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Effect of insect pollinators on quantitative yield parameters of okra (*Abelmoschus esculentus*) in mid-Himalayan region

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ABSTRACT

Okra [*Abelmoschus esculentus* (L.) Moench] is an often cross-pollinated crop with up to 19–42% of cross pollination assisted by insects and planned pollination may improve the economic fruit yield and biological parameters. The present study was carried out during rainy (*kharif*) seasons of 2021 and 2022 at Research Farm of ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Hawalbagh, Almora, Uttarakhand to assess the pollinator diversity and possible results (both biological and economical) of planned bee pollination. The study on floral visitors of okra recorded 28 insect spp. belonging to four insect orders, among which five spp., viz. *Apis cerana indica* (Fabricius) 1798*, Apis mellifera* (Linnaeus) 1758*, Bombus haemorrhoidalis* (Smith) 1852, *Lithurgus atratus* (Smith) 1853 and *Xylocopa latipes* (Drury) 1773 were predominant. The foraging activity and pollination behaviour showed that two non-*Apis* bee species (*X. latipes* and *B. haemorrhoidalis*) were swift flyers and visited more numbers of flowers per unit time. It was noticed that, peak period of pollinator's visitation was between 9.00–11.00 h accounting to 113.76±7.65 insects/m^2/10 min, during which stigma receptivity and pollen germination were at its peak. Assessment of yield related parameters of insect pollinated flowers showed superior quality fruits with better capsule length (17.4–20.9 cm), capsule girth (6.56–7.84 cm), seeds/capsule (51.4–60.6), test weight of 100-seeds (7.05–8.38 g) and even the seed yield (1.86–3.04 tonnes/hectare) than closed control and hand pollination (emasculated and cross pollinated). In conclusion, ecological engineering of okra fields enhances the pollination rate and ultimately the yield and seed quality.

Keywords: Insect pollinators, Okra, Pollination behaviour, Seed germination, Yield enhancement

Okra [*Abelmoschus esculentus* (L.) Moench] is an important vegetable crop cultivated throughout the world (Azoo *et al.* 2011, Patil *et al.* 2013, Patil 2017). India is the major producer of okra, contributing to 72% of total world production (Angbanyere and Baidoo 2014, Ray *et al.* 2020, Pal 2020). But the productivity per unit area has not seen much improvement over the years in India (Patil 2017). In the year 2010–11, the average productivity of the country was 11.6 tonnes/ha (NHB database 2011), whereas, in the year 2019–2020 the productivity rose to just 12.30 t/ha (Indiastat 2021), which is far lower than the world productivity of 15 t/ha. The reasons for lower productivity of okra in India can be attributed non-availability of quality hybrid seeds, poor seed replacement, practicing of traditional methods of hand emasculation and most importantly failure to explore an efficient commercial seed production system through insect pollination (Brar *et al.* 2020).

Okra produces attractive flowers containing both female and male reproductive organs within the same blossom, rendering it a hermaphroditic and self-compatible plant. (Chandra and Bhatnagar 1975, Al-Ghzawi *et al.* 2003). Even though, self-pollination is a common phenomenon in okra, presence of attractive golden yellow flowers and nectaries attract insect pollinators resulting in some degree of cross pollination (19–42%) (Hasnat *et al.* 2015, Nandhini *et al.* 2018). Several authors have investigated the role of insect pollinators in okra plants and their importance in enhancing the yield and yield related quantitative parameters (Azoo *et al.* 2011, Angbanyere and Baidoo 2014, Hasnat *et al.* 2015, Nandhini *et al.* 2018, Perera and Karunaratne 2019). But not many studies were conducted to evaluate the foraging behaviour and pollination efficiency of different pollinators. In view of this, an experiment was conducted to estimate the pollinator diversity, pollination behaviour and foraging activity of important insect pollinators, yield enhancement by artificial introduction of bees in okra in mid-Himalayas of Uttarakhand, India.

