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Impact of *Apis mellifera* linnaeus in augmenting seed yield of broccoli, *Brassica oleracea* var. *Italica* Plenck

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Abstract

The studies on seed yield augmentation of broccoli cv. Palam Samridhi revealed that there was no silique and seed setting in pollinators' exclusion. Numbers of siliquae per plant in open pollination were 28.3 per cent higher than in bee pollination. Silique from open pollinated and bee pollinated plants had 12.5 and 10.5 mean number of seeds, respectively. Seed yield per hectare was 51.6 per cent higher in open pollinated plots than in bee pollinated plots. Further studies on the effect of number of *Apis mellifera* visits on flowers on the seed yield parameters revealed that flowers which received five bee visits resulted in the highest mean number of seeds per silique (11.95), thereby contributing 29.35 mg mean seed weight per silique. The results conclude that *A. mellifera* has tremendous contribution in broccoli seed production, however, other insect pollinators also play significant role in broccoli pollination.

Keywords: *Apis mellifera*, broccoli, diversity, pollinators

Introduction

In the flowering plants pollinators plays an important role in their reproduction and fruit setting [3]. It has been observed that self-sterile crops like cucurbits, crucifers, alfalfa, clover, almonds, safflower and number of fruit crops do not give their maximum yield in the absence of bees even if practiced with any amount of fertilizer, any cultural practices and irrigation [6]. Broccoli is a cross-pollinated crop due to self-incompatibility [4], therefore, requires insect pollinators. Broccoli is an exotic crop in the Punjab and belongs to family Brassicaceae. It is the richest source of glucosinolates (72-100 mg/100 g) in the human diet and has been reported to protect our body against colon, rectum and thyroid cancers [1]. In the previous study, average seed yield of cauliflower was 22.60, 14.50 and 5.61 g per plant under open pollination, crop caged with honey bees and crop caged without bees, respectively [8]. Managed pollination of cauliflower by *Apis cerana* Fabricius resulted in 129 per cent increase in seed yield over pollinator's exclusion [11]. The pollination of cabbage by *A. cerana* resulted in 28.0, 35.0 and 40.0 per cent increase in cabbage pod setting, seed setting and seed weight, respectively [17]. According to Extension economics estimate, honey bees and other pollinators contribute \$153 billion annually to the world agriculture, which represented 9.5 per cent value of world agricultural production used for human food in 2005 [5]. Bees (Hymenoptera: Apoidea) are diverse taxa with 20139 species known worldwide [2]. However, Honey bees are the most important pollinators which can be hived in artificial structures as large colonies and can be managed in any number, place and time, as per the requirement. There appears to be a considerable scope for increasing the seed yield of broccoli by introducing colonies of honey bees. Considering these factors, study on the diversity of pollinators, role of *Apis mellifera* Linnaeus and other pollinators in augmenting seed yield of broccoli was planned.

Material and Methods

Seed yield augmentation assessment through cage studies: Broccoli cultivar Palam Samridhi was grown at Experimental Area, Seed Technology Centre Punjab Agricultural University (PAU), Ludhiana and University Seed Farm (USF), Ladhawal during the year 2016-17, as per the recommendations of the PAU [1] in Randomized Block Design. In these experiments, there were three treatments; namely open pollination, intensive pollination and pollination exclusion. In open pollination (T₁), there was no installation of any cage and the

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crop was accessible to the all-natural pollinators. In intensive pollination (T_2), a four bee-frame strength colony of *A. mellifera* was placed on stand inside the crop covered with the nylon netting cage (6×3×3 m). In Pollinators' exclusion (T_3), the crop was caged with nylon net cage (6×3×3 m) to exclude all pollinators. There were six replications of every treatment. In every replication of every treatment, five plants were randomly selected and tagged at the start of flowering for recording data and sampling later on. At crop maturity, following yield parameters were recorded:

Number of siliquae per plant: The number of siliquae per plant was counted from the tagged five plants from every replication plot in every treatment and the mean number of siliquae per plant was calculated for all the treatments.

