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Pollination efficiency of important insect visitors on pomegranate (*Punica granatum*) under mid hill conditions of Himachal Pradesh

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ABSTRACT

Insect pollinators are found to be highly significant in enhancing the quality fruit production and productivity. The experiment was conducted during April–May 2020 and 2021 at Dr Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh to determine the pollination indices of significant insect pollinators visiting pomegranate (*Punica granatum* L.). The data on pooled basis from two years revealed that *Bombus haemorrhoidalis* visited most number of flowers during the day (4.96 flowers/min) followed by *Apis mellifera* (4.24), *Apis cerana* (3.95), *Apis dorsata* (3.76) and *Episyrphus balteatus* (3.09). The time spent on each flower was maximum by *A. cerana* (17.10 sec/flower) followed by *E. balteatus* (17.04 sec), *A. dorsata* (14.20 sec), *A. mellifera* (13.07 sec) and *B. haemorrhoidalis* (5.36 sec) irrespective of different day hours. *A. mellifera* (5.43 bees/m^2/2 min) activity was maximum followed by *A. cerana* (5.06), *E. balteatus* (2.91 visitors/m^2/2 min), *B. haemorrhoidalis* (1.72) and *A. dorsata* (1.37). Data on loose pollen grains for the two years revealed that *A. dorsata* adhered maximum number of loose pollen grains (357.00 thousand) followed by *B. haemorrhoidalis* (283.00 thousand), *A. mellifera* (98.00 thousand), *A. cerana* (41.00 thousand) and solitary bees (28.00 thousand). *A. mellifera* scored highest pollination index and *A. dorsata* scored least pollination index. *A. mellifera*, *A. cerana* and *B. haemorrhoidalis* are the primary pollinators that increase pomegranate pollination efficiency whereas *A. dorsata*, *E. baltaeatus* and solitary bees are supplementary insects improving pollination in various crops including pomegranate. *A. mellifera*, *A. cerana*, *B. haemorrhoidalis* and *A. dorsata* were main foragers of pomegranate flowers ensuring effective and efficient pollination.

Keywords: *Apis mellifera*, *Apis cerana*, Foraging behaviour, Pollination, Pomegranate

Pomegranate (*Punica granatum* L.) is an economically significant fruit crop of the tropical as well as subtropical area (Jalikop 2003). It is the member of family *Punicaceae* and genus *Punica* (Chatterjee and Randhawa 1952). It is a vital export-oriented crop of Indian subcontinent (Chandra and Jadhav 2008). According to Meena (2018) pomegranate tree needs hot and dry climate having temperature of 38°C amid the period of fruit development and ripening and is a cross pollinated crop (Nalawadi *et al.* 1973). Because of the heaviness of the pollen in pomegranate, there may be little or no wind dispersal of the pollen and as a result the bugs are primarily liable for the shipping of pollen among the flowers (Halder *et al.* 2019). Pollination by bee is ought to enhance the fruit setting and an appreciable increase in pomegranate fruit weight in comparison to natural pollination (Derin and Eti 2001, Tao *et al.* 2010). Visit rate by bees was higher at 21ºC, but worse on rainy, cloudy or foggy days. The feeding of bees increased at 1200 h. Bee pollinized fruits are large in diameter, deep red, juicy, very delicious and full of juice, and have the highest sweetness, followed by naturally pollinated fruits. According to Halder *et al*. (2019), cross pollination in pomegranate was mainly done by *Apis* spp., *Camponotus* spp., and *Papilio* spp. When devouring the flowers, beetles from the genera *Cetonia* and *Trichodes* cross-pollinate and self-pollinate the flowers. It is unclear if the plant will self-pollinate or whether it needs pollen transfer inside its own flower, between flowers, or between plants. There is a lack of quantitative information with respect to contribution of honey bees in pomegranate pollination (Haldhar *et al.* 2018). There is lack of data regarding pollination efficiency of various insect visitors on pomegranate so the current study was planned to investigate the pollination efficiency of *Apis mellifera*, *Apis cerana*, *Bombus haemorrhoidalis*, *Apis dorsata*, *Episyrphus balteatus* with respect to their relative abundance, foraging rate and speed, and loose pollen grains adhering upon their body.

