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## *Apis mellifera* Linnaeus contribution in augmenting seed yield of *Brassica carinata* A. Braun

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### Abstract

The studies on seed yield augmentation of African sarson cv. PC-6 revealed that in pollinators' exclusion treatment had significantly lower seed setting and siliqua formation. Numbers of siliqua per plant in open pollinated and bee pollinated were 34.0 and 26.2 per cent, respectively higher than pollinators' exclusion. Seed yield, per plant and per hectare was 141.2 and 131.4 per cent higher in open pollinated and bee pollinated over pollinator's exclusion plots. Further studies on the effect of number of *A. 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 siliqua (11.45), thereby contributing 32.1 mg mean seed per siliqua. Overall results showed that the significance of bees in seed yield enhancement in *B. carinata*. however, other insect pollinators also play significant role in African sarson pollination.

**Keywords:** *Apis mellifera*, African sarson, diversity, pollinators

### Introduction

Pollinator's plays a vital role in reproduction and fruit setting of many flowering plants [5]. Beekeeping has positive ecological consequences. Bees visit plants for searching food in the form of nectar and pollen. In this process of pollen collection, bees visit the number of flowers as resulted pollination of horticultural and agricultural crops and also wild plants. It increases yield in terms of seed yield and also improves the quality of seeds/fruits and vegetable. It has been seen that self-sterile harvests like cucurbits, crucifers, alfalfa, clover, almonds, safflower and number of natural product crops don't give their greatest yield without honey bees regardless of whether rehearsed with any measure of manure, any social practices and water system [7]. African sarson is a cross-pollinated crop therefore, requires insect pollinators. African sarson belongs to family Brassicaceae, being a new cultivar, have much improved now over the earlier ones and have determinate growth, relatively shorter stature and maturity duration, better harvest index, higher oil content, etc., and thus, the crop is a better option for cultivation under abiotic (drought, high temperature) and biotic (resistance against white rust, downy mildew, powdery mildew; tolerance against alternaria blight and stem rot) stresses. Managed pollination of *B. juncea* by *Apis mellifera* Fabricius resulted in 49.9 per cent increase in seed yield over pollinator's exclusion [12]. It was also reported that mean number of seeds per pod (25.36) and mean seed weight per pod (106.09 mg) increased significantly with the increase in bee visits and was significantly the maximum at eight bee visits in *B. napus* [10]. Similar results on *B. juncea* was reported, in that mean number of seeds per pod (20.44) and mean seed weight per pod (98.58 mg) was significantly the maximum up to eight bee visits [10]. Extension economics estimate suggested that contribution of the honey bees and other pollinators to the world agriculture is worth over \$ 153 billion annually, which represented 9.5 per cent value of world agricultural production used for human food in 2005 [6]. 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 African sarson by introducing colonies of honey bees. Considering these factors, study on the diversity of pollinators, role of *Apis mellifera* and other pollinators in augmenting seed yield of African sarson was planned.

## Material and Methods

### Seed yield augmentation assessment through cage studies:

African sarson cultivar PC-6 was grown at Experimental Area, University Seed Farm (USF), Ladhawal during the year 2016-17 and 2017-18, as per the recommendations of the PAU<sup>[1]</sup> in Randomized Block Design. In these experiments, there were three treatments; namely open pollination, intensive pollination and pollination exclusion. In open pollination (T<sub>1</sub>), there was no installation of any cage and the crop was accessible to the all-natural pollinators. In intensive pollination (T<sub>2</sub>), 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<sub>3</sub>), 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. 1000 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 m<sup>2</sup>) 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<sup>o</sup> 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.

**Oil content:** Oil content of ten gram seed (per replication) was extracted with petroleum ether (solvent) using Soxhlet Extraction apparatus at 80 °C. At the end of the extraction process, which lasted for sixteen hours, the flask containing the solvent and the sample was removed. The solvent in the flask was then evaporated and the remaining oil was weighed. The percentage of oil on the basis of the weight of the initial sample was then calculated.

### Number of bee visits to the blooms of the African sarson 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 visits. 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 African sarson 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 oil content shows significant differences under different treatments in African sarson seed production (Table 1). Each treatment did not vary significantly between the two years. African sarson is a cross-pollinated crop and thus, requires insect vectors for its pollination.

The number of siliquae per plant in open pollinated crop (262.0±0.2) and crop caged with *A. mellifera* colony (246.8±3.4) were significantly higher than pollinators' exclusion (195.5±1.8) (Table 1). Similar results have also been reported on *B. Napus*<sup>[10]</sup>.