Siliqua length: From five tagged plants under each replication, 20 siliquae per plant were selected randomly and their length was measured using a scale. The average siliqua length was then calculated for every replication plot and treatment.

Number of seeds per siliqua: Seeds from these 20 randomly selected siliquae (3.2.1.2) from all the randomly selected plants were counted separately for every replication in every treatment to determine the average number of seeds per 20 siliquae.

Thousand seed weight: Seeds from five tagged plants from every replication were collected and mixed. Thousand seeds were counted using the seed counter and 1000 seed weight was recorded by using of monopan electronic balance.

Seed yield (per plant and per plot): The mean seed yield (g) per plant was calculated on the basis of seed yield per plant recorded from five randomly selected and tagged plants per replication. Seed yield per plot (18 m²) was calculated on the basis of seed yield from the six replications in all the treatments.

Per cent seed germination: Between-paper method was used to test the seed germination of cultivars of both the crops. There were three treatments with three replications each. Hundred seeds were taken in each replication. Seed from five plants from each replication was collected and mixed. After that 100 seeds were randomly selected from the bulk sample and were arranged in rows at regular intervals on moist towel paper. These seeds were then covered with another sheet of moist paper towel and the paper was rolled loosely from the opposite end of label. These rolls were kept in an incubator in which 25^o C temperature and 80 per cent relative humidity was maintained. Germination data was recorded at weekly interval after the incubation. The seedlings were classified into normal, abnormal and dead seedlings. Average seed germination percentages for every treatment were calculated.

Number of bee visits to the blooms of the broccoli cultivar for maximizing seed setting: To study impact of every single bee visit on seed yield, six treatments (0, 1, 2, 3, 4 and 5 visits of *A. mellifera* on flowers) were evaluated and every treatment was replicated 10 times. For these studies, five flower buds per plant were tagged just before their opening on each of the 25 randomly selected plants. Out of these 125

tagged buds, 10 buds were bagged to study the effect of zero bee visit on seed setting. The next day, honey bees' visitation was observed on these tagged newly opened flowers and ten such flowers were bagged after they received single bee visit each. Then another ten flowers, each visited twice by *A. mellifera*, were bagged. Similarly, another three sets of ten flowers, each set visited by three, four and five bees, respectively were bagged. These bags were removed after the bagged flowers withered. At crop maturity, the tagged siliquae were harvested individually and seeds were taken out, weighed and counted. The observations recorded included mean number of seeds per siliqua, mean seed weight per siliqua and weight per seed for every number of bee visit.

Statistical analyses were done using Completely Randomized Design. Means and standard errors were worked out for comparison using Least Significant Difference at 5 per cent level of significance.

Results and Discussion

Seed yield augmentation assessment through cage studies

The broccoli was sown in Randomized Block Design. There were three treatments; open pollination, pollination by *A. mellifera*, and pollinators' exclusion. The data from the two locations revealed that yield parameters such as number of siliquae per plant, seeds per siliqua, seed yield per hectare, siliqua length, thousand seed weight, seed germination and seed vigour shows significant differences under different treatments in broccoli seed production (Table 1). Each parameter in every treatment differed significantly at each location. However, each treatment did not vary significantly between the two locations. Broccoli is a cross-pollinated crop due to self-incompatibility requires insect vectors for its pollination, was confirmed by zero seed setting on the plants which were not accessible to pollinators.

The number of siliquae per plant in open pollinated crop (461.2±9.0) was significantly higher than in crop caged with *A. mellifera* colony (359.5±10.9) whereas in pollinators' exclusion number of siliquae per plant was nil which shows that broccoli is totally self-incompatible. As we see mode of pollination indicated higher number of siliquae per plant in crop having access to insect pollinators at blooming stage than the crop deprived of insect pollination (Table 1). Similar results have also been reported on cauliflower^[18, 16, 15].