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MATERIALS AND METHODS

The experiment was conducted during April–May of 2020–21 and 2021–22 at Dr Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh (located 30.86˚ N, 77.16˚ E and at an elevation of 1300 m amsl). The study was carried out using a factorial randomized block design (F-RBD) with the following 5 treatments, viz. *Apis cerana*, *Apis mellifera, Bombus haemorrhoidalis*, *Apis Dorsata* and *Episyrphus balteatus* and 3 replications (at different day hours). The treatments were compared at 5% level of significance. The important inects visitor in pomegranate are given in Supplementary Fig. 1.

Foraging rate: Foraging rate of *A. mellifera, A. cerana*, *B. haemorrhoidalis*, *A. dorsata*, *E. balteatus* were recorded by counting the number of flowers visited by the insect/min, by using stop watch. Three replications were taken at 1000, 1200 and 1500 h. Accordingly, the observations were recorded for 15 days at full flower bloom stage.

Foraging speed: Foraging speed of *Apis* spp., *B. haemorrhoidalis*, *A. dorsata*, *E. balteatus* were recorded as time spent on flower by the insect visitors using stop watch and 3 replications were there at 1000, 1200 and 1500 h. The observations were recorded for 15 days at full flowering stage. Plants of similar canopy size and vigour were selected for observations on relative abundance of pollinators.

Relative abundance: The study of the insect pollinator abundance on flowers was recorded by counting number of insects/2 min/m^2 of flower bloom at 1000, 1200, and 1500 h and were replicated thrice using stopwatch during full flower bloom for 10 days.

Loose pollen grains: For counting loose pollen grains, individuals were seized with a forceps and preserved in 70% alcohol in 5 ml glass vials after amputating the legs of the forager. The vials were then shaked and rinsed to dislodge the pollen. From the supernatant, 0.02 ml aliquot was taken for the count of the pollens by haemocytometer and observed under microscope (lab experiment). Efficiency in pollination was assessed through relative abundance of insect pollinators and their foraging behaviour. The rankings were determined by statistical analysis of the data. Values that differed considerably were assigned different scores. Mean efficiency rating that was obtained was multiplied by average pollinator (average number of pollinator/m^2/2 min) during a day

Pollination index was calculated as per Bohart and Nye (1960) In the current investigation pollination indices were calculated only for highly frequent visitors which included *A. cerana*, *A. mellifera, B. haemorrhoidalis* and *A. dorsata*.

RESULTS AND DISCUSSION

Foraging rate: In the year 2020, the results dipicted that irrespective of different hours *Bombus haemorrhoidalis* and *A. mellifera* visited more flowers in 1 min i.e. 5.03 and 4.23, respectively followed by *Apis cerana* (3.93), *Apis dorsata* (3.73) and *Episyrphus balteatus* (3.03). *A. cerana* and *mellifera* visited more flowers in a minute at 1500 h (5.62 and 5.43, respectively) than at 1000 h (3.82 and 4.77, respectively). *E. balteatus* and *B. haemorrhoidalis* was more active at 1000 h (4.05 and 7.28, respectively) than at 1500 (3.05 and 3.38, respectively) and 1200 h (1.98 and 4.42, respectively) which can be validated by earlier research done by Bakshi *et al*. (2018) who stated that syrphid (*Episyrphus balteatus*) visited maximum flowers at 1000 h (4.47) and least at 1500 h (3.03). This might be attributed to low floral reward such as nectar sugar during evening hours which resulted in lesser time on each inflorescence and more number of flower visit in a minute. Regardless of the time of day, *A. cerana* (3.93 number of flowers visited/min) was statistically on par with *A. dorsata* (3.73). *A. dorsata* visited maximum flowers at 1500 h (4.65) followed by 1000 h and 1200 h (2.77). During 2021, *B. haemorrhoidalis* (4.89) visited maximum flowers that were followed by *A. mellifera* (4.16), *A. cerana* (3.97) and *A. dorsata* (3.78) and *E. balteatus* (3.16) at all the day hours in study. The present investigation revealed that foraging rate was maximum at 1000 h (4.73) than at 1500 h (4.46) and 1200 h (2.78) for all insect visitors. The above results are in accordance with Yankit (2016) who recorded the maximum rate of foraging in morning and minimum during noon on tomato. Similarly, pooled data indicated that foraging rate (irrespective of day hours) was maximum for *B. haemorrhoidalis* (4.96) (Fig. 1). The maximum and minimum number of flowers visited by the bumble bee was at 1000 h and 1500 h, respectively. The present studies on foraging rate in context with *Bombus haemorrhoidalis* were confirmed by Nayak *et al*. (2019) who investigated

