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Floral biology and pollinator activity on parental lines of bottlegourd

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ABSTRACT A two year experiment was conducted at Indian Agricultural Research Institute, New Delhi to investigate floral biology and honeybee activity on parental lines of bottlegourd, Pusa hybrid-3. The crop was sown in *Spring-summer* and *kharif* seasons, with three planting ratios (female: male) 2:1, 3:1 and 4:1. Observations on bee abundance, their foraging modes, time spent per flower, nectar and sugar contents were recorded during both the seasons. Results obtained revealed that *Apis mellifera* was the most abundant pollinator followed by *Apis dorsata, Apis cerana indica* and *Apis florea*. Bee abundance was more in *kharif* season and honey bee activity was found to be more between 12.00 and 14.00 h in *kharif* season, whereas in *Spring-summer* season, it was more at 12.00 and 16.00 h. Pollen gatherers out numbered nectar collectors. Honey bees showed significant difference between movements. Male to male parental line movement was higher than other movements. *Apis mellifera* spent more time (57.26 sec) per flower followed by *Apis dorsata* (49.03 sec), *Apis cerana indica* (40.65 sec) and *Apis florea* (38.83 sec). The time spent by *Apis cerana indica* and *Apis florea* was on par with each other, whereas it had significant difference between *Apis mellifera* and *Apis dorsata*. Nectar content (in µl) and Nectar sugar content (in mg) were more in male parental line than in female parental line. Nectar content was higher at 8.00 h, whereas the least nectar content was recorded at 14.00 h. Nectar sugar was the least at 8.00 h and highest at 12.00 and 14.00 h.

Keywords: Floral biology, honeybee behavior, bottle gourd, *Lagenaria siceraria*, nectar, pollen

Bottlegourd (*Lagenaria siceraria* M.), an important vegetable crop is grown for its immature fruits. Its uses and nutritive values and other uses are described in detail [1-2]. It is used for culinary purposes and in preparation of different types of sweets in India. This crop is commercially cultivated in the Indo-gangetic plains of northern India as a summer crop and is cultivated in plains, lower hills and plateau regions mainly in Uttar Pradesh, Punjab, Gujarat, Assam, Meghalaya and Rajasthan. Bottlegourd is grown in around 4 lakh ha area that comprises 30% of the total world area [3].

A new hybrid, Pusa Hybrid-3 was developed with better performance over the earlier released variety/hybrids. This hybrid can be cultivated both in spring-summer and *kharif* seasons. The demand for hybrid bottlegourd seed is very high as hybrids are more profitable and nutritious with 40-45% higher fruit yield than open pollinated varieties. Bottlegourd is monoecious, cross-pollinated crop with male and female flowers borne separately on the same plant. For successful fruit set, honeybee pollination is needed for transfer of pollen from male line to female line. Effective pollination and fertilization are the prime pre-requisites for bumper harvest in bottlegourd. Earlier, behavior of pollen gatherers of *Apis cerana indica* and *Apis mellifera* in cauliflower was studied [4]. Among pollinators, *Apis* species constituted 79.0 to 83.3% in cauliflower during a three year study [5]. In another study, it was found that proportion of bee foraging for pollen on cauliflower varied from 3.8 to 23.3% of the population during the day and pollen was mostly collected in afternoon [6]. Among different honeybees, *Apis cerana indica, Apis dorsata, Apis mellifera* constituted 8, 33.2 and 30.3%, respectively in cauliflower [7]. Similar

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observations were also made by some other workers [8-10].

In cucumber, the dependency of fruit set on insect pollination is well established. This is exemplified by monoecious flowering condition in which both male and female flowers are borne on the same plant. It was reported that the percentage of marketable melons increased with the number of pollinator visit a flower received (up to about 10 visits) but additional visit made little difference [11].

The presence of sticky pollen grain and adhesive stigma further demonstrate the need for active transfer of pollen from male to female flower. Anthesis and production of high grade nectar reach their highest to coincide with anther dehiscence (pollen shedding) and increased honey bee visit. It was observed that pistillate flowers produce 1.5 and 2.3 times more nectar than staminate flowers but due to higher concentration of sugar in staminate flowers, more honeybees were attracted towards the staminate flowers [12-13]. Similar observations were also reported by others [14-16].

The synchrony of bee visit with optimum stigma receptivity increases the fruit set. Foraging behavior of honeybees is influenced by weather conditions, like temperature, sunshine hours and relative humidity. In this background, the present investigation was carried out to understand the floral biology and foraging behavior of honeybees on parental lines of hybrid bottle gourd Pusa hybrid-3.

