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In Search of the Best Native Pollinators for Bitter Gourd (*Momordica charantia* L.) Pollination in Multan, Pakistan

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Abstract.- In order to assess the pollinator community and the best native pollinators for bitter gourd (*Momordica charantia* L., Cucurbitaceae), an experiment was performed at the Vegetable Research Station in Multan, Pakistan. We measured the abundance of pollinators, their diurnal and seasonal dynamics along with their floral visitation rates and single-visit efficacy in terms of reproductive success. The pollinator community was composed of 15 insect species in 3 orders and 10 families. Bees were the most dominant (435 individuals) floral visitors followed by butterflies (345 individuals) and flies (248 individuals) while moths and wasps were observed occasionally. *Apis florea*, *Parnara guttata* and *A. dorsata* were the most abundant pollinators. *A. florea* and *A. dorsata* also exhibited the highest visitation rates and frequencies. Five major pollinators were tested for their single-visit efficacy, showing that *A. dorsata* was the most effective pollinator, along with *A. florea* and *Eristalinus laetus.* Conserving and enhancing these pollinators may boost *M. charantia* production in Pakistan.

Key words: *Momordica charantia*, pollinator community, visitation rate, single visit efficacy, reproductive success.

INTRODUCTION

Many horticultural crops are dependent on insect pollination, and better pollination results in higher yields (McGregor, 1976; Free, 1993; Klein *et al.*, 2007), more uniform ripening and improved plant vigor (Stoddard and Bond, 1987). A diverse array of insects like bees, butterflies, wasps, flies, beetles and moths are responsible for providing this essential ecosystem service of crop pollination (Buchmann and Nabhan, 1996; Kevan, 1999). Crops differ in their pollination requirements and hence their dependence on insect pollinators (Morse and Calderone, 2000).

With its generalized monoecious flower, bitter gourd always requires pollinating insects for effective pollination and better fruit and seed setting (Ashworth and Galetto, 2002; Lenzi *et al.*, 2005). Flowers that are not visited by pollinators do not set fruit. Rodelina and Cervancia (2009) reported honey bees (*Apis mellifera* and *A. cerana*) as a major floral visitors of bitter gourd in the Philippines along with some solitary bees (*Trigona* sp. and *Halictus* sp.).

The visitation activity and behavior of pollinators in flowers is influenced by environmental factors such as temperature and relative humidity (Lundberg, 1980; Willmer, 1983; Stone *et al.*, 1988; Stanton and Galen, 1989; Herrera, 1995). This dependence of insects on abiotic factors thus significantly inhibits the process of cross-pollination in crops (Eisikowitch and Galil, 1971; Martinez del Rio and Burqurez, 1986; Bergman *et al.*, 1996).

Visitation rate is an important component of the effectiveness of any pollinator (Proctor *et al.*, 1996): the more visits occur, the more efficient a pollinator will be. Various studies have shown spatial and temporal variations in the visitation rate of pollinators (Herrera, 1988; Horvitz and Schemske, 1990; Traveset and Saez, 1997; Fenster and Dudash, 2001; Ivey *et al.*, 2003), probably as a result of environmental variation in temperature, light intensity, wind speed and relative humidity (Primack and Inouye, 1993), as well as plant characteristics including floral structure and the spatial and temporal arrangement of flowers (Thompson, 2001; Mitchell *et al.*, 2004). Temporal variation in the production of the reward (nectar and pollen) also influences the rate of visitation (Thomson and Thomson, 1989). Differences in visitation rates among pollinators are probably related to both pollinator efficiency and effectiveness (Fishbein and Venable, 1996; Ivey *et al.*, 2003).

Besides visitation rates of pollinators, other

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parameters for measuring their effectiveness are the pollen depositing ability, number of grains needed for well-formed fruit and adequate seed set (Dafni, 1992; Kearns and Inouye, 1993), floral preference (male or female flower), foraging habits (timing of visits in relation to receptivity, nectar or pollen collection) and physical attributes (tongue length, size, pollen carrying structures) (Dafni, 1992; Free, 1993; Buchmann and Nabhan, 1996; Delaplane and Mayer, 2000; Adler and Irwin, 2006).

