl) and the volume calculated by using the Cruden and Hermann (1983) method. The sugar concentration of each flower was measured by placing a drop of five microliter of nectar in the hand held refractometer.

Data analysis

The data of time spent on staminate and pistillate flowers, visitation rate, pollen deposition and harvesting per pollinator species per visit and fruit set per single visit were analyzed by using analysis of variance (ANOVA). Means were compared by Fisher's test at P= 0.05. Paired sample T-test was applied to check the differences in visitation frequencies of pollinator species, nectar volumes and sugar concentrations between the two floral types. The statistical analysis was performed on computer software XLSTAT (XLSTAT, 2012).

RESULTS

Pollinator community

The pollinator community of *C. pepo* was composed of 12 bee species (Hymenoptera), 4 fly species (Diptera) and 2 butterfly species (Lepidoptera). Only 8 bee and 2 syrphid fly species were encountered during our systematic observations (Table I).

Among the bees, *A. dorsata*, *Nomia* sp., *Halictus* sp., *Lasioglossum* sp.2, *Lasioglossum* sp.1, *Ceratina sexmaculata*, *Xylocopa* sp., and Halictidae sp. came under systematic observations while *Amegilla* sp., *Dieunomia* sp., *Agapostemon* sp. and *Megachile* sp. were rarely seen. Halictidae was the dominant family with seven species followed by the Apidae with four species.

Apis dorsata, *Nomia* sp. and *Halictus* sp. were the most frequent floral visitors with a total abundance of 399, 189 and 117 individuals respectively. Their average visitation frequency was also the highest among all the observed bee species, *i.e.* 3.33, 1.58 and 0.99 individuals per flower per minute respectively (Table I).

Among the syrphid flies, *Ischiodon scutellaris* and *Episyrphus balteatus* were the occasional visitor and only recorded as a part of pollinator community. The two butterfly species, *Pieris brassicae* (Pieridae) and *Danaus chrysippus* (Danadidae) were also rarely seen.

Population dynamics

The diurnal dynamic pattern revealed that all the pollinators started their activity on or slightly before 07:00 h and the peak activity was observed between 8:00 to 09:00 h. *Apis dorsata* and *Nomia* sp. foraged throughout the observation period in both staminate and pistillate flowers while the activity of other pollinators largely ceased at 10:00 h in pistillate flowers (Fig.1A,B).

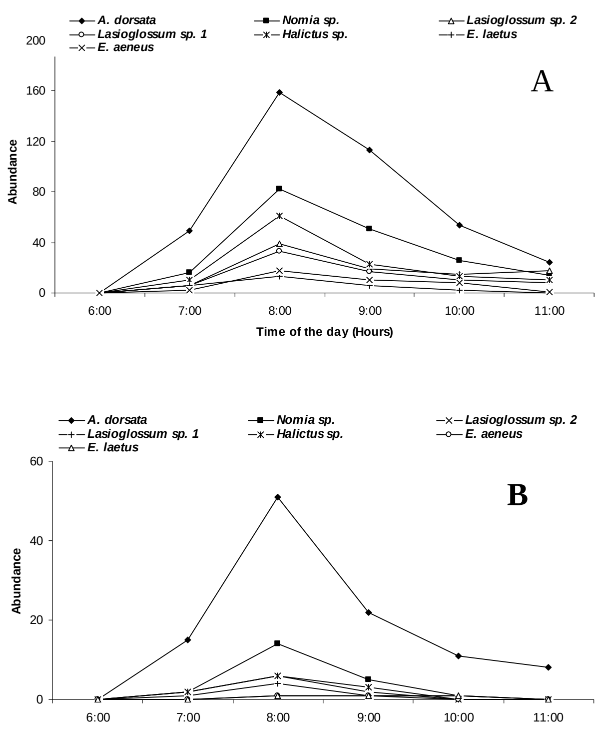

Fig. 1 Diurnal dynamic pattern of pollinators in (A) staminate and (B) pistillate flowers of *C. pepo* at Multan, Pakistan during Oct-Nov, 2011.