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MATERIALS AND METHODS

Study area and crop cultivation: The present study was carried out during rainy (*kharif*) seasons of 2021 and 2022 at Research Farm of ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Hawalbagh, Almora, Uttarakhand. Okra seeds of variety VL Bhindi-2 were sown in the field of 200 m^2 area at a spacing of 45 cm × 20 cm during May, 2021 [18^{th} standard meteorological week (SMW)] and 2022 (19^{th} SMW). The crop was raised with recommended package of practices (Mahanta *et al.* 2021).

Floral biology studies of VL Bhindi-2: In order to understand the floral biology of the cultivated variety VL Bhindi-2, the following floral characters like, type of flower, colour of the flower, opening and closing time of the flowers, length of the style, number of stamens and the diameter of calyx were analysed for 30 numbers of flowers during peak flowering period (55–60 DAS).

Stigma receptivity and pollen germination studies: To test the time frame of highest pollen germination and stigma receptivity, 30 freshly opened flowers were collected from the fields and carried to laboratory at five-time intervals of the day (7.00–9.00 h, 9.00–11.00 h, 11.00–13.00 h, 13.00–15.00 h and 15.00–17.00 h) during peak flowering period for five consecutive days. Hydrogen peroxide method proposed by Zeisler (1933) and Paschapur *et al.* (2022a) was followed to assess the stigma receptivity. For estimating the pollen germination, the methodology used by Abdelgadir *et al.* (2012) and Paschapur *et al.* (2022a) was followed.

Pollinator density, behaviour and foraging activity: The insect species visiting okra flowers during peak flowering period (55–60 DAS) were recorded from 07.00–17.00 h for 5 consecutive days in 4 plots of 1 m^2 each. *In-situ* method of sampling was followed to collect one representative specimen of each insect visitor using a hand net. The specimens were identified up to species level using taxonomic keys. To calculate the peak period of bee visitation, four quadrants of 1 m^2 area were marked in the field and number of insects visiting the okra flowers in a span of 10 min in the demarcated area during five consecutive days (55–60 DAS) was counted at five-time intervals of the day (7.00–9.00 h, 9.00–11.00 h, 11.00–13.00 h, 13.00–15.00 h and 15.00–17.00 h). Moreover, to assess the foraging activity and pollination behaviour like, flower handling time and flower visitation rate by five major insect pollinators of okra (*A. c. indica, A. mellifera, B. haemorrhodalis, L. atratus* and *X. latipes*), was calculated at three-time frames of the day (8.00 h, 10.00 h and 12.00 h) during both the years.

Pollination methods and pollinator recruitment for artificial pollination: Immediately after the floral initiation (42–47 DAS), approximately 11–12 okra plants in an area of 1 m^2 were covered with a nylon mesh to prevent natural bee visitation. A total of seven treatments (Supplementary Table 1) were set up with three replications each. On the previous day at 16.00 h, up to 25 flowers per each treatment and each replication were marked, emasculated to avoid self-pollination and covered with paper bags in order to pollinate on the next day. The individual pollinators actively foraging in the okra fields with pollen debris over the body were collected by using a test tube (50 ml capacity) and released on to the marked emasculated flowers by enclosing them in 1 litre capacity nylon mesh and allowed to pollinate for a period of 15 min (09.00–09.15 h).

Impact of planned bee pollination on okra yield: To estimate the yield improvement in okra, the capsules from each treatment were harvested separately and the quantitative parameters like capsule length, girth, fresh weight, number of seeds, seed weight/capsule were recorded separately for 30 capsules under each replication. For calculation of test weight of 100-seeds, the seeds obtained from 30 capsules were pooled and 100-seeds were selected randomly and weighed separately for each treatment. To estimate the seed yield/hectare, seeds obtained from all the capsules in 1 m^2 area were weighed and the values were extrapolated to tonnes per ha.