Although honey bees (*A. mellifera*) are considered to be the most efficient pollinators of the world crops (Free, 1993) and are used to provide managed pollination services in many countries, in Pakistan the apiary industry has been limited mainly because of unrestricted use of pesticides in vegetable cultivated areas. Furthermore, farmers are unable to rent honey bees because they cannot afford them, and therefore the services of wild pollinators may be of key importance (Klein *et al.*, 2007; Kremen *et al.*, 2007).

Conserving wild native pollinators seems to be a good choice (Sajjad *et al*., 2008) and basic studies on the biology (nest site location and alternate foraging resources) of these species must be undertaken. The scope of the current study is widespread throughout Indian sub-continent, Southeast Asia and some parts of central and West Asia, since many wild native bee species are common here, which, unlike *A. mellifera*, effectively survive and pollinate under very hot summer conditions.

The present study was intended to determine the diurnal and seasonal trends in the native pollinator diversity of bitter gourd, the effect of environmental conditions on their visitation rates and their single-visit efficacy in order to pave the way for the conservation of effective pollinators of bitter gourd and other cucurbits.

MATERIALS AND METHODS

Study site

The study area was located at the vegetables research farm of the Cotton Research Station (CRS), Multan, about 10 km south of the city of Multan. The experimental material was *Momordica charantia* (Bitter melon). The crop was sown at in area of ½ hectare on 27 February 2009, and started flowering on *ca.* 10 April 2009: the study started on 23 April 2009. Nearby crops included mango, citrus and date-palm orchards, along with a mixed vegetable culture was also grown *i.e.* chilies, garlic, pumpkin and eggplant. The climate is Subtropical with extreme conditions of hot summers and cold winters. The mean monthly temperature ranges between a maximum of 35°C to 40°C and a minimum of 10°C to 20°C. The extreme maximum temperature of the region varies between 45°C and 51°C during the months of May and June, while the lowest minimum temperature varies between 0°C to -5°C during the month of January. The mean monthly summer and winter rainfalls are the same at *ca.* 18 mm (Khan *et al.,* 2010).

Pollinator abundance

To measure the abundance of pollinators, a quadrate of 1m² was thrown randomly five times in the field of *M. charantia* during one census. The number of individuals per insect visitor species per 5 minutes was recorded in this specified area. Observations were made on the hour at 07:00, 08:00, 10:00, 12:00, 14:00, 16:00 and 18:00 (local time), at intervals of seven days throughout the flowering season. The temperature and relative humidity was also measured during each census. Insects were collected with a sweep net and identified in the laboratory for later identification. Pollinators were identified by the experts (see acknowledgements). Voucher specimens were deposited in the Agricultural Museum of the University College of Agriculture, Bahauddin Zakariya University, Multan.

Floral visitation rate

Visitation rate (number of flowers visited per minute) of flower visitors was recorded by using a stop watch. Weekly observations were made at three time intervals of the day *i.e.* 08:00, 14:00 and 16:00, since different insects had different diurnal and seasonal dynamics.

Reproductive success

To confirm the effectiveness of pollinators in depositing pollen during a single visit, we caged female-stage floral buds with butter paper bags before they opened and re-caged them after a single visit had been made by a particular pollinator species. We did this during the peak activity time of the pollinators between 08:00 to 12:00. Before the onset of flowering, three pollinator's exclusion cages (mosquito nets of $1m^2$) were placed randomly over vines of *M. charantia* plants in the fields. Three patches of $1m^2$ were also marked for unrestricted open-pollination. The resulting fruits were harvested upon ripening, and fruits and seeds were weighed on an electronic-balance. The number of seeds were also counted and then subjected to a germination test.

Data analysis

The data of fruit weight, number of seeds per fruit and seed weight per fruit were subjected to statistical analysis using analysis of variance (ANOVA). Means were compared by Least Significant Difference (LSD) with a threshold of significance set at $P = 0.05$. Regression analysis was used to determine the relationship between pollinator visitation rates and abiotic factors (temperature and relative humidity). Statistical analyses were performed using XLSTAT (XLSTAT, 2008).