The mean siliqua length was the highest in open pollinated plots (5.9±0.2 cm) followed by that in plots caged with *A. mellifera* colony (5.3±0.0 cm) at PAU Ludhiana. Similar at USF Ladhawal, significantly the highest mean siliqua length was recorded in open pollinated plots (6.5±0.2 cm) followed by that in plots caged with *A. mellifera* colony (6.0±0.1 cm). The overall mean of the two locations revealed that significantly highest mean siliqua length was recorded in open pollinated plots (6.2±0.3 cm) followed by that in plots caged with *A. mellifera* colony (5.6±0.3 cm).

Seed setting in open pollinated plots (12.1±0.5 seeds/siliqua) was significantly higher than in plots caged with *A. mellifera* colony (10.2±0.7 seeds/siliqua) at PAU Ludhiana. At USF Ladhawal, seed setting in open pollinated plots (12.9±0.5 seeds/siliqua) was significantly higher than in plots caged with *A. mellifera* colony (10.7±0.7 seeds/siliqua). Overall mean number of seeds in open pollination (12.5 seeds/siliqua) was 18.3 per cent higher than in bee pollination (10.5 seeds/siliqua).

Table 1: Effect of *Apis mellifera* and other insect pollinators on *Brassica oleracea* var. *italica* seed yield parameters at PAU, Ludhiana and University Seed Farm, Ladhawal during 2017

Treatment	Mean number of siliquae per plants*			Mean siliqua length (cm)			Mean number of seeds per siliqua*			Mean thousand seeds weight (g)		
	Ludhiana	Ladhawal	Overall mean	Ludhiana	Ladhawal	Overall mean	Ludhiana	Ladhawal	Overall mean	Ludhiana	Ladhawal	Overall mean
Open pollination	452.2±9.8 (21.3)	470.1±33.3 (21.6)	461.2±9.0 (21.5)	5.9±0.2	6.5±0.2	6.2±0.3	12.1±0.5 (2.6)	12.9±0.5 (3.7)	12.5±0.4 (3.7)	2.8±0.1	2.8±0.1	2.8±0.0
Pollination by <i>A. mellifera</i>	348.6±21.9 (18.7)	370.3±33.3 (19.2)	359.5±10.9 (19.0)	5.3±0.0	6.0±0.1	5.6±0.3	10.2±0.7 (3.3)	10.7±0.7 (3.4)	10.5±0.3 (3.4)	2.5±0.1	2.5±0.0	2.5±0.0
Pollinators' exclusion	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.0±0.0	0.0±0.0	0.0±0.0
L.S.D. (p=0.05)	(1.1)	(2.0)	(0.9)	0.3	0.3	1.2	(0.2)	(0.2)	(0.2)	0.2	0.2	0.1

Treatment	Mean seed yield /plant (g)			Mean seed yield q/ha			Mean seed germination (%)**			Mean seed vigour index		
	Ludhiana	Ladhawal	Overall mean	Ludhiana	Ladhawal	Overall mean	Ludhiana	Ladhawal	Overall mean	Ludhiana	Ladhawal	Overall mean
Open pollination	11.6±0.8	12.9±0.6	12.3±0.7	4.6±0.3	4.8±0.2	4.7±0.1	75.6±1.3 (60.4)	75.2±1.5 (60.1)	75.4±0.2 (60.2)	440.3±27.2	425.3±22.8	432.8±7.5
Pollination by <i>A. mellifera</i>	8.1±0.7	7.4±0.4	7.8±0.4	3.3±0.3	2.8±0.2	3.1±0.3	70.3±0.8 (56.9)	71.1±1.1 (57.4)	70.7±0.4 (57.2)	350.0±26.5	320.5±17.9	335.3±14.8
Pollinators' exclusion	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.0±0.0	0.0±0.0	0.0±0.0
L.S.D. (p=0.05)	1.8	1.3		0.7	0.5	0.7	(1.6)	(1.9)	(0.7)	62.2	47.2	43.2

*Figures in parentheses are the means of $\sqrt{n+1}$ transformations

**Figures in parentheses are the means of arc sine $\sqrt{\text{Percentage}}$ transformations

Similar result was reported on cauliflower, where seeds per siliqua on were the maximum (13.25) in natural pollinated crop followed by bee pollination (9.53) and minimum in self-pollinated crop (8.07) [15].