The population dynamic pattern of pollinators during the full flowering season revealed *A. dorsata* and *Nomia* sp. as the most dominant and unevenly regular floral visitors of both staminate and pistillate flowers. In contrast, the abundance of syrphid flies was too low on pistillate flowers to conclude its seasonal occurrence (Fig. 2A,B).

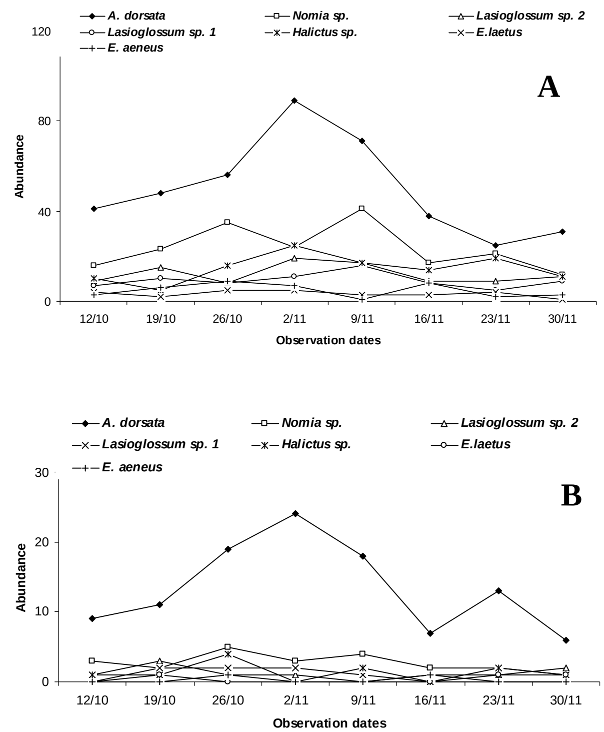

Fig. 2. Fluctuation in population of pollinators in (A) staminate and (B) pistillate flowers of *C. pepo* at Multan, Pakistan during Oct-Nov, 2011.

Visits to staminate and pistillate flowers

Staminate flowers received more visits (117.75±13.69) as compared to pistillate flowers (20.25±2.89) (F=48.52, df =1.0, p<0.0001). The t-test (Alpha=0.05) further confirmed that all the species were significantly higher on staminate flowers (Fig. 3).

Apis dorsata visited a higher percentage of pistillate flowers (21%) compared to the other abundant bees *i.e. Nomia* (10%) and *Halictus* sp. (9%). *Halictus* sp. visited a higher percentage of staminate flowers (91%) followed by *Nomia* sp. (89%) and *A. dorsata* (78%).

Table I.- **Insect species in *Cucurbita pepo* along with their abundance, visitation frequency and foraging behavior in staminate and pistillate flowers.**

Order	Family	Genus/Species	Staminate abundance	Visitation frequency (Individuals/ staminate flower/minute)	Pistillate abundance	Visitation frequency (Individuals/ pistillate flower/minute)	Foraging task (N/P)*
Hymenoptera	Apidae	*Apis dorsata*	399	3.33	107	0.89	N
		Xylocopa sp.	09	0.08	0	0	N
		Ceratina sexmaculata	11	0.09	0	0	N/P
	Halictidae	*Nomia* sp.	189	1.58	22	0.18	N/P
		Halictidae sp.	13	0.11	3	0.03	N/P
		Lasioglossum sp.1	74	0.62	7	0.06	N/P
		Lasioglossum sp.2	97	0.81	10	0.08	N/P
		Halictus sp.	117	0.98	11	0.09	N/P
Diptera	Syrphidae	*Eristalinus laetus*	27	0.23	3	0.02	N/P
		Eristalinus aeneus	39	0.33	2	0.03	N/P

*N/P= Nectar/Pollen

In *C. pepo*, pistillate flowers produced on an average almost twice the nectar volume (74.35±19.33) than the staminate flowers (41.20±7.88) and also of higher sugar concentration (35.40±1.87: 32.10±2.02%). The paired sample t-test also revealed the significant differences in nectar volume (t = 2.47, p = 0.03) and sugar concentration (t = 2.54, p = 0.03) between the two flowering types.