RESULTS

Pollinator community

The visitor community to *M. charantia* was composed of four bee, two wasp, three butterfly, one moth and five fly species. Bees were among the most abundant floral visitors with a total abundance of 435 individuals, followed by the butterflies (345) and true flies (248). Moths and wasps were the rarest floral visitors with 83 and 36 individuals, respectively. *A. florea* (Fabricius, 1787) (Hymenoptera) was the most frequent floral visitor (214 individuals) followed by *Parnara guttata* (195) (Lepidoptera) and *A. dorsata* (156) (Hymenoptera). Syrphids (*Eristalinus aeneus* and *Eristalinus laetus*) (Diptera) were the most abundant among flies (Table I).

Diurnal and seasonal population trends:

The diurnal pattern of bee visits showed that foraging activity started early in the morning (08:00) and continued throughout the day (to 18:00) with a peak between 08:00 and 10:00 and a sharp decline 10:00-12:00. *Lasioglossum* sp. was recorded only during the first half of the day (Fig. 1A). Like bees, butterflies, moths, true flies and wasps also foraged throughout the day and exhibited a similar diurnal activity pattern *i.e.*, their peak activity between 08:00 and 10:00 followed by a sharp decline up to 12:00 (Fig. 1B, C, D). Thereafter, different species exhibited different dynamic patterns until 18:00 but visitation levels remained below the peak between 08:00 and 10:00.

The seasonal pattern showed *A. dorsata* and *A. florea* to be the dominant and most regular floral visitors to *M. charantia* among the bees (Fig. 2A). Their activity peaked between the 2nd week of April and the middle of May. *Lasioglossum* sp. appeared in the 3rd week of May and remained active at low abundance throughout the remaining time. *C. sexmaculata* appeared in the 1st week of May and thereafter showed a variable abundance.

The two syrphid flies (*E. anenus* and *E. laetus*) were the dominant fly visitors. *E. aeneus* gradually decreased in abundance until the 3rd week of May and was rarely seen afterward. However, *E. laetus* appeared and peaked during 2nd week of May, and sustained visitation throughout the observation dates. The other two true flies recorded (Anthomyiidae sp. and *Sarcophaga* sp.) were only occasional visitors (Fig. 2B).

Butterflies and moths were very frequent floral visitors in high fluctuating numbers in *P. guttata* and *Tarucus* sp. while *Eurema hecabe* and *Utetheisa* sp. showed a gradual decrease in population throughout the observation dates. The two wasps, *Polistes olivaceus* and *Vespa* sp. were rarely seen (Fig. 2D).

Floral visitation and abiotic factors

Temperature was only positively related with the visitation rates of bees ($y = 0.71-0.13x$; $r^2 = 0.073$; $P = 0.06$; n=104), flies ($y = 0.21-8.35x$; $r^2 = 0.06$; $P = 0.02$; n=163) and butterflies ($y = 0.35-4.26x$; $r^2 = 0.07$; $P = 0.06$; n=102) whereas for wasps ($y = 5.53-6.29x$; $r^2 = 0.04$; $P = 0.11$; n=70) it was only negatively related. All the insect groups were negatively related with the relative humidity however, this relationship was strongest in flies *i.e.* bees ($y = 7.00-0.5.37x$; $r^2 = 0.04$; $P = 0.04$; n=104),

Table I.- Insect species in *Momordica charantia* flowers along with their total abundance, visitation frequency and visitation rate.

