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Research Article

POLLINATOR INSECTS AND THEIR IMPACT ON CROP YIELD OF MUSTARD IN KUSMA, PARBAT, NEPAL

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ABSTRACT

The diversity of insect pollinators and their impact on crop yield of mustard were studied in Kusma, Parbat, Nepal from December 2018 to April 2019 in four blocks with each having 12 m^2 areas. Two plots; treatment and control, were established in each block. Insect diversity was observed from 8 to 16 hrs, with the interval of an hour for three consecutive months (Jan-Feb). Eighty mustard plants were randomly selected, 40 from each plot just before flowering to find the impact of insect pollination on crop yield and these selected plants were examined for various qualitative and quantitative parameters. Altogether 16 species of pollinator insects belonging to five orders and nine families were recorded. Hymenoptera (36 %) was the most abundant order visiting mustard flowers followed by Diptera (34 %), Coleoptera (17 %), Lepidoptera (12 %) and Heteroptera (1 %). The most abundant family was Apidae (35.64 %), followed by Syrphidae (31.84 %). *Apis cerana* and *Eristalis* sp. were the most important pollinator insects of mustard. Seven species were found foraging both on pollen and nectar, four species foraging only on nectar and remaining five as casual visitors. The peak foraging activities of majority of the insects were observed between 12 hr to 14 hr. A significant difference was observed in the number of pods (59.80 ± 1.967 and 70.47 ± 2.431), fruit set (70.55 ± 1.362 and 80.94 ± 0.638), number of seeds per pods (16.70 ± 0.248 and 19.30 ± 0.330), diameter of seed (0.133 ± 0.2547 and 0.275 ± 0.0051) and weight of 100 dry seeds (0.33 ± 0.058 and 0.48 ± 0.023) in control and treatment plots whereas, the difference was non-significant in case of pod length between control and treatment plots (P=0.163).

Keywords: Hymenoptera, Mustard, Mustard productivity, Pollinator diversity, Syrphidae

INTRODUCTION

Brassica campestris L. var *toria* belongs to the Cruciferae family, also known as mustard (APG, 2009). It has green foliage, leaves glabrous or slightly hispid when young, and the upper leaves are partially clasping the stem. Branches originate in the axial of the highest leaves on the stem, and each terminates in an inflorescence. The inflorescence is an elongated raceme; the flowers are pale yellow, densely clustered at the top with open flowers borne at or above the level of terminal buds, and open upwards from the base of the raceme (Downey *et al*., 1980). The mustard flower has four petals and six stamens of which two stamens are shorter than the style, but four others are longer. Nectar is excreted at the bases of the short stamens and ovary. Mustard is one of the important cash crops of Nepal, which occupies about 85 % of the total oilseed area in the country and it is a dominant winter season oilseed crop (Basnet, 2005), and the second-largest oilseed crop that plays a vital role to sustain the human consumption of cooking oil. It is prominent source of fats, protein and vitamins as compared to cereals and legumes in Nepalese diet (Chaudhary, 2001). It is mostly grown after monsoon maize in upland and after early rice in the lowland of Terai, inner Terai and mid-hills (Ghimire *et al.*, 2000).

Agricultural production forms one of the most important economic sectors where the quality of most crops is increased by pollination (Klein *et al.*, 2007). Pollination is an essential process in maintaining a healthy and bio-diverse ecosystem. Pollination not only improves the yield of the crop, but it also contributes to uniform and early pod setting (Abrol, 2007). A wide variety of organisms can act as pollinators, including birds, bats, other mammals and insects (Willmer *et al*., 1994), with insects being the most common. Pollination is an essential supporting ecosystem service required by the majority of flowering plants; it has been estimated that 87.5 % of angiosperms require biotic pollination (Ollerton *et al.*, 2011) and that 62 % of these flowering species are limited in reproduction by the amount of pollen they receive (Burd, 1994). Insects constitute one of the primary groups of pollinating agents, since the association between insects and flowers is well established.

Mustard is a cross-pollinated crop and requires sufficient pollinating agents for better pollination and seed production. The mustard flower attracts a wide range of insect species (Stanley *et al.*, 2013) and especially pollen and nectar-feeding insects due to its bilateral, bright yellow flowers (Abrol, 2007). Various pollinating insect groups of mustard crops belong to the orders Hymenoptera, Diptera, Coleoptera, Lepidoptera, Thysanoptera, Hemiptera and Neuroptera (Mitra *et al.*, 2008). Studies have shown that insect pollination increased pollen deposition in mustard crops leading to expanded fruit set and seed production per plant, and decreased the variance of seed sets, and also enhanced better quality, uniform ripening and plant vigour (Thapa, 2006; Klien *et al*., 2007).

Pollination in mustard by honeybees *Apis cerana* and *A. mellifera* increased oil content by 3.17 % and 1.44 %, respectively, over open pollination and by 6.86 % and 5.07 % over caged plants (Dhakal, 2003). Several field investigations have reported that honeybee pollination on mustard significantly elevated the pod set and productivity, which hymenopteran insects account for 92.3 % of all the visiting insects, and 99.8 % of which are *Apis mellifera* (Annelise *et al.*, 2011; Shakeel & Inayatullah, 2011).