The mean siliqua length was the highest in open pollinated plots (5.8±0.2 cm) followed by that in plots caged with *A. mellifera* colony (5.2±0.2 cm) and the lowest in pollinators' exclusion (3.1±0.2 cm) in 2016-17. Similar results have been recorded next year with mean siliqua length in open pollinated plots (6.1±0.2 cm) followed by that in plots caged with *A. mellifera* colony (4.9±0.2 cm), which were higher than pollinators' exclusion (2.9±0.2 cm). The overall mean of the two years revealed that significantly highest mean siliqua length was recorded in open pollinated plots (5.9±0.3 cm) followed by that in plots caged with *A. mellifera* colony (5.0±0.2 cm), which were higher than pollinators' exclusion (3.0±0.1 cm).

Seed setting in open pollinated plots ( $12.2 \pm 0.6$  seeds/siliqua) was higher than in plots caged with *A. mellifera* colony ( $11.8 \pm 0.6$  seeds/siliqua) followed by the lowest in pollinators' exclusion ( $4.9 \pm 0.3$  seeds/siliqua) during 2016-17. Similarly seed setting in open pollinated plots ( $12.3 \pm 0.5$  seeds/siliqua) was higher than in plots caged with *A. mellifera* colony ( $11.7 \pm 0.6$  seeds/siliqua) followed by the lowest in pollinators' exclusion ( $5.2 \pm 0.2$  seeds/siliqua). Overall mean number of seeds in open pollination and in bee pollination were 141.2 per cent and 131.3 per cent higher than in pollinators' exclusion. Similar result was reported on *B. juncea* [10], where seeds per siliqua on were the maximum (14.06) in natural pollinated crop followed by bee pollination (12.32) and minimum in self-pollinated crop (9.96). This study is also supported by another in which it was reported that the *B. napus* plants visited by insect pollinators yielded 472 pods per plant with mean of 14 seeds per pod. Plants caged to exclude insects yielded only 37 pods per plant containing only three seeds per pod [3].

Mean thousand seed weight was higher in open pollinated plots ( $2.7 \pm 0.2$  g) followed by caged with *A. mellifera* colony ( $2.4 \pm 0.1$  g) and lowest in pollinators' exclusion plots ( $1.7 \pm 0.2$  g) during 2016-17. Similarly during next year, mean thousand seed weight was recorded in open pollinated plots ( $2.8 \pm 0.0$  g) followed by plots caged with *A. mellifera* colony ( $2.5 \pm 0.1$  g) and the lowest in pollinators' exclusion plots ( $1.8 \pm 0.2$  g). Combined result from two years revealed that mean thousand seed weight were more in open pollinated plants and plots caged with *A. mellifera* colony by 64.7 per cent and 47.1 per cent from plots devoid of pollinators. The present study is in line with the findings of [10] who reported significantly higher 1000 seed weight (4.66 g) of *B. juncea* in open pollinated plants as compared to bee pollination (3.96 g) and control (3.13 g). Seed weight of honey bees pollinated *B. napus* was 26 g against 9.3 g without pollination [14]. The studies uncovered that pollinators additionally improve nature of seed as progressively strong seeds were obtained in open fertilization than under confined conditions.

The mean seed yield in open pollinated plots ( $19.8 \pm 0.3$  q/ha) was significantly higher than that in plots caged with *A. mellifera* colony ( $15.9 \pm 0.5$  q/ha) whereas seed yield was significantly lower in plots caged devoid of any pollinators ( $3.6 \pm 0.5$  q/ha). Following year, results were obtained, where, significantly higher seed yield was in open pollinated plots ( $20.2 \pm 0.4$  q/ha) than in plots caged with *A. mellifera* colony ( $16.4 \pm 0.3$  q/ha) followed by the lowest yield in plots devoid pollinators ( $4.1 \pm 0.2$  q/ha). Overall mean of two years revealed that mean seed yield in open pollination was the maximum ( $20.0 \pm 0.1$  q/ha) followed by the crop pollinated by *A. mellifera* ( $16.2 \pm 0.3$  q/ha), whereas seed production was significantly lowest in the crop devoid of any pollinators ( $3.9 \pm 0.3$  q/ha) (Table 1). Similar results were reported by [10] that seed yield of *B. juncea* crop was 92.8 per cent 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%) [17]. Similarly, there was 46 per cent more seed production in the presence of *A. mellifera* as compare to absence of bees in *Brassica* crops [15]. In another study, an increase in seed yield of mustard to the extent of 67.7 and 28.08 per cent in open pollinated and caged crop with honey bees, respectively over the control (caged to exclude all pollinators) [4].