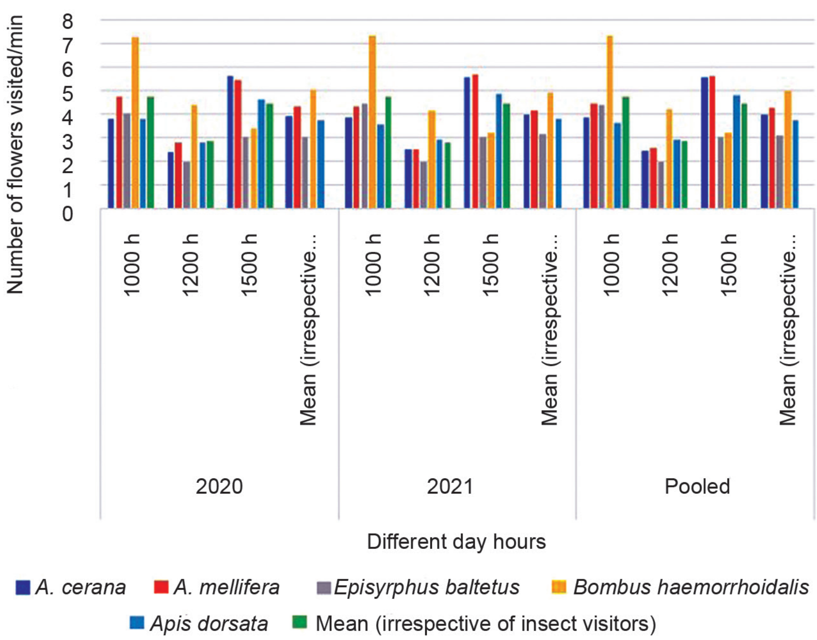

Fig. 1 Foraging rate (number of flowers visited/minute) of insect visitors on pomegranate.

that in kiwi fruit inside cage conditions with one and two colonies of bumble bees the foraging rate had a peak value at 1000–1200 h (7.60 flowers/min and 7.84 flowers/min, respectively) whereas the minimum rate of foraging was during 1200–1400 h (2.11 flowers/min and 2.29 flowers/min, respectively).

Foraging speed: During 2020, observations on the speed of foraging by different insect visitors were recorded which showed that irrespective of the day hours, *A. cerana* (17.09 sec) spent significantly more time on each flower followed by *E. balteatus* (16.79 sec), *A. dorsata* (14.18 sec), *A. mellifera* (13.04 sec) and *B. haemorrhoidalis* (5.41 sec) (Fig. 2). Irrespective of different day hours, *A. cerana* (17.09) was statistically at par with *E. balteatus* (16.79). Irrespective of the insect visitors, foraging speed was maximum at 1200 h (16.08 sec.) followed by 1000 h (12.52 sec) and 1500 h (11.31 sec). *E. balteatus* and *A. dorsata* spent more time per bloom at 1200 h (21.65 sec and 14.72 sec, respectively). Foraging speed of *B. haemorrhoidalis* was maximum at 1000 h (6.38 sec) and least at 1500 h (3.98 sec). The present investigations in context with *B. haemorrhoidalis* were confirmed by Nayak *et al*. (2019) who found that *B. haemorrhoidalis* spend a maximum of 7.82 and 8.02 sec/flower at 0800–1000 h and a minimum of 3.42 and 3.62 sec/flower at 1200–1400 h on kiwifruit with one and two colonies of bumble bee placed inside cage, respectively. During 2021, data on foraging speed showed the dominance of *E. balteatus* (17.30 sec) as it spent maximum time/flower (irrespective of different day hours) followed by *A. cerana* (17.12 sec), *A. dorsata* (14.24 sec), *A. mellifera* (13.11 sec) and *B. haemorrhoidalis* (5.31) (Fig. 2). Pooled data on foraging speed showed that *A. cerana* (17.10 sec) spent significantly more time per bloom compared to other insect visitors (Fig. 2). The above findings are in line with Bakshi *et al*. (2018), who reported that foraging speed of *A. cerana* (10.7 sec) was significantly more when compared with other insect visitors on cherry. Foraging speed of *A. mellifera* at 1200 h was 15.15 sec/flower which was more as compared to 1000 h (12.10 sec) and 1500 h (11.96 sec). Studies on foraging speed in the present investigation are in conformity with Nayak *et al*. (2019) who delineated that the speed of foraging of *A. mellifera* was the most in1000–1200h (11.50 sec/flower) and least (9.09 sec/flower) during 0600–0800 h. The present findings are similar to the foraging speed investigations in tomato by Yankit (2016). According to which the time spent in tomato flower was maximum (6.82 sec/flower) at 0800–0900 h. Comparatively higher foraging speed at 1200 h might have been due to more nectar sugar in each flower during 1200–1300 h due to which bees took more time to collect the forage