MATERIAL AND METHODS

The experiment was conducted during *spring* and *kharif* seasons at the Indian Agricultural Research Institute, New Delhi located at 2804 North latitude and 7701 East longitude at an altitude of 216 m above mean sea level. The climate of this region is sub-tropical with mean temperature ranging between 34-39^{0}C in May and June and 32-35^{0}C in August and September months. Average rainfall is around 745 mm. Parental lines of Pusa hybrid-3 were used in the present research work. In *spring season* crop, was sown in middle of February and in *kharif* season crop was sown in first fortnight of July. In both the seasons, female and male lines were sown with the planting ratio of 4:1, 3:1 and 2:1. All the planting ratios were separated from each other by 600 m to avoid out-crossing. The observations were recorded on floral biology and activity of honey bees.

Floral biology

Nectar quantity and dry nectar- sugar content were measured at 8.00, 10.00, 12.00, 14.00, 16.00 and 18.00h. Nectar production in freshly opened flower at each mentioned hour was measured (in µl per flower) with the help of micropipettes (one lambda capacity). To estimate the dry nectar-sugar content (mg per flower), freshly opened flowers were put in to the separate glass vial containing 5 ml distilled water. After thorough shaking, the vials with nectar dissolved in water (NDW) were kept in a refrigerator. The NDW was analyzed for dry nectar sugar as per Dubois method (17). Nectar-sugar content (mg/ flower) was calculated with the help of standard curve prepared from the known concentrations of glucose.

Stigma receptivity

Stigma receptivity was determined by selectively hand pollinating the plants before anthesis at different hours and observing for the fruit set. Number of fruits set was recorded 6 to 8 days after pollination in each case and the percentage of fruit set was calculated as number of fruit set out of total number of flowers pollinated multiplied by 100.

Bee abundance

Frequency of bee visit was recorded for two minutes at two hourly intervals starting from 8 AM to 6 PM *i.e.* at 8.00, 10.00, 12.00, 14.00, 16.00 and 18.00 h. Bee visit was recorded on five randomly selected plants from male and

female parents at different intervals. Total pollinators were classified into two groups *viz.* honey bee and others. Bees with pollen load on its body were recorded as pollen gatherer (P) and without pollens were considered as nectar collector (N). Such observations were taken for four days (as four replications) during peak flowering period.

Movement of Honeybee

Movement of honey bee for pollen as well as nectar on male and female parental lines was observed individually. From female lines, male flower was pinched off regularly during flowering period.

The movement of bees was classified in to four categories *viz.*, male to male (M_1), male to female (M_2), female to male (M_3) and female to female (M_4). Five bees from each *Apis* species and other pollinating insects were observed for two minute everyday for 4 days in each planting ratio during peak hour (12.00 - 14.00 h). The percentage of pollen gathering (P) or nectar collecting (N) or total P+N honeybees showing a particular movement was calculated using the under mentioned formula:

$$P = [P_1 / (P_1 + P_2 + P_3 + P_4)] \times 100$$
$$N = [N_1 / (N_1 + N_2 + N_3 + N_4)] \times 100$$

Where, P_1, P_2, P_3 and P_4 are the number of pollen gathering bees in M_1, M_2, M_3 and M_4, respectively.

Time spent by honeybee on flower

Time spent (in seconds) by a bee from its first landing on a flower till it flies away after foraging was recorded with the help of a stop watch. These observations were made at 2 h intervals viz. 800, 1000, 1200, 1600, and 1800 h. In each period 5 plants were selected and observed for 2 minute to note time spent by pollinating insect.

RESULTS AND DISCUSSION

Floral biology

i. Nectar content

In both the years, nectar content was recorded more in male than female parent. It was 2.05 and 1.90 µl in male flowers whereas in female flower the nectar content was 1.83 and 1.71 µl per flower in first and second year, respectively. It was also observed that, nectar content in *Kharif* season was significantly higher (2.11 and 1.96 µl), than *spring-summer* season (1.77 and 1.64 µl). The highest and least amount of nectar content was recorded at 0800 and 1400 h, respectively.