Mean thousand seed weight in open pollinated plots (2.8±0.1 g) was significantly higher than in plots caged with *A. mellifera* colony (2.5±0.0 g) at PAU Ludhiana. Similarly at USF Ladhawal, significantly higher mean thousand seed weight was recorded in open pollinated plots (2.8±0.1 g) than in plots caged with *A. mellifera* colony (2.5±0.1 g). The results from the two locations revealed that mean thousand seed weight was more in open pollinated plants (2.8±0.0 g) than in plots caged with *A. mellifera* colony (2.5±0.0 g). The present study is in line with the findings of Kakkar *et al.* [8] who reported significantly higher 1000 seed weight (3.73 g) of cauliflower in open pollinated plants as compared to bee pollination (2.36 g) and control (1.34 g). The studies revealed that pollinators also improve quality of seed as more bold seeds were obtained in open pollination than under caged conditions.

The mean seed yield in open pollinated plots (4.6±0.3 q/ha) was significantly higher than that in plots caged with *A. mellifera* colony (3.3±0.3 q/ha) whereas seed yield was nil in plots caged devoid of any pollinators at PAU Ludhiana. At USF Ladhawal also significantly higher seed yield was obtained in open pollinated plots (4.8±0.2 q/ha) than in plots caged with *A. mellifera* colony (2.8±0.2 q/ha). Overall mean of two locations (Ludhiana and Ladhawal) revealed that mean seed yield in open pollination was the maximum (4.7±0.1q/ha) followed by the crop pollinated by *A. mellifera* (3.1±0.3q/ha), whereas seed production was nil in the crop devoid of any pollinators (Table 1). Similar results were reported by Sihag [16] that seed yield of cauliflower crop was 47.50 times more in open pollination than under pollinators' exclusion. In the another study, honey bees, *A. mellifera*, proved to be a good source of pollination of *Brassica campestris* resulting in maximum seed yield (19.9 q/ha), 1000 seed weight (3.8 g) and seed germination (95.8%) [14]. Similarly, there was 46 per cent more seed production in the presence of *A. mellifera* as compare to absence of bees in Brassica crops [13].

Pollinators other than *A. mellifera* also contributed towards

significant improvement in seed germination as well as seed vigour. Overall mean seed germination results from the two locations (Ludhiana and Ladhawal) the data revealed significantly higher seed germination in open pollination (75.4±0.2%) than in plots caged with *A. mellifera* colony (70.7±0.4%). Likewise, seed germination of *B. napus* was the highest in open pollinated plots (88.62±1.07%) followed by in plots caged with *A. mellifera* colony (84.75±1.36%) and plots caged to exclude pollinators (77.79±1.73%) [9]. Overall mean seed vigour was significantly higher in open pollinated plots (432.8±7.5) than in plots caged with *A. mellifera* colony (335.3±14.8). These findings are supported by Kumari [9] who reported that seeds obtained from open pollinated plots of *B. napus* exhibited the maximum seed vigour (666.02±5.34), followed by the plots under pollination by *A. mellifera* alone (570.09±5.15) and the plots caged to exclude pollinators' exhibited the minimum vigour (378.74±3.08).

In open pollinated broccoli plants were significantly better than in those plants pollinated by *A. mellifera* alone. All yield contributing parameters, probably because of contribution by several different insect pollinators under the open field conditions. However, crop caged with *A. mellifera* colony revealed the significant role of *A. mellifera* alone in seed production of broccoli as there was no seed setting in absence of pollinators. In bee-pollinated plots, caging might have affected on the microclimate of crop, growth of plants and activity of bees. The combination of several complementary pollinator species differing in flower-visiting behaviour could be of more importance for high fruit set than only pollinator abundance [10]. The per cent increase in broccoli seed yield in the presence of pollinator's emphasis the need to protect insect pollinators at blooming stage.