Pollinators other than *A. mellifera* also contributed towards

significant improvement in seed germination as well as oil content. Overall mean seed germination results of two years revealed that significantly higher seed germination in open pollination ( $77.6 \pm 1.1\%$ ) than in plots caged with *A. mellifera* colony ( $72.4 \pm 0.8\%$ ) than pollinators' exclusion ( $50.8 \pm 1.1\%$ ). Likewise, seed germination of *B. juncea* was the highest in open pollinated plots ( $91.00 \pm 0.38\%$ ) followed by in plots caged with *A. mellifera* colony ( $86.46 \pm 1.45\%$ ) and plots caged to exclude pollinators ( $81.77 \pm 0.17\%$ ) [10]. Pollinated plants, plants caged with honey bees and plants from which pollinators were excluded, had germination rates of 96.3, 95.0 and 82.9 per cent, respectively [9]. Seed germination ( $72.70\%$ ) was significantly higher in bee pollinated radish plants over open-pollination [18].

Overall mean oil content was higher in open pollinated plots ( $40.2 \pm 0.1\%$ ) than in plots caged with *A. mellifera* colony ( $39.3 \pm 0.2\%$ ) than pollinators' exclusion ( $24.9 \pm 1.0\%$ ). These finding are supported by [10] who reported that seeds obtained from open pollinated plots of *B. juncea* exhibited the maximum oil content ( $33.48 \pm 1.08\%$ ), followed by the plots under pollination by *A. mellifera* alone ( $29.08 \pm 1.02\%$ ) and the plots caged to exclude pollinators' exhibited the minimum oil content ( $26.09 \pm 1.05\%$ ). Similar study suggested that oil yield in *B. napus* was 9.76 and 1.55 times higher under open pollination and net caging, respectively, than under muslin bagging [13].

In open pollinated African sarson 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 African sarson as there was less 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 behavior could be of more importance for high fruit set than only pollinator abundance [10]. The per cent increase in African sarson 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 african sarson 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  $4.4 \pm 0.2$  seed formation in siliqua when flower was devoid of pollinators. The number of seeds per siliqua in one bee visit was  $6.9 \pm 0.1$  only and increased significantly with every additional bee visits subsequently to reach  $11.45 \pm 0.03$  seeds per siliqua in siliqua which received 5 bee visits (Table 2). The mean weight per seed in one bee visit was  $2.3 \pm 0.01$  mg only and increased significantly with every additional bee visits subsequently to reach  $2.8 \pm 0.01$  mean weight per seed in siliqua which received 5 bee visits (Table 2). The seed weight per siliqua in one bee visit was  $7.7 \pm 0.4$  mg only and increased significantly with every additional bee visits subsequently to reach  $32.1 \pm 0.2$  mg seed weight per siliqua in siliqua which received 5 bee visits (Table 2). The seed per siliqua, weight per seed and seed weight per siliqua doesn't differed significantly among different years. Similarly, In *B. juncea*, it has been reported that zero, one, two and five bee visits per flower by *A. mellifera* resulted in 57.35, 65.5, 82.5 and 88.4 per cent pod setting, respectively in Ludhiana [11].

**Table 1:** Effect of *Apis mellifera* on *Brassica carinata* seed yield parameters at University Seed Farm, Ladhawal during 2016-17 and 2017-18

Treatment	Mean number of silique per plants*			Mean siliqua length (cm)			Mean number of seeds per siliqua*			Mean thousand seeds weight (g)		
	2017	2018	Overall mean	2017	2018	Overall mean	2017	2018	Overall mean	2017	2018	Overall mean
Open pollination	262.2±10.5 (16.2)	261.8±12.3 (16.2)	262.0±0.2 (16.2)	5.8±0.2	6.1±0.2	5.9±0.2	12.2±0.6 (3.6)	12.3±0.5 (3.6)	12.3±0.0 (3.6)	2.7±0.2	2.8±0.1	2.8±0.0
Pollination by <i>A. mellifera</i>	243.4±25.3 (15.6)	250.2±15.5 (15.8)	246.8±3.4 (15.7)	5.2±0.1	4.9±0.2	5.0±0.2	11.8±0.6 (3.5)	11.7±0.6 (3.6)	11.8±0.1 (3.5)	2.4±0.1	2.5±0.1	2.5±0.0
Pollinators' exclusion	193.7±21.1 (13.9)	197.3±17.7 (14.0)	195.5±1.8 (13.9)	3.1±0.2	2.9±0.2	3.0±0.1	4.9±0.3 (2.4)	5.2±0.4 (2.5)	5.1±0.2 (2.4)	1.7±0.2	1.8±0.2	1.7±0.1
L.S.D. (p=0.05)	(1.7)	(1.2)	(1.5)	(0.1)	(0.1)	(0.1)	(0.2)	(0.2)	(0.1)	(0.1)	(0.1)	(0.1)