Order	Family	Genus/Species	Total abundance	Visitation frequency (No. of visits/flower/5min.) (N=50)	Visitation rate (No. of flowers visited/min.) (N=40)
Hymenoptera	Apidae	*Apis dorsata* Fabricius, 1793	158	0.48±0.26	5.11±0.97
		Apis florea Fabricius, 1787	214	0.65±0.46	5.70±0.72
		Ceratina sexmaculata Smith, 1879	51	0.15±0.18	2.15±0.61
		Lasioglossum sp. Curtis, 1833	12	0.03±0.05	2.00±0.75
	Vespidae	*Vespa dorylloides* Saussure, 1853	6	0.01±0.01	3.16±0.81
		Polistes olivaceus De Gee, 1773	30	0.09±0.04	2.74±0.81
Lepidoptera	Pieridae	*Eurema hecabe* Linnaeus, 1758	91	0.28±0.14	1.16±0.40
	Lycaenidae	*Tarucus* sp. Moore, 1881	59	0.18±0.08	1.83±0.42
	Hesperiidae	*Parnara guttata* Bremer & Grey, 1852	195	0.51±0.20	1.01±0.30
	Noctuidae	*Utethesia* sp. Hubner, 1819	83	0.25±0.12	1.92±0.45
Diptera	Syrphidae	*Eristalinus aeneus* Scopoli, 1763	104	0.37±0.07	3.76±0.91
		Eristalinus laetus Wiedemann, 1830	116	0.35±0.23	4.09±1.13
	Sarcophagadae	*Sarcophaga* sp. Meigen, 1826	8	0.02±0.02	3.01±0.75
	Anthomyiidae	*Anthomyiidae* sp.	20	0.06±0.02	0.57±0.20
	Tephritidae	*Bactrocera zonata* Bezzi, 1913	59	0.18±0.08	3.30±0.87

*Mean values (± S.E.)