Relative abundance of insects: Data on relative abundance of insect pollinators during 2020 showed that irrespective of different day hours, *A. mellifera* (5.41 bees/m²/2 min) was the most relatively abundant insect pollinator followed by *A. cerana* (5.09 bees/m²/2 min), *E. balteatus* (2.89 insect visitors/m²/2 min), *B. haemorrhoidalis* (1.70 bees/m²/2 min) and *A. dorsata* (1.38 bees/m²/2 min) (Table 1). Significantly more number of insects visited the pomegranate flowers at 1000 h (4.26 insect visitors) than 1200 h (3.08 insect visitors) and 1500 h (2.55 insect visitors). *E. balteatus* peaked its activity at 1000 h (3.73 insect visitors /m²/2 min) and was least active at 1200 h (1.83 insect visitors). *B. haemorrhoidalis* was observed in abundance at 1500 h (2.90 bees) and is least active at 1200 h (0.60 bees). The dominance of *B. haemorrhoidalis* during 1500–1600 h gets support from the finding of Nayak *et al*. (2019) who recorded maximum abundance of *B. haemorrhoidalis* during 1400–1600 h and 1600–1800 h. *A. dorsata* was another insect visitor whose population was maximum at 1200 h (2.16 insect visitors) and least at 1500 h (0.57 insect visitors). Similarly, during 2021, *Apis mellifera* (5.45 bees/m²/2 min) and *Apis cerana* (5.09 bees/sq m/2 min) were the most abundant pollinating visitors and *A. dorsata* (1.37 insect visitors) was the least abundant (Table 1). At 1000 h (4.27 insect visitors) significantly more insect visitors visited pomegranate flower when compared to 1200 h (3.12 insect visitors) and 1500 h (2.53 insect visitors). Pooled data of relative abundance of insect visitors showed that *A. mellifera* (5.43 bees/m²/2 min) was most abundantly recorded insect visitor at all hours of the day and *A. dorsata* (1.37 insect visitors) was least abundant (Table 1). The abundance of insect visitors turned maximum in morning hours (1000–1100 h) compared to afternoon and evening hours. The investigations on relative abundance gets support from the findings of earlier workers (Neelema and Kumar