Statistical analysis: All the experiments were set up in a completely randomized block design (CRBD) with 7 treatments and 3 replications. The quantitative yield data for two years was pooled and analysed by calculating the

Table 1 Diversity of insect pollinators visiting okra flowers

Order of insect pollinators	List of insect pollinators visiting okra flowers
Hymenoptera (11 spp.)	*Apis cerana indica* Fabricius, 1798 (18.28%); *Apis mellifera* Linnaeus, 1758 (10.45%); *Apis dorsata* Fabricius, 1793 (2.61%); *Bombus haemorrhoidalis* Smith, 1852 (6.34%); *Xylocopa latipes* Drury, 1773 (4.85%); *Lithurgus atratus* Smith, 1853 (8.58%); *Andrena hilaris* Smith, 1853 (1.87%); *Nomia incerta* Pérez, 1902 (2.99%); *Halictus sexcinctus* Fabricius, 1775 (1.49%); *Ceratina smaragdula* Fabricius, 1798 (2.99%); *Sceliphron curvatum* Smith, 1870 (2.61%)
Lepidoptera (8 spp.)	*Papilio demoleus* Linnaeus, 1758; *Danaus chrysippus* Linnaeus, 1758; *Pieris rapae* Linnaeus, 1758; *Euploea core* Cramer, 1780; *Aglais caschmirensis* Kollar, 1844; *Vanessa cardui* Linnaeus, 1758; *Celastrina argiolus* Linnaeus, 1758; *Colias ladakensis* Felder & Felder, 1865 (All the butterflies accounted to 6.34%)
Diptera (6 spp.)	*Episyrphus balteatus* (De Geer, 1776); *Metasyrphus corollae* (Fabricius, 1794); *Eupeodes luniger* (Meigen, 1822); *Eristalis tenax* (Linnaeus, 1758); *Macronomia* spp., *Sarcophaga carnaria* Linnaeus, 1758 (Syrphids + Drone flies = 16.79%, Other dipterans = 9.70%)
Coleoptera (3 spp.)	*Oxycetonia versicolor* (Fabricius, 1775); *Clinteria klugi* (Hope, 1831); *Chiloba acuta* (Gahan, 1891) (All the beetles accounted to 2.99%)

ANOVA through SPSS software for WINDOWS version 16.0 (SPSS Inc, Chicago) at $P<0.05$ level of significance and comparison of statistically significant yield data in different treatments was done through post-hoc, Tukey's-B test. Further, the data obtained for stigma receptivity and pollen germination studies and pollinator density, behaviour and foraging activity were analysed at $P<0.001$ significance level and F-values, SE(m) and CV (coefficient of variation) values were calculated through SPSS software to compare the mean values.

RESULTS AND DISCUSSION

Floral biology of VL Bhindi-2: Okra plants of variety VL Bhindi-2 produced solitary hermaphrodite flowers located at axillary positions with terminal capitate stigma. The flower colour ranged from yellowish to creamish with light violet colouration at the flower base. The flowers usually opened every day between 7.00–7.45 h and remained open throughout the day and closed during evening hours between 17.25–18.15 h. At bud stage, flowers were protected by calyx, whose diameter ranged from 1.90–2.80 cm (with mean 2.62±0.23 cm). The length of style ranged from 3.7–4.6 cm (4.12±0.29 cm) and the capitate stigma was 4–8 lobed with an average of 6.10±1.14 number of staminal columns per style (values were average of 30 flowers selected in random) (Supplementary Table 2). Similar results were reported by Srivastava and Sachan (1973), Chandra and Bhatnagar (1975) and Al-Ghzawi *et al.* (2003). Although passive self-pollination is the main character, but some degree of cross pollination through insects is still possible in okra (Angbanyere and Baidoo 2014, Hasnat *et al.* 2015, Nandini *et al.* 2018).