Interaction between sowing seasons and parental lines in both the years was found to vary significantly (Table 1). By and large, nectar content (µl) was more (2.22 and 2.00 µl) in *Kharif* than spring summer season (1.88 and 1.66 µl)of male and female flowers. In the second year, nectar content recorded in male flower (1.73 µl in *spring summer* and 2.07 µl in *Kharif*) was significantly more than female flowers (1.56 µl in *spring summer* and 1.86 µl in *Kharif*). Significant interaction also existed

Table 1. Nectar content in parental lines of bottle gourd Pusa hybrid-3 in different seasons and hours of the day

Factors	*Spring-summer*	*kharif*	*Spring-summer*	*kharif*
Parental line				
Female	1.66	2.00	1.56	1.86
Male	1.88	2.22	1.73	2.07
SEm±	0.06	0.04		
CD (p=0.05)	0.20	0.14		
Hour (h) of the day				
8.00	2.52	2.70	2.39	2.59
10.00	1.96	2.20	1.82	2.04
12.00	1.29	1.65	1.00	1.50
14.00	0.82	1.30	0.74	1.15
16.00	2.08	2.57	2.08	2.39
18.00	1.97	2.30	1.87	2.14
SEm±	0.14		0.12	
CD (p=0.05)	0.43		0.38	

between sowing season and period of day with respect to nectar content. In both the seasons, nectar content was the highest at 0800h. In *kharif* (first year), more nectar was recorded 0800 h (2.70 µl), followed by 16 00 h (2.57 µl), 18 00 h (2.30 µl), 10 00 h (2.2 µl), 12 00 h (1.65 µl) and 14 00 h (1.30 µl).

Similar trend was also observed in *spring-summer* season of the consecutive year also. In 1st year, nectar content recorded at 8.00 h was significantly higher (2.52 µl) than nectar content recorded at 10.00 (1.96 µl), 12.00 (1.29 µl), 14.00 (0.82 µl), 16.00 (2.08 µl) and 18.00 h (1.97 µl). The trend was similar with the observations recorded in the crop sown in second year. Nectar content in *kharif*, at 8.00 h was significantly higher (2.59 µl) than the nectar content recorded in *spring-summer* at 8 00 h (2.39 µl) and the results were similar for observed nectar content at different hours as in the 1st year (Table 1). Earlier, it was reported that nectar sugar content in cauliflower was less at morning and it gradually increased in the noon [18-19].

ii. Nectar-sugar content

In case of sugar content, there was no significant difference among parental lines, sowing seasons and hours of the day in both the years.

On yearly basis, nectar and nectar sugar content varied slightly between parental lines. Overall, male parents had higher content of both nectar and sugar (Table 2 and Fig. 1).

i. Stigma receptivity

Stigma receptivity was found to vary at different periods of starvation, seasons and their interaction in both the years. Season (*Kharif* and *Spring summer*) data means (14.74, 12.92 fruit set per 30 flowers) showed significant difference in the first year. In the second year also, season means (13.40, 11.12 fruit set per 30 flowers) showed significant difference from one another. Starvation period also recorded significant difference with each other in both the years. The number of fruit set was higher on the day of anthesis of flower followed by 24 hour after anthesis of flower, 24 hour before anthesis of flower and 48 hour after anthesis of flower in both the seasons (Table 3). This contradicts one earlier report [20], wherein it was observed that stigma became receptive 36 hours before anthesis and declined 48 hours after anthesis.

Honeybee activity

Among total insect pollinators, *Apis* species constituted 89.15 and 86.28% in first and second year, respectively. Pollen gatherers were 57.46 and 52.25% for the reported years, respectively. Earlier, honeybees were reported as major pollinating insects in entomophilous vegetable seed crops like cauliflower [5, 7, 21]. By and large, nectar collectors were less in number as compared to pollen gatherers. They constituted 42.54 and 47.75% of the bees, respectively in both the years. Mean number of pollen gatherers per plant per two minutes was significantly higher (5.78 and 5.13) than nectar collectors (4.28 and 4.69) in both the years. Thus, proportion (57%) of pollen gatherers was more as compared to nectar collectors (47%). In general, mean number of honey bee visiting/ plant/ two minutes was higher in *kharif* than *spring-summer* seasons. In first and second year, an average of 5.27 and 5.18

Table 2. Interaction of parental lines of bottle gourd with hours of the day and nectar content per flower

Hour (h) of day	Nectar content		Nectar sugar content	
	Female	Male	Female	Male
8.00	2.44	2.67	0.28	0.35
10.00	1.83	2.16	0.43	0.54
12.00	1.24	1.49	0.64	0.74
14.00	0.94	1.07	0.89	0.95
16.00	2.21	2.36	0.57	0.69
18.00	1.99	2.15	0.36	0.48

honeybees visited crop in *kharif* season, while in *spring-summer* season, it was 4.79 and 4.64 honey bee/plant/2 minutes. There was no significant difference in honey bee visit between different hours of the day (Table 1). However, more pollen gatherers visiting at 1400 h followed by 1200 and 1600 h in cauliflower [22]. Mean number of honey bees visiting per plant/two-minute on parental lines showed significant difference in the first year. It was also observed that honey bee visit was more on male parent (5.12) than on female (4.94). In the second year, honey bee visit on both the parental lines was on at par (Table 1). This report was in conformity with the earlier finding [16], wherein it was reported that honeybees were attracted more towards male flower due to high sugar concentration than female flower in cucumber.