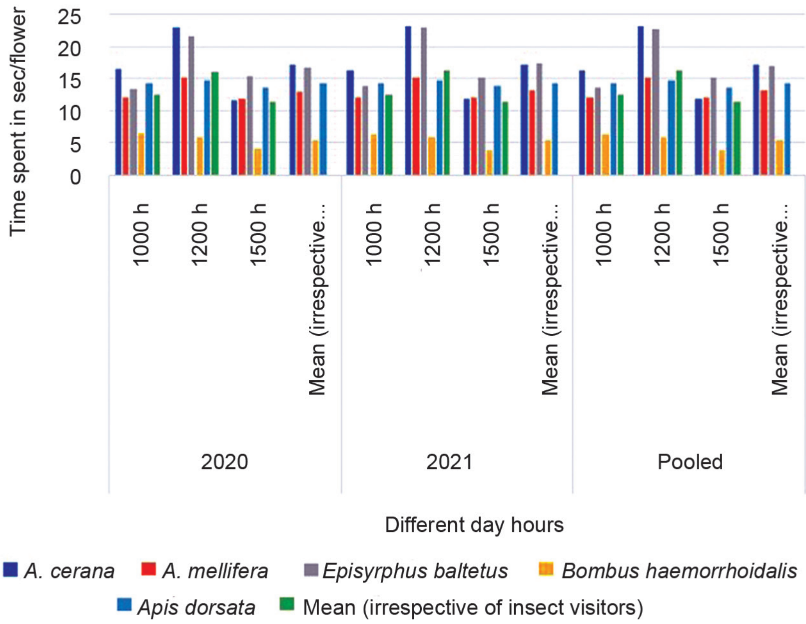

Fig. 2 Foraging speed (time spent in sec/flower) of insect visitors on pomegranate flowers.

Table 1 Relative abundance of insect visitors on pomegranate

Insect visitors	Activity of insect visitors (number/m^2/2 min)											
	2020				2021				Pooled			
	1000 h	1200 h	1500 h	Mean	1000 h	1200 h	1500 h	Mean	1000 h	1200 h	1500 h	Mean
Apis cerana	7.43 (2.90)*	4.93 (2.44)	2.93 (1.98)	5.09 (2.44)^d	7.36 (2.89)*	5.00 (2.39)	2.90 (1.97)	5.09 (2.42)^d	7.40 (2.89)*	4.85 (2.42)	2.92 (1.98)	5.06 (2.43)^d
Apis mellifera	7.16 (2.85)	5.86 (2.62)	3.20 (2.05)	5.41 (2.51)^d	7.23 (2.86)	5.96 (2.63)	3.16 (2.04)	5.45 (2.51)^e	7.20 (2.86)	5.92 (2.63)	3.18 (2.05)	5.43 (2.51)^e
Episyrphus balteatus	3.73 (2.17)	1.83 (1.68)	3.13 (2.03)	2.89 (1.96)^c	3.63 (2.15)	1.80 (1.67)	3.33 (2.07)	2.92 (1.96)^c	3.68 (2.16)	1.82 (1.68)	3.22 (2.05)	2.91 (1.96)^c
Bombus haemorrhoidalis	1.60 (1.61)	0.60 (1.26)	2.90 (1.97)	1.70 (1.61)^b	1.70 (1.64)	0.63 (1.25)	2.80 (1.96)	1.71 (1.62)^b	1.65 (1.62)	0.62 (1.27)	2.88 (1.97)	1.72 (1.62)^b
Apis dorsata	1.40 (1.55)	2.16 (1.78)	0.57 (1.25)	1.38 (1.53)^a	1.43 (1.55)	2.20 (1.78)	0.47 (1.21)	1.37 (1.51)^a	1.42 (1.55)	2.18 (1.78	0.52 (1.23)	1.37 (1.52)^a
Mean	4.26 (2.22)^c	3.08 (1.96)^b	2.55 (1.86)^a		4.27 (2.22)^c	3.12 (1.94)^b	2.53 (1.85)^a		4.27 (2.22)^c	3.08 (1.96)^b	2.54 (1.86)^a	
CD (*P*=0.05)	Insects (0.07), Day hours (0.05), Insects × Day hours (0.13)				Insects (0.08), Day hours (0.06), Insects × Day hours (0.14)				Insects (0.05), Day hours (0.04), Insects × Day hours (0.09), Insects × Years × Day hours (NS)			

*Figures in the parentheses are $\sqrt{x+1}$ transformed values.

1997, Sharma *et al*. 2000, Dev 2010) who've additionally discovered greater activity of *Apis mellifera* and *A. cerana* during the morning hours. However, the existing findings differed from the investigations of Dashad (1989) who has mentioned greater insect visitors to apple bloom during 1200–1300 h than 0900–1000 and 1500–1600 h. These variations could be because of the fact that the timing of observation varied for both the studies.