Stigma receptivity and pollen germination studies: The per cent stigma receptivity and pollen germination was recorded in okra at five different time intervals. Statistically significant pollen germination percentage of 81.75±4.57 and 85.25±5.32 were noticed in the year 2021 and 2022, respectively between 9.00–11.00 h. As the time passed in a day, both the stigma receptivity and pollen germination declined drastically and at time interval of 15.00–17.00 h, significantly lowest stigma receptivity of 7.75±1.71% and 9.50±1.29% were recorded in the year 2021 and 2022, respectively (Supplementary Fig. 1). For efficient pollination and fruit set, stigma receptivity and pollen germination should occur simultaneously (Priya *et al.* 2009, Singh *et al.* 2010, Korat *et al.* 2018). In the present study, peak pollen germination and stigma receptivity was recorded between 7.00–9.00 h. This is in close confirmation with the studies by Angbanyere and Baidoo (2014), Perera and Karunaratne (2019) and Sruthi *et al.* (2020).

Pollinator density, behaviour and foraging activity: A total of 28 spp. of insect floral visitors belonging to four insect orders (Coleoptera, Diptera, Hymenoptera, and Lepidoptera) were reported to visit okra flowers. Hymenopterans were the major floral visitors of okra with 11 spp., followed by Lepidopterans (8 spp.), Dipterans (6 spp.) and Coleopterans (3 spp.) (Table 1). The peak period of pollinators' visitation was analysed. The time duration between 9.00–11.00 h was observed as the peak period of bee visitation, wherein, up to 113.76±7.65 (F cal= 99.32, $P<0.01$) numbers of insects were recorded to visit okra flowers in 1 m^2 area in a span of 10 min, followed by 74.35±4.38 insects during 7.00–9.00 h and 32.75±3.69 insects during 11.00–13.00 h. However, the pollinators' density declined drastically as time lapsed during the day. In the afternoon hours, their population was 15.75±1.98 insects during 13.00–15.00 h, while, least population density was recorded in the evening hours between 15.00–17.00 h with

Table 2 Pollination behaviour and foraging rate of five insect pollinators of okra during 2021 and 2022 under mid-Himalayan region

Treatment	Time spent/flower in the year 2021 (in sec)			Time spent/flower in the year 2022 (in sec)			Number of flowers visited/ min 2021			Number of flowers visited/ min 2022		
	8.00 h	10.00 h	12.00 h	8.00 h	10.00 h	12.00 h	8.00 h	10.00 h	12.00 h	8.00 h	10.00 h	12.00 h
A. c. indica	38.75 ± 3.33^{b}	41.75 ± 1.11^{b}	46.50 ± 1.32^{b}	39.25 ± 2.50^{b}	41.25 ± 1.84^{b}	49.75 ± 1.38^{b}	1.75 ± 0.25^{d}	1.50 ± 0.29^{c}	1.00 ± 0.00^{d}	1.50 ± 0.29^{d}	1.25 ± 0.25^{d}	1.00 ± 0.00^{d}
A. mellifera	35.75 ± 1.49^{c}	38.00 ± 1.08^{c}	45.75 ± 2.43^{b}	35.75 ± 1.65^{c}	37.50 ± 1.71^{c}	43.25 ± 1.03^{c}	2.00 ± 0.00^{c}	1.50 ± 0.29^{c}	1.75 ± 0.25^{c}	1.75 ± 0.25^{c}	1.75 ± 0.25^{c}	1.75 ± 0.25^{c}
B. haemorrhodalis	21.75 ± 1.80^{d}	23.00 ± 1.23^{d}	19.50 ± 1.94^{c}	21.25 ± 2.29^{d}	22.50 ± 1.56^{d}	19.00 ± 1.47^{d}	3.50 ± 0.29^{b}	3.25 ± 0.25^{b}	4.25 ± 0.48^{b}	3.50 ± 0.29^{b}	3.25 ± 0.25^{b}	3.75 ± 0.25^{b}
Lithurgusatratus	61.50 ± 2.47^{a}	66.25 ± 2.87^{a}	80.25 ± 1.38^{a}	64.25 ± 2.49^{a}	65.75 ± 2.69^{a}	75.00 ± 2.58^{a}	1.25 ± 0.25^{e}	1.00 ± 0.00^{d}	1.00 ± 0.00^{d}	1.00 ± 0.00^{e}	1.00 ± 0.00^{e}	1.00 ± 0.00^{d}
Xylocopalatipes	11.50 ± 1.19^{e}	14.50 ± 1.32^{e}	9.50 ± 0.65^{d}	11.75 ± 0.75^{e}	13.75 ± 1.18^{e}	11.25 ± 0.85^{e}	4.50 ± 0.29^{a}	4.25 ± 0.48^{a}	6.50 ± 0.29^{a}	4.25 ± 0.25^{a}	4.50 ± 0.29^{a}	5.75 ± 0.48^{a}
CD	6.418	4.96	5.62	6.32	5.09	5.14	0.68	0.95	0.84	0.68	0.74	0.91
SE (m)	2.06	1.59	1.81	2.03	1.64	1.65	0.22	0.31	0.27	0.22	0.24	0.29
CV	12.172	8.67	8.96	11.77	9.05	8.32	16.83	26.62	18.62	18.24	20.19	22.06
F-value	84.72*	156.14*	233.81*	97.5*	148.68*	238.86*	38.22*	20.47*	79.97*	40.83*	39.22*	49.98*
CD (P=0.05)	0.0000014	0.0000004	0.0000008	0.0000003	0.0000014	0.0000009	0.0000007	0.0000001	0.0000013	0.00000013	0.000008	0.00000017