Pollination by insects identifies as an essential ecosystem service. Although many studies on the diversity of pollinator insects of mustard and other crops have been carried out, very little information is available on the extent to which insects contribute to crop seed via pollination. Low pollinator abundance and diversity have been appearing in different parts of the world (Kasina *et al*., 2015). Knowledge of specific pollinators of mustard crops is limited in the context of Parbat district although its pollination requirements have been studied in other parts of the country.

It is expected that the present study would provide baseline information to plan future research in the field of pollination. Moreover, the result of this study will ultimately be helpful for the farmers of the study area to increase the production of mustard through the management and conservation of pollinator insects. Therefore, this study was carried out to explore insect pollinators of mustard and their impacts on crop yield.

MATERIALS AND METHODS

Study area

The study was conducted in the mustard field at Thulipokhari-12 Kusma, Parbat ($83^0$42' E and $28^0$11' N) district from December 2018 to April 2019. This region covers the mid-hill area, which is 1449 m asl.

Observation and collection of insects

Insect diversity was observed from 8 hr to 16 hr at the interval of an hour, after the onset of flowering for three consecutive months (Jan-Mar). Insects were captured using the sweeping net as well as a handpicking method. Four spots of 1 m^2 area were selected randomly to know the foraging activities of insects, and the numbers of insects visiting each square meter area were counted for a minute for each period as adopted by Soliman *et al.* (2015). The insect was categorized into three groups based on their foraging sources. Pollen and nectar-feeding insects have pollen in their body parts such as wings, legs, mouth while nectar-feeding insects lack pollen in their body parts and a casual visitor insects visit mustard flower irregularly and accidentally transfers pollen.

Experimental design

Within the mustard field, four blocks (replication) each of size 12 m^2 were established by purposive sampling technique considering homogenous and continuous crop cover maintaining one meter distance between replication. Each block was divided into two plots, treatment and control. Plot size was maintained as 3×2 m. One treatment plot was left open so that flowers were accessible for self, wind and insect pollination while control plot was enclosed by mosquito net made of nylon having mesh size 1.2 mm to prevent insect pollination, so the flowers were accessible to self and wind pollination only.

Analysis for crop yield of mustard

All together 80 mustard plants were selected randomly, 40 from each experimental plot to see the impact of insect pollination on crop and tagged them just before flowering. After the completion of flowering, all nets were removed, and tagged plants were left open in the field to ripen. Finally, all the plants from each plot were examined for various qualitative and quantitative parameters (i.e., number of pods per plant, percentage of fruit set, length of the pod, number of seed in each pod, the diameter of seed and mean weight of 100 dry seeds) before the farmer thresh the field.

The number of open flowers was counted from each of the tagged plants from all experimental plots during each field visit to estimate the number of flowers per plant. The topmost open flowers were marked with a coloured plastic tag at each visit to keep track of the flowers that have already been counted. This process was continued until the end of flowering. The number of seed per pod was counted before harvest to estimate the effect of pollination on the number of seeds per pod and to find the effect of pollination on fruit set following formula as shown in equation (1) was used as adopted by Devi *et al*. (2017).

$$\text{Fruit set \%} = (\text{No. of pods}/\text{No. of flowers}) \times 100\ \% \qquad (1)$$

The diameter of seed was measured using Axminster vernier calliper made from stainless steel with clear metric and imperial graduations. Similarly, the mean weight of 100 dry seeds was measured using an electrical balance of pan size 180×160 mm (0.1 g/ 15 kg)

Preservation and identification of specimens

The collected specimens were killed in killing jar containing ethyl acetate and preserved in 90 % alcohol. Butterflies were caught using a sweeping net and pinched in the thorax and were kept in an envelope made of tracing paper. The specimens were brought to the Central Department of Zoology, Entomology laboratory where setting and pinning of specimens was done for further identification. Pollinator insects other than butterflies were identified following Thapa (2015), Mitra *et al*. (2008), Kapur (1958), Borrer *et al.* (1964), Richards and Davies (1977). Butterflies were identified following Smith (2011).

Statistical analysis

The diversity of pollinator insect species was calculated using the Shannon-Weaver diversity index (H) (Shannon and Weaver, 1949)

$H = -\sum pi \times \ln(pi)$.

Where, pi = ni /N, ni is the number of individuals of the species and

$N = \sum ni$. ln = the natural log $\sum$ = the sum of calculations

To find the evenness of species Pielou's species evenness index (J) was used (Pielou, 1966).

$J = H/Hmax$

Where, $H = -\sum pi \times \ln (pi)$

Hmax = ln (n), n is the total species richness.

Relative abundance was used to show the family-wise and order-wise composition of pollinator insects.

Relative abundance (%) = (n/N) × 100

Where, n= Number of each individual

N= Total number of individual.