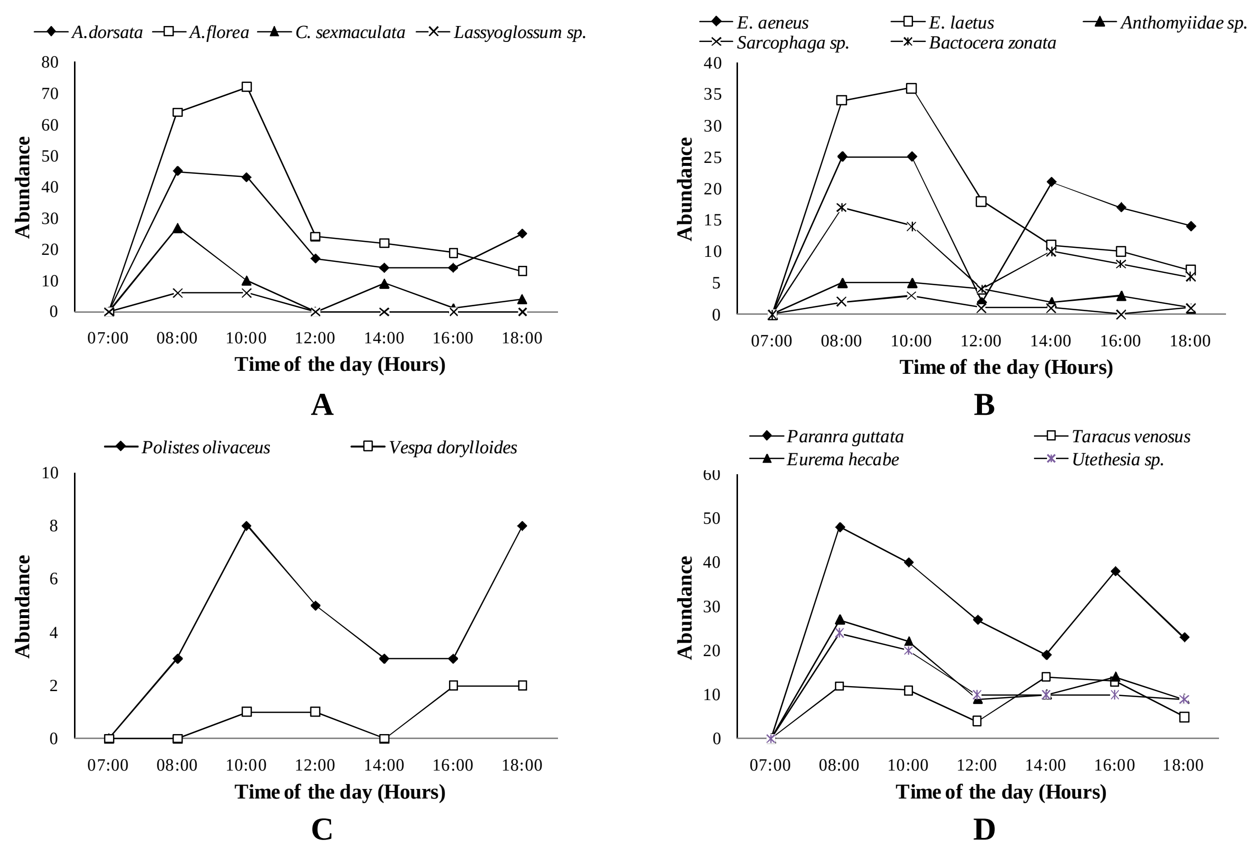

Fig.1. Diurnal dynamic pattern of (A) bees (B) flies (C) wasps (D) butterflies and moths in bitter gourd field at CRS, Multan, Pakistan during April-July, 2009.