Loose pollen grains: Studies showed that *A. dorsata* had significantly more pollen (357.00 thousand) adhered to hairy body in comparison to the other insect visitors (Table 2). It was followed by *B. haemorrhoidalis* (283.00 thousand). The least pollen was recorded on solitary bees (28.00 thousand). *A. mellifera* had significantly more number of loose pollen grains (98.00 thousand) attached to its body than *A. cerana* (41.00 thousand). In similar investigations by Sharma and Rana (2000) in cherry *A. mellifera* held more loose pollen grains which ranged from 1225–2230 whereas in *Apis cerana* it ranged from 1450–1913. During 2020, *A. cerana* (38.00 thousand) was statistically at par with *A. mellifera* (92.00) and solitary bees (27.00) whereas, *A. dorsata* (360.00) which carried maximum loose pollen grains was statistically at par with *B. haemorrhoidalis* (296.00). During 2021, *A. cerana* (44.00 thousand) was statistically at par with solitary bees (29.00) whereas, *A. dorsata* (354.00) was statistically at par with *B. haemorrhoidalis* (270.00). Pooled data showed that *A. cerana* (41.00 thousand) and solitary bees (28.00) was statistically on par with each other. Free and Williams (1972) reported that the average pollen detected on honey bees gathering pollen and collecting nectar on sweet cherry blossoms were 13,357 and 5790, respectively.

Table 2 Number of loose pollen grains on the body of important insect visitors

Number of loose pollen grains adhering to the body of important insect pollinators			
Insect visitors	Values in thousand (000)		pooled
	2020	2021	
A. mellifera	92.00 (09.30)*^{b}	104.00 (09.80)^b	98.00 (09.55)^b
A. cerana	38.00 (05.92)^{ab}	44.00 (06.54)^a	41.00 (06.23)^a
B. Haemorrhoidalis	296.00 (16.98)^c	270.00 (16.33)^c	283.00 (16.66)^c
A. dorsata	360.00 (18.97)^c	354.00 (18.79)^c	357.00 (18.88)^d
Solitary bees	27.00 (05.17)^a	29.00 (05.39)^a	28.00 (05.28)^a
CD (*P*=0.05)	3.45	3.15	2.11

*Figures in the parenthesis are $\sqrt{x+1}$ transformed values.

Pollination index: *Apis mellifera* had the highest pollination index (12.67) followed by *A. cerana* (10.96), *B. haemorrhoidalis* (4.87) and *A. dorsata* (3.65) on pomegranate bloom (Table 3). Bohart and Nye (1960) and Bohart *et al*. (1970) allocated an efficiency rating for insect visitors based on pollen carrying capacities. This factor was further merged with size, hairiness and insect activity pattern. The present results are partially in accordance with Dashad (1989) and Sharma and Gupta (1993) who reported higher pollination index for *A. cerana* on apple. Similar to their observation the determining factor for maximum pollination index of *A. mellifera* and *A. cerana* was their high population. The studies showed that in addition to *A. mellifera* and *A. cerana*, *B. haemorrhoidalis* and *A. dorsata* also played significant role in pomegranate pollination.

Table 3 Pollination index of important pollinators of pomegranate

Pollinators	Foraging rate (number of flowers visited/min)	Foraging speed (time spent in sec/ flower)	Loose pollen grains (number in thousand)	Relative abundance (number of insects/ m^2/2 min)	Pollination index
Apis cerana	3.95 (1.5)	17.10 (1.5)	41.00 (1)	5.06	10.96
Apis mellifera	4.24 (3)	13.07 (3)	98.00 (2)	5.43	12.67
Bombus haemorrhoidalis	4.96 (4)	5.36 (4)	283.00 (3.5)	1.72	4.87
Apis dorsata	3.76 (1.5)	14.20 (1.5)	354.00 (3.5)	1.37	3.65

*Figures in parantheses are ranks assigned.

Rate of foraging of *A. mellifera* was maximum and foraging speed of *A. cerana* was maximum, this indicated the dominance of hive bees among all other foragers. Pollen carrying capacity of *Bombus haemorrhoidalis* and *A. dorsata* was more than others. Pollination index of *A. mellifera* and *A. cerana* was higher when in comparison to other bees which indicated their importance in pollinating pomegranate. *E. baltaeatus* is crucial for assisting in the pollination of pomegranate.

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