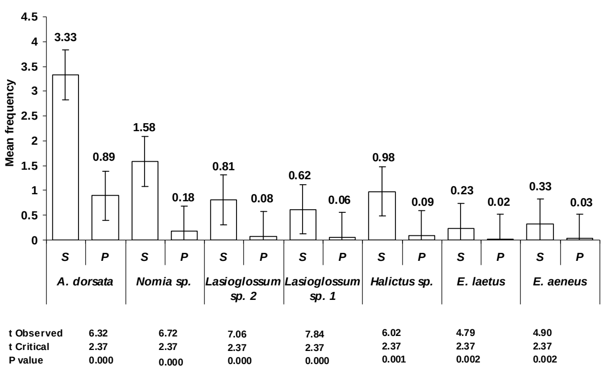

Fig. 3. Results of the paired sample t-test for comparing frequency of pollinators in staminate (S) and pistillate (P) flowers of *C. pepo*. Error bars show standard errors.

Foraging behavior

Nectar and pollen were the primary foraging task of all the bee and syrphid fly species except *Xylocopa* sp. and *A. dorsata* which fed on nectar alone (Table I).

There was significant difference among the pollinators in terms of their stay time on both the staminate (F=51.31, df =6.0, p<0.0001) and pistillate (F=37.10, df =6.0, p<0.0001) types. Stay time was highest for *Halictus* on both floral types (90.87±6.81 for pistillate; 77.37±3.28 for staminate) followed by *Lasioglossum* sp.1 (79.84±4.56; 63.31±2.84) and *Lasioglossum* sp.2 (71.36±4.64; 54.29±2.87). It was lowest for *E. aeneus* (07.05±0.42; 17.56±1.30) (Table II).

Pollinator species also varied significantly in terms of their visitation rate (F=147.93, df =6.0, p<0.0001). *Nomia* sp. visited the highest number of flowers (02.49±0.61) followed by *A. dorsata* (02.05±0.03) and *Lasioglossum* sp. 2 (01.56±0.05). Contrary to their highest stay time on both staminate and pistillate flowers, *Halictus* sp. (01.10±0.03) and *Lasioglossum* sp. 1 (01.31±0.04) showed the lowest visitation rate (Table II).

Pollination effectiveness

Pollen harvest differed statistically among the seven tested pollinator species (F=126.24, df =6.0, p<0.0001); *Nomia* sp. harvested the maximum number of pollen grains (3121.50±199.54) comprising 9% of the average pollen production (33668±1970) followed by *A. dorsata* (2090.40± 139.03; 6%) and *Halictus* sp. (798.45±109.39; 2%) (Table III).

Table II.- Pollination effectiveness of seven pollinators in terms of visitation rates and stay time.

Pollinator species	Stay time (S)/flower/visit (n=100)		Visitation Rate No. of flowers visited/min (N=100)
	Staminate	Pistillate	
Apis dorsata	30.02±2.39 d	67.11±4.67 cd	02.05±0.03 b
Nomia sp.	49.50±2.99 c	58.20±4.29 d	02.49±0.05 a
Lasioglossum sp.1	63.31±2.84 b	79.84±4.56 ab	01.31±0.04 d
Lasioglossum sp.2	54.29±2.87 c	71.36±4.64 bc	01.56±0.05 c
Halictus sp.	77.37±3.28 a	90.87±6.81 a	01.10±0.03 e
Eristalinus laetus	23.37±1.51 de	10.55±0.60 e	01.39±0.05 d
Eristalinus aeneus	17.56±1.30 e	07.05±0.42 e	01.24±0.05 de

Means followed by the same letters in a column are not statistically different according to Fisher at 5% level (±S.E.).