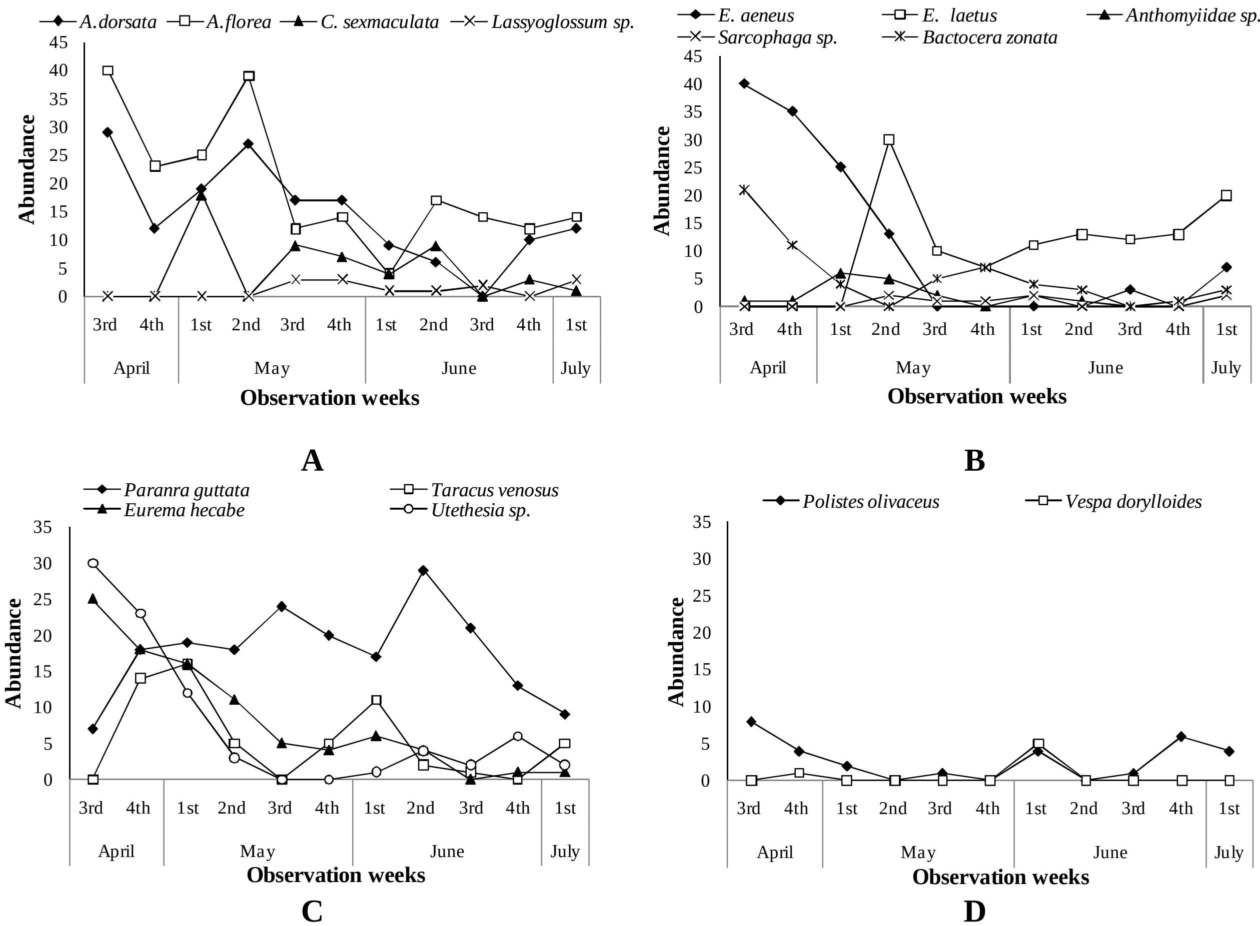

Fig.2. Seasonal dynamics of (A) bees (B) flies (C) butterflies and moths (D) wasps in bitter gourd field at CRS, Multan, Pakistan during April-July, 2009.

flies ($y = 5.95\text{-}6.22x$; $r^2 = 0.10$; $P = 0.0001$; n=163), butterflies ($y = 1.71\text{-}7.46x$; $r^2 = 0.08$; $P = 0.39$; n=102) and wasps ($y = 3.71\text{-}1.62x$; $r^2 = 0.02$; $P = 0.24$; n=70).

Pollinator's abundance and visitation rates

Among bees, *A. florea* showed maximum abundance, followed by *A. dorsata*. *P. guttata* was the most abundant butterfly species followed by *E. hecabe* whereas *E. aeneus* and *E. laetus* were the most abundant fly species (Table 1). Visitation rates of social bees (*A. florea* and *A. dorsata*) were higher than those of solitary bees (*Lasioglossum* sp and *C. sexmaculata*). *A. florea* visited at the maximum recorded rate, followed by *A. dorsata* and *E. laetus*. In contrast to its highest abundance, *P. guttata* showed the lowest visitation rate (1.01± 0.30 flower per minute) among butterflies. Butterflies stayed on a flower for longer than bees and flies (Table II).

Single visit efficacy

The fruits produced though unrestricted open-pollination exhibited the highest weight, number of seeds and seed weight while, no fruit set at all results from caged flowers due to flower abortion. The single visit efficacy in terms of fruit weight showed that *A. dorsata* was the best pollinator, statistically indistinguishable from open-pollinated fruits (Table II). The other four tested pollinators were statistically indistinguishable from one another. The fruit resulting from a visit of *A. dorsata* also produced the maximum number of seeds followed by those from visits by *A. florea*, *E. laetus* and *E. aeneus*. Seed weight was greater in flowers visited by *A. dorsata* and *A. florea* (Table II).

Table II.- Fruit weight (g), number of seeds and seed weight (g) resulted in single visit by five tested pollinators.

Pollinator species	Fruit weight (g)	No. of seeds	Seed weight (g)
Apis dorsata	50.60±15.12 a	15.11±1.42 b	1.63± 0.16 b
Apis florea	50.07± 9.42 b	12.64± 2.26 bc	1.47± 0.23 b
Eristalinus aeneus	29.80± 4.1 b	6.25± 1.1 bc	0.52± 0.32 cd
Eristalinus laetus	47.45± 7.32 b	11.75± 0.79 bc	0.92± 0.11 c
Ceratina sexmaculata	36.97± 13.86 b	7.67± 2.85 cd	0.32± 0.21 cd
Open pollinated	76.29± 4.32 a	22.89± 1.5 a	2.37± 0.13 a
Caged pollinated	0.00± 0.00 c	0.00± 0.00 d	0.00± 0.00 d

Mean values with similar letters in represent non-significant difference according to Tukey at 5% level (± S.E.)

DISCUSSION

M. charantia is monoecious with large pollen grains well suited to insect pollination. Male flowers produce both nectar and pollen; female flowers do not produce nectar (Lenzi *et al.*, 2005). Both attract a wide array of pollinators specially bees (Nidagundi and Sattagi, 2005). In this study 14 floral visitor species were recorded from 3 insect orders within a single experimental plot.