Treatment	Mean seed yield /plant (g)			Mean seed yield q/ha			Mean seed germination (%)**			Per cent oil content**		
	2017	2018	Overall mean	2017	2018	Overall mean	2017	2018	Overall mean	2017	2018	Overall mean
Open pollination	8.9±0.2	9.1±0.1	9.0±0.1	19.8±0.3	20.2±0.4	20.0±0.1	78.6±0.9 (62.4)	76.5±1.4 (61.0)	77.6±1.1 (61.7)	40.1±0.7 (39.3)	40.3±0.5 (39.4)	40.2±0.1 (39.3)
Pollination by <i>A. mellifera</i>	7.2±0.3	7.4±0.3	7.3±0.1	15.9±0.5	16.4±0.3	16.2±0.3	71.5±0.7 (57.7)	73.2±1.4 (58.8)	72.4±0.8 (58.3)	39.2±0.9 (38.7)	39.5±0.7 (38.9)	39.4±0.2 (38.8)
Pollinators' exclusion	1.6±0.1	1.8±0.1	1.7±0.1	3.6±0.2	4.1±0.2	3.9±0.3	51.8±1.2 (46.0)	49.7±1.2 (44.8)	50.8±1.1 (45.4)	24.6±0.7 (29.7)	25.3±1.2 (30.2)	24.9±1.0 (29.9)
L.S.D. (p=0.05)	(0.5)	(0.4)	(0.1)	(0.9)	(0.8)	(0.8)	(1.4)	(2.1)	(1.5)	(1.1)	(1.1)	(1.1)

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

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

**Table 2:** Influence of number of *Apis mellifera* visits on *Brassica carinata* blooms on seed yield parameters at University Seed Farm, Ladhawal during 2016-17 and 2017-18

No. of bee visits per bloom	Mean number of seeds per siliqua*			Mean weight per seed (mg)			Mean seed weight per siliqua (mg)		
	2017	2018	Overall mean	2017	2018	Overall mean	2017	2018	Overall mean
0	4.3±0.1 (2.30)	4.5±0.1 (2.34)	4.4±0.2 (2.32)	1.7±0.2	1.8±0.2	1.7±0.0	7.3±0.4	8.1±0.1	7.7±0.4
1	6.9±0.3 (2.80)	7.0±0.2 (2.82)	6.9±0.1 (2.81)	2.2±0.1	2.3±0.1	2.3±0.01	15.2±0.4	16.1±0.2	15.7±0.4
2	9.1±0.2 (3.17)	8.9±0.3 (3.14)	9.0±0.1 (3.16)	2.5±0.2	2.5±0.2	2.5±0.02	22.8±0.2	22.3±0.2	22.6±0.3
3	10.8±0.2 (3.43)	10.5±0.3 (3.39)	10.6±0.3 (3.41)	2.7±0.3	2.7±0.3	2.7±0.02	29.2±0.3	28.4±0.4	28.8±0.4
4	11.3±0.3 (3.50)	11.1±0.3 (3.47)	11.2±0.2 (3.49)	2.8±0.2	2.8±0.1	2.8±0.01	31.6±0.4	31.1±0.3	31.4±0.2
5	11.5±0.1 (3.53)	11.4±0.2 (3.51)	11.45±0.03 (3.52)	2.8±0.2	2.8±0.1	2.8±0.01	32.2±0.6	31.9±0.6	32.1±0.2
Overall mean (Treatment)	8.98±3.12	8.9±3.11	8.94±3.11	2.45±0.2	2.48±0.2	2.39±0.01	23.05±0.4	22.98±0.3	23.01±0.3
L.S.D. (p=0.05)	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1

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

## Conclusion

Seed yield in the absence of pollinators was less as compared to Open pollination and plots caged with *A. mellifera*. 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 African sarson seed production, however, other insect pollinators need to be conserved for obtaining further higher yield.

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