Pollen deposition followed a similar pattern as pollen harvest (F=42.23, df =6.0, p<0.0001). *Nomia* sp. proved the best pollen depositor (376.60± 23.01) followed by *A. dorsata* and *Halictus* sp. (204.15±20.63 and 177.85±16.31, respectively). Syrphid flies did not prove to be efficient pollen harvesters and depositors (Table III).

Single visit efficacy in terms of fruit set percentage (F=47.99, df =8.0, p<0.0001) revealed *Nomia* sp. as the best pollinator (36.66) followed by *A. dorsata* (23.33) and *Halictus* sp. (20.0) while the remaining pollinator species were statistically non-significant. All the flowering buds receiving unrestricted insect visitation set fruit while no fruit set at all resulted from caged buds due to flower abortion (Table III).

DISCUSSION

The large and monoecious flowers of pumpkin (*C. pepo*) produce copious nectar and pollen and attract a wide array of insect visitors, particularly bees (Canto-Aguilar and Parra-Tabla, 2000; Nicodemo *et al*., 2009). In this study 18 floral visitor species from 3 insect orders and 6 families were observed foraging in our single experimental plot.

The diurnal dynamic pattern revealed the peak activity of pollinators between 08.00 and 09.00 h. *Apis dorsata* and *Nomia* sp. foraged throughout the observation period in both staminate and pistillate flowers while the activity of other pollinators largely ceased at 10:00 h in pistillate flowers. In Indian Punjab, the foraging activity of insect visitors in *C. pepo* has been documented from 07:00 to 10:30 a.m. when the flowers began to close (Atwal, 1970). Several factors have been documented to influence the foraging activity of the pollinators including the temperature light levels, wind speed and relative humidity (Primack and Inouye, 1993) and these can alter the most abundant and effective pollinators of a crop (Kremen *et al*., 2002).

Table III.- Pollination effectiveness of seven pollinators in terms of pollen harvest and deposition along with fruit set per single visit.

Pollinator species	Pollen harvesting (n=50)	Number of pollen grains deposited/ stigma/visit (n=30)	Single visit fruit set (%) (n=30)
Apis dorsata	2090.40±139.03b	204.15±20.63 b	23.33 bc
Nomia sp.	3121.50±199.54a	376.60±23.01 a	36.66 b
Lasioglossum sp.1	324.05±33.36de	116.00±14.11 d	10.0 cde
Lasioglossum sp.2	582.80±80.22cd	138.45±13.05 cd	13.33cde
Halictus sp.	798.45±109.39c	177.85±16.31 bc	20.0 cd
Eristalinus laetus	153.55±12.74e	99.10±09.14 de	6.66 de
Eristalinus aeneus	106.65±12.56e	70.95±07.64 e	6.66 de
Open pollinated	-	-	100.0 a
Caged pollinated	-	-	0.00 e

Means followed by the same letters in a column are not statistically different according to Fisher at 5% level (±S.E.)

A. dorsata visited a significantly higher percentage of pistillate flowers among all the observed pollinators. This is because *Apis* bees preferentially collect nectar rather than pollen from *C. pepo* (Michelbacher *et al*., 1964) and a two fold greater nectar volume along with higher sugar concentration was available in pistillate than in staminate flowers in our studies. Similar preference of *A. mellifera* for pistillate flowers has also been documented by Artz and Nault (2011). In contrast, the lower number of visits to pistillate flowers by the other bee species in this study might be due to their dependence on pollen required for larval development and adult maintenance (Michener, 2000) which is a limiting factor in pistillate flowers. These perhaps only visit pistillate flowers when the nectar rewards in staminate flowers become limiting.