Data obtained in the present study were subjected to analysis of variance (ANOVA) with Tukey's post hoc test for mean separation for different parameters ($P < 0.05$).

RESULTS

Diversity, foraging sources and activities of insects

The pollinator insects of mustard included 16 species belonging to five different insect orders and nine families. Among them, Hymenoptera (36 %) was the most abundant insect order visiting mustard flowers followed by Diptera (34 %), Coleoptera (17 %), Lepidoptera (12 %) and Heteroptera (1 %), as shown in Fig. 1. Two hymenopteran families; Apidae (35.64 %) and Bombidae (0.51 %), two dipteran families; Syrphidae (31.84 %) and Muscidae (2.41 %), one coleopteran family Coccinellidae (16.67 %), three lepidopteran families; Nymphalidae (6.66 %), Pieridae (3.53 %) and Lycaenidae (1.23 %) and one heteropteran family Pentatomidae (1.38 %) were recorded.

The Shannon-Weaver diversity index (*H*) was calculated to be 2.2283, which indicate high diversity of pollinator insects in the mustard field, with Pielou's species evenness (*J*), estimated to be 0.8037. Among the recorded 16 species, seven species were found foraging on both pollen and nectar of mustard flowers. Of them, hymenopterans were the most common, followed by dipterans. Pollen and nectar-feeding insects comprised of *Apis cerana, A. florea, Bombus* sp., *Eristalis* sp., *Episyrphus balteatus, Musca domestica* and *Aglais caschmirensis*. Four species were found foraging on nectar such as *Pieris canidia, Vanessa cardui, Junonia lemonias* and *Lampiides boeticus.* The remaining five species were recorded as a casual visitor of the mustard flowers which comprised of *Coccinella undecimpunctuta, C. septempunctata*, *Neptis hylas*, *Eurema hecabe* and *Eurydem*a sps (Table 1).

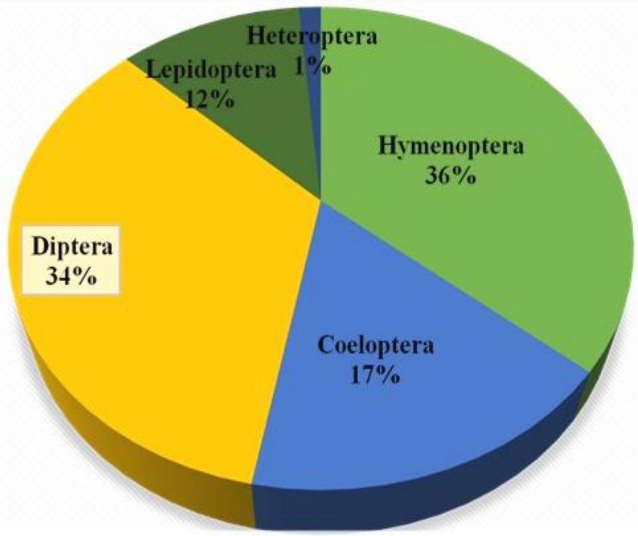


Fig. 1. Order-wise composition of insects

All the insect orders were found active throughout the day, but peak foraging activity timing was different for different orders. The peak foraging activities of the members of Hymenoptera and Diptera were observed between 12 hr to 14 hr, which was 12.88 individuals/1m^2/min and 13 individuals/1m^2/min, respectively. Butterflies were less active in the morning, and their activities reached peak at 14 hr with 2.55 butterflies/1m^2/min. Foraging activities of coleopteran insects remain relatively constant throughout the day, while that of heteropterans recorded peak at 14 to 16 hr (Fig. 2).

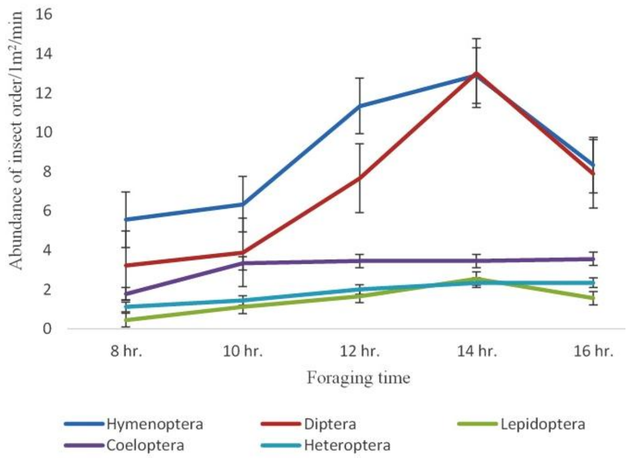
Effect of insect pollination on crop yield of mustard

A significant difference was observed in the number of pods ($F_{1,78}$=11.651, P=0.001), fruit set percentage ($F_{1,78}$=47.87, P=0.001), number of seeds per pod ($F_{1,78}$=39.526, P=0.001), diameter of seed ($F_{1,78}$= 498.339, P=0.002) and weight of 100 dry seeds between the seeds obtained from treatment and