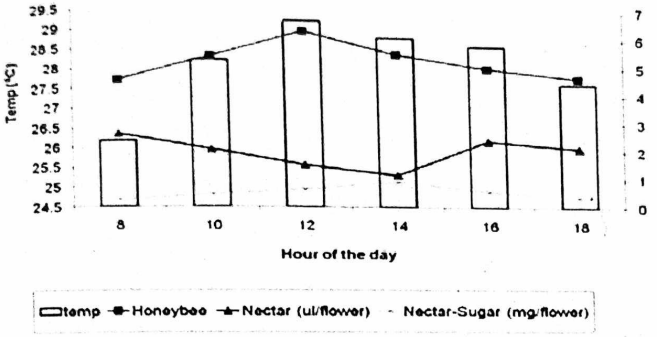

Fig 1. Nectar content (µl) and nectar -sugar content (mg/flower) at different hours of the day against temperature.

Significant differences were observed between different *Apis* species visiting the seed plots which with *Apis mellifera* was the most abundant (6.41) pollinator followed by *Apis dorsata* (5.80), *Apis cerana indica* (4.51) and *Apis florea* (3.40) in first year (Fig. 2). The same trend was observed in the second year also with respect to different species visiting the seed plots. *Apis mellifera* was second highest bee visiting cauliflower followed by *Apis dorsata* in cauliflower crop [22]. This showed

Table 3. Effect of different sowing season on stigma receptivity of female parent of Pusa Hybrid 3

Factors	Starvation period (hour) Number of fruit set/30 flowers				
	24 h BOF	24 h AOF	48 h AOF	72 h AOF	Mean
Sowing season					
Year I					
Spring	10.14	28.3	18.36	2.19	14.74
kharif	8.25	26.41	15.28	1.756	12.92
Mean	9.19	27.35	17.02	1.97	
CD (p=0.05)					
Sowing season (s)	1.07				
Starvation period (H)	2.15				
S x H	3.05				
Year II					
Spring	8.71	25.85	17.48	1.56	13.4
kharif	5.1	24.76	13.26	1.38	11.12
Mean	6.9	25.3	15.37	1.47	
CD (p=0.05)					
Sowing season (s)			1.57		
Starvation period (H)			1.6		
S x H			2.26		

that bee behavior may change from one crop to another.

Movement of honeybees between two parental lines is possible in four combinations *viz.* Male to male (M_1), Male to female (M_2), Female to male (M_3) and Female to female (M_4). Among all such movements, M_2 was the most important from pollination point of view. In first year, only M_1 and M_2 had shown significant differences with M4 movement. In second year, all the movements were at par with each other. This indicated that honey bees do not discriminate between parental lines. The reports are in accordance with the earlier finding [22].