The attractiveness of pollinators to the *C. pepo* depends upon the nectar quantity and sugar concentration in its flowers (Karp *et al*., 2004). The higher nectar volume prolongs the time spent by the pollinators on a flower and increases the chances of pollen deposition and successful pollination (Manetas and Petropoulou, 2000). The increased time spent by the bees on pistillate flowers in our study could be due to their two fold higher nectar volume and sugar concentration than the staminate flowers. However, Girish (1981) did not find any significant difference between the time spent by the bees on both floral types probably because the staminate flowers had slightly more nectar than the pistillate ones but of similar sugar concentration.

Nomia sp. visited higher numbers of flowers than did *A. dorsata*. The solitary bee (*Peponapis pruinosa*) has been reported foraging faster than honey bees *A. mellifera* (Tepedino, 1981). Visitation rate is an important measure used in many pollination studies (Proctor *et al*., 1996) and it is generally considered that the more visits made the more efficient is the pollinator. However, this depends upon the amount of pollen transferred by a pollinator per visit to the pistillate flowers (Herrera, 1989).

Pollen harvest and deposition per single visit revealed *Nomia* sp. as the best pollinator followed by *A. dorsata* and *Halictus* sp. The squash bee (female *P. limitaris*) and bumble bee (*Bombus impatiens*) have also been documented as better pollinators than the managed honey bee species (*A. mellifera*) in terms of harvesting and depositing pollen per single visit (Canto-Aguilar and Parra-Tabla, 2000; Artz and Nault, 2011). The high quantity of pollen harvested by the *Nomia* sp. in our studies could be due to its dependence on the cucurbit pollen and it could serve as a pollen source for its larval cells as has been previously reported for *P. limitaris* (Willis and Kevan, 1995). In general, the magnitude of pollen removal and its deposition may vary with the behaviour of the pollinator (either collecting nectar or collecting pollen) at flowers (Goodell and Thomson, 1996; Freitas and Paxton, 1998; Williams and Thomson, 2003) and the degree of grooming pollen from their bodies (Rademaker *et al*., 1997). The fruit and seed quality in *C. pepo* has been found to increase with the pollen load size (Melendez-Ramirez *et al*., 2000).

The previous studies on pollination biology of pumpkin suggest that percent fruit set increased with the increase in number of visits (Nicodemo *et al*., 2009; Vidal *et al*., 2010). Therefore, we focused on single visit efficacy of different pollinator species aiming to determine the best pollinator in terms of fruit set percentage. *Nomia* sp. proved the best pollinator with the highest fruit set (35%) followed by *A. dorsata* (22%) and *Halictus* sp (19%). However, Tepedino (1981) found 22% fruit set in either the single visit of the squash bee, *P. pruinosa* or the honey bee, *A. mellifera*. The increased fruit set by the *Nomia* sp. in our studies could be due to its higher pollen deposition (376) per single visit compared to the pollen deposition (200) of *P. pruinosa* in the Tepedino study. The high pollen depositing ability resulting in increased fruit set has also been previously reported for *B. impatiens* by Artz and Nault (2011).

In conclusion, *Nomia* sp., *A. dorsata* and *Halictus* sp. proved to be the best pollinators for *C. pepo* production. Conserving and enhancing these native pollinators may boost *C. pepo* production in Pakistan. Future studies should evaluate the effectiveness of these native pollinators for other cucurbit crops together with basic studies on their biology such as nesting site locations and alternate nectar resources.

ACKNOWLEDGEMENTS

The study was funded by Higher Education Commission of Pakistan. We are grateful to Dr. Claus Claussen (Twedter Holz 12 D-24944 Flensburg, Germany) for his help in identification of syrphid fly species.

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XLSTAT, 2012. XLSTAT. (http://xlstat.com/en/download) Date of access: 20 Mar. 2012.

(Received 8 January 2014, revised 3 March 2014)