only 9.50±1.29 (F-cal= 99.32, P<0.001) (Supplementary Fig. 2). Our results were in close concurrence with the studies of Hasnat *et al.* (2015), who concluded that, between 9.00–11.00 h the maximum numbers of insect pollinators were observed to visit okra flowers in Dhaka, Bangladesh. Moreover, studies by Perera and Karunaratne (2019) on non-*Apis* bees in Sri Lanka reported that, the bees frequently visited flowers between 9.00–12.00 h during the day. Additionally, Hasnat *et al.* (2015) and Nandhini *et al.* (2018) reported that hymenopterans were the most dominant insect pollinators of okra in Dhaka, Bangladesh and Bengaluru, India respectively.

Pollination behaviour and foraging activity are two important factors for analysing the efficiency and suitability of any pollinator in a particular crop (Bajiya *et al.* 2017, Nandhini *et al.* 2018). The pollination behaviour studies (flower handling time) of five major insect pollinators of the region showed that (Table 2), a megachilid leaf cutter bee (*Lithurgus atratus*) spent statistically significant amount of time per flower during all the three-time frames in both the years (61.50±2.47 to 80.25±1.38 sec). This was followed by *A. c. indica, A. mellifera* and *B. haemorrhodalis.* Whereas, the carpenter bee (*Xylocopa latipes*), being a swift flyer spent significantly least amount of time per flower (Table 2). In the present study, we also observed that, as the time lapsed in a day, the pollinators (*A. c. indica, A. mellifera* and *L. atratus*) spent more time per flower for collection of pollen and nectar. To decipher the foraging rate (number of flowers visited/min) of the targeted pollinators, it was noted that, *X. latipes* and *B. haemorrhodalis* visited highest number of flowers during all the three-time frames of the day in both the years ranging between 3.25±0.25 to 6.50±0.25. Whereas, *A. c. indica, A. mellifera* and *L. atratus* were comparatively slow foragers and became less active as time progressed in a day which led to very less number of flower visits per unit time (as low as 1.00±0.00 flower/min). As per the literature search, this was the first study of its kind to assess the pollination behaviour and foraging activity of important insect pollinators of okra.