Number of bee visits to the blooms of the broccoli for maximizing seed setting:

Increase in number of bee visits from 0-5 resulted in increase in the mean number of seeds per siliqua, weight per seed and seed weight per siliqua. There was no seed formation in siliqua when flower was devoid of pollinators. The number of seeds per siliqua in one bee visit was 4.59±0.04 only and increased significantly with every additional bee visits subsequently to reach 11.95±0.03 seeds per siliqua in siliqua which received 5 bee visits (Table 2).

The mean weight per seed in one bee visit was 2.99 ± 0.01 mg only and increased significantly with every additional bee visits subsequently to reach 3.02 ± 0.01 mean weight per seed in siliqua which received 5 bee visits (Table 2). The seed weight per siliqua in one bee visit was 9.32 ± 0.13 mg only and increased significantly with every additional bee visits subsequently to reach 29.35 ± 0.18 mg seed weight per siliqua

in siliqua which received 5 bee visits (Table 2). The seeds per siliqua, weight per seed and seed weight per siliqua differed significantly among the treatments at each location. However, these yield parameters did not vary significantly between the locations. Similarly, siliqua setting in cauliflower increased significantly (35-58%) as the number of bee visits increased from one to five per flower [17].

Table 2: Influence of number of *Apis mellifera* visits on *Brassica oleracea* var. *italica* blooms on seed yield parameters at PAU, Ludhiana and University Seed Farm, Ladhawal during 2017

No. of bee visits per bloom	Mean number of seeds per siliqua*			Mean weight per seed (mg)			Mean seed weight per siliqua (mg)		
	Ludhiana	Ladhawal	Overall mean (Location)	Ludhiana	Ladhawal	Overall mean (Location)	Ludhiana	Ladhawal	Overall mean (Location)
0	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.0±0.0 (1.0)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.00	0.0±0.0	0.0±0.0
1	4.55±0.39 (2.34)	4.62±0.24 (2.36)	4.59±0.04 (2.36)	2.65±0.02	2.67±0.04	2.66±0.01	9.19±0.85	9.44±0.55	9.32±0.13
2	8.66±0.26 (3.10)	8.71±0.27 (3.11)	8.69±0.03 (3.11)	2.77±0.05	2.80±0.04	2.79±0.02	19.10±0.91	19.43±0.86	19.27±0.17
3	9.97±0.35 (3.30)	10.02±0.33 (3.31)	10.00±0.03 (3.31)	2.87±0.03	2.91±0.03	2.89±0.02	22.90±0.75	23.42±0.78	23.16±0.26
4	11.08±0.35 (3.47)	11.11±0.25 (3.48)	11.10±0.02 (3.48)	2.95±0.03	2.97±0.03	2.96±0.01	26.43±0.95	26.69±0.85	26.56±0.13
5	11.92±0.29 (3.59)	11.98±0.27 (3.60)	11.95±0.03 (3.60)	3.01±0.04	3.03±0.04	3.02±0.01	29.17±0.87	29.53±0.76	29.35±0.18
Overall mean (Treatment)	7.70±1.87	7.74±1.87	7.72±1.87	2.38±0.48	2.40±0.48	2.39±0.48	17.80±4.55	18.09±4.61	17.94±4.58
L.S.D. (p=0.05)	(0.14)	(0.11)	L: (NS) T: (0.09) L x T: (NS)	0.09	0.10	L: NS T: 0.06 L x T: NS	2.25	1.88	L: NS T: 1.45 L x T: NS

*Figures in parentheses are the means of $\sqrt{n+1}$ transformations

However, they reported no significant increase in the number of siliqua set as the number of bee visits increased from five to nine per flower probably due to transfer of sufficient number of pollen grains by 5 bee visits. The single bee visit transferred 53 pollen grains whereas 12 bee visits transferred 1253 pollen grains in *Cucurbita pepo* flowers [19].

Conclusion

Seed yield in the absence of pollinators was nil. Seed yield in open pollinated plants was significantly better than crop caged with *A. mellifera* colony probably because under the open field conditions pollination might have been accomplished by several different insect pollinators whereas under caged conditions *A. mellifera* was the only pollinator species. *Apis mellifera* contribute significantly in broccoli seed production, however, other insect pollinators need to be conserved for obtaining further higher yield.

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