Both the diversity and abundance of pollinators are functions of temporal and spatial variability (Ollerton and Louise, 2002). The temporal variation of pollinators at any location depends on the availability of floral resources and seasonal changes (Sajjad *et al.*, 2010), while spatial variation is influenced by the availability of nesting sites, floral resources and hibernating places (Cunningham, 2000). Bees were the dominant floral visitors, followed by the butterflies and flies, while moths and wasps were rare. Lenzi *et al.* (2005) reported beetles (Coleoptera, Chrysomelidae) from Brazil while Deyto and Cervancia (2009) reported bees (*A. mellifera, A. cerana*, *Trigona* spp. and *Halictus* spp.) from the Philippines as the most dominant pollinators of *M. charantia*.

An array of pollinators could be involved in pollination process (Buchmann and Nabhan, 1996; Kevan, 1999). A floral visitor may not actually be a pollinator at all, and different pollinators may differ in their pollination efficiency (Sajjad *et al.*, 2008). Knowledge of pollinator efficiency and its relationship with abiotic factors is useful for making future conservation strategies of the most efficient pollinators (Lenzi *et al.*, 2005).

Apis florea and *A. dorsata* visited the maximum number of flowers and their visitation frequency was also highest among the floral visitors of bitter gourd. In general, pollination efficacy increases with increasing visitation rate, affected by a number of other factors including foraging behaviour, the type and quantity of floral rewards (Rao and Suryanarayana, 1990; Rao, 1991), floral structure (Free, 1993), length of proboscis (Inouye, 1980), and the time of day (Sajjad *et al.*, 2008).

The diurnal and seasonal activity of the most frequent and reliable floral visitors (bees, butterflies and flies) varied during the day and the season. There were few relationship between abiotic variables and pollinator visits here, although elsewhere foraging activity can be affected by temperature, light levels, wind speed and relative humidity (Primack and Inouye, 1993) which can cause alteration in the most abundant and effective pollinators of a crop (Kremen *et al.*, 2002).

A. dorsata proved to be the best pollinators because a single visit resulted in maximum fruit weight and number of seeds. *A. dorsta* has already been documented as most efficient pollinator for *Allium cepa* (Sajjad *et al*., 2008), *Brassica napus* (Ali *et al.*, 2011) and *Sesbania sesban* (Sajjad *et al*., 2009b) in southern Punjab of Pakistan. Single visit efficacy has been used to test the efficiency of pollinators, and shows that floral visitors vary in pollination efficiency and preferences for different floral traits (Lau and Galloway, 2004).

Managed honey bees (*A. mellifera*) and squash bees (*Peponapis pruinosa*) have been regarded as the important pollinators of cucurbits (Jaycox and Elbert, 1982; Girish, 1981). Although squash bees make more contact with flower reproductive parts and work faster, they are considered no more efficient than honey bees in setting fruit (Tepedino, 1981). However, a recent study (Williams *et al.,* 2009) demonstrated the importance of squash bees in Ohio (USA) because they were 90% more abundant than honey bees.

A Syrphid, *E. laetus*, also showed good potential for bitter gourd pollination. *E. laetus* is a saprophagus species with aquatic "rat-tailed" maggots, particularly in water bodies loaded with decaying vegetation, such as rot holes, ditches or drains (Sajjad and Saeed, 2009a). Managed honey bees are not successful pollinators in most of the southern Punjab due to intensive use of insecticides and high temperatures (Sajjad *et al*., 2008). Therefore conserving alternative native pollinators is a good option. *A. dorsata* and *A. florea* cannot be managed and therefore can be considered as wild honey bees. Knowledge of the biology and ecology of the most efficient pollinator species is helpful in planning their conservation and utilization in agro-ecosystems.

In conclusion, *A. dorsata* can be the most important pollinator of *M. charantia* along with *A. florea* and *E. laetus* in southern Punjab, Pakistan. Conserving and enhancing these pollinators may boost cucurbit production in Pakistan. Future research should develop conservation strategy for these most efficient pollinators in such a way that the other less abundant or less efficient pollinators may also benefit.

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