Impact of planned bee pollination on okra yield: A total of seven pollination treatments were imposed and their role in enhancing the crop yield and yield related parameters were analysed for two consecutive years through pooled data analysis (Table 3). In both the years, the capsules formed through interaction pollination (*A. c. indica* + *A. mellifera*) showed significantly superior yield parameters like, capsule length (20.9±0.44 cm), capsule girth (7.84±0.16 cm), number of seeds/capsule (60.6±1.56), weight of each capsule (11.48±0.49 g), test weight of 100-seeds (8.38±0.12 g) and seed yield/hectare over other treatments (3.04±0.97 tonnes/ha). Thus, indicating that, interaction of two honey bees is the most efficient pollination method in the present study. Additionally, other insect pollinators like *A. c. indica, B. haemorrhodalis* and *A. mellifera* yielded very encouraging results. Moreover, the insect pollinated flowers recorded superior yield parameters than those flowers which were self-pollinated or hand pollinated (emasculated and cross pollinated). Yield enhancement is one of the very important aspects of every cross-pollination study (Choudhury and Anothai Choomsai 1970, Patricio *et al.* 2012, Bodlah and Waqar 2013, Das *et al.* 2018, FAO 2018, Paschapur *et al.* 2022b). In the present study, we noticed that, the pollination by insects improved yield related parameters. Similarly, several studies reported that, the seeds obtained from the insect pollinated flowers in okra yielded longer capsules with higher number of seeds which were significantly superior in weight and germination in comparison to self-pollinated flowers (Al-Ghzawi *et al.* 2003, Angbanyere and Baidoo 2014, Sruthi *et al.* 2020).

Finally, the insect assisted cross pollination studies carried out for two consecutive years, showed that insect pollinated flowers yielded the highest seed yield/pod/unit

Table 3 Effect of pollination methods on enhancing the quantitative yield parameters of okra (Pooled data of 2021 and 2022)

	Capsule length (cm)	Capsule girth (cm)	No. of seeds/ capsule	Weight of seeds/capsule (g)	Weight of each capsule (g)	Test weight of seeds (g)	Seed yield (t/ha)
A. c. indica	19.3±0.21^{b}	6.93±0.12^{b}	57.3±1.96^{a}	5.37±0.27^{ab}	10.12±0.35^{a}	7.99±0.03^{ab}	2.63±0.25^{ab}
A. mellifera	18.3±0.43^{b}	6.74±0.13^{bc}	54.5±1.67^{ab}	4.97±0.13^{cde}	9.32±0.25^{bc}	7.32±0.09^{cd}	2.38±0.35^{bcd}
B. haemorrhodalis	18.8±0.47^{b}	6.81±0.19^{b}	57.6±0.89^{a}	5.11±0.16^{bc}	9.57±0.41^{ab}	7.85±0.15^{ab}	2.61±2.60^{bc}
Interaction (*A. c. indica* + *A. mellifera*)	20.9±0.44^{a}	7.84±0.16^{a}	60.6±1.56^{a}	5.86±0.16^{a}	11.48±0.49^{a}	8.38±0.12^{a}	3.04±0.97^{a}
Hand	17.4±0.41^{b}	6.78±0.20^{bc}	51.4±1.78^{b}	4.38±0.25^{de}	7.84±0.29^{c}	7.05±0.15^{d}	1.86±0.53^{d}
Open	18.6±0.39^{b}	6.56±0.16^{b}	54.2±2.36^{a}	5.33±0.22^{bcd}	8.98±0.49^{bc}	7.31±0.07^{bc}	2.17±0.65^{bcd}
Close	15.4±0.37^{c}	5.83±0.16^{c}	45.5±1.29^{c}	3.92±0.12^{e}	7.12±0.30^{c}	6.46±0.19^{e}	1.88±0.86^{cd}
CD	1.25	0.53	3.64	0.63	1.16	0.37	4.88
SE(m)	0.44	0.18	1.41	0.22	0.35	0.12	1.78
CV	9.38	10.21	13.71	16.45	17.09	2.72	9.35
F-value	10.33*	15.48*	13.97*	11.84*	17.73*	23.41*	12.04*
CD (P=0.05)	0.00001	0.00007	0.0007	0.00001	0.00001	0.00005	0.00003

area (ha). Thus, indicating the advantages of employing *A. c. indica* and *A. mellifera* in commercial seed production systems. This study concludes that, maintenance of *Apis* bee colonies and ecological engineering of fields to enhance the activity of non-*Apis* bees can improve the pollination rate and thus, enhance the yield of okra plants and their F_1 populations as well.

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