Female to female (M_4) movement as pollen collector also plays crucial role in

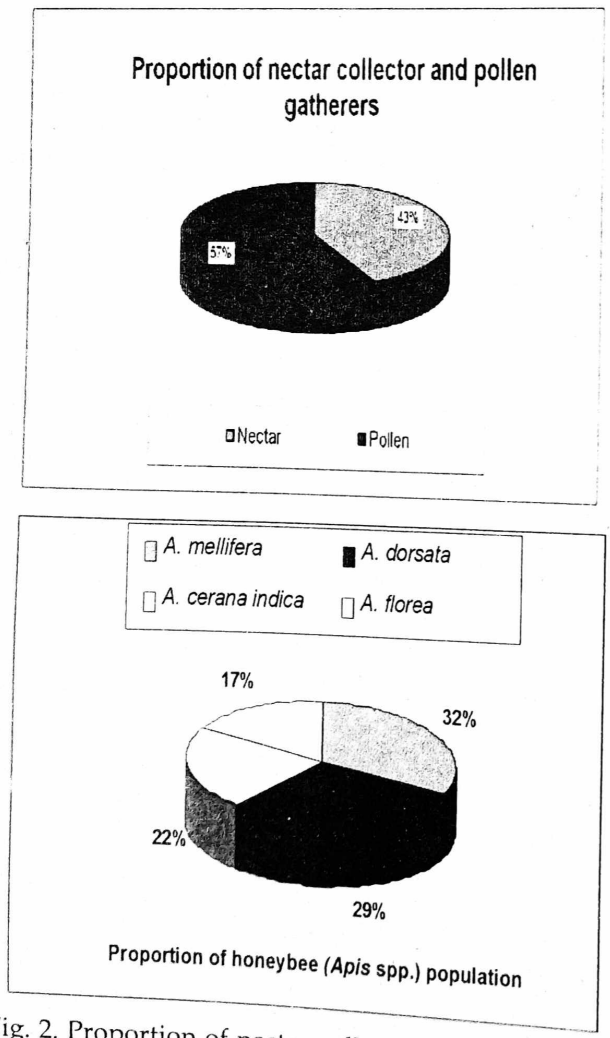

Fig. 2. Proportion of nectar collector/pollen gatherers and honey bee species visiting the flower in bottle gourd.

Table 4. Number of honeybees (per two minutes per plant) visiting parental lines of bottlegourd Pusa Hybrid-3

Factor	Year I	Year II
Foraging mode		
Nectar gatherer	4.28 (2.18)	4.69 (2.27)
Pollen collector	5.78 (2.50)	5.13 (2.37)
SEm±	0.02	0.02
CD (p=0.05)	0.06	0.05
Sowing season		
Spring	4.79 ()2.30	4.64 (2.26)
Kharif	5.27 (2.40)	5.18 (2.38)
Sem±	0.01	0.03
CD (p=0.05)	0.04	0.06
Hour (h) of the day		
8.00	4.75 (2.29)	4.49 (2.23)
10.00	5.09 (2.36)	5.02 (2.34)
12.00	5.6 (2.46)	5.68 (2.36)
14.00	5.05 (2.35)	4.79 (2.30)
16.00	5.13 (2.37)	4.92 (2.32)
18.00 h	4.54 ()2.24	4.55 (2.24)
CD (p=0.05)	NS	NS
Parental line		
Female	4.94 (2.33)	4.87 (2.31)
Male	5.12 (2.37)	4.95 (2.33)
Sem±	0.01	0.03
CD (p=0.05)	0.03	NS
Honey bee		
A. mellifera	6.41 (2.62)	6.20 (2.58)
A. dorsata	5.80 (2.50)	5.95 (2.53)
A. cerana indica	4.51 (2.23)	4.34 (2.20)
A. florea	3.40 1.97	3.15 (1.91)
Sem±	0.04	0.03
CD (p=0.05)	0.94	0.10

Figures in parenthesis are the transformed [Square root (X+0.5)]

Table 5. Time spent (in seconds per plant) by a honeybee visiting seed production plot of bottlegourd Pusa Hybrid-3

Factor	1st year	2nd year
	Time spent (in seconds per plant)	
Hour (h)	1st year	2nd year
8.00	41.96	39.50
10.00	47.38	44.44
12.00	52.31	49.33
14.00	48.23	45.53
16.00	47.44	45.40
18.00	41.34	39.67
SEm±	2.50	2.62
CD (p=0.05)	5.36	5.50
Honey bee		
A. mellifera	57.26	52.86
A. dorsata	49.03	45.65
A. ceranaindica	40.65	39.02
A. florea	38.83	37.41
SEm±	1.54	1.39
CD (p=0.05)	4.63	4.37
Sowing season		
Spring	43.85	44.53
Kharif	49.04	47.48
Sem±	0.76	0.69
CD (p=0.05)	2.41	2.15

transferring pollen which was collected from male flowers in earlier visits. These results explain that pollen dispersal is not only from male to female but it is also possible from female to female line. Such observations were also made by other workers [23].

Time spent (in seconds) by honeybee

In both the years, time spent per plant was more at 1200 h. In the first year, time spent at 0800 h (41.96 sec) and 1800 h (41.34 sec) were at par with each other. The time spent at different hours was 1000 h (47.38 sec), 1200 h (52.31 sec), 1400 h (48.23 sec) and 1600 h (47.44 sec). Similar trend was recorded in the second year. *Apis mellifera* spent more time (57.26 sec) followed by *Apis dorsata* (49.03 sec), *Apis cerana indica* (40.65 sec) and *Apis florea* (38.83 sec) in both the years (Table 5). The time spent by *Apis mellifera* and *Apis dorsata* was significantly more than *Apis cerana indica* and *Apis florea*, which were at par with each other. Among the seasons, time spent was significantly more in *kharif* season. It was 49.04 and 47.48 seconds for *kharif* and 43.85 and 44.53 seconds for *spring-summer* in the first and second years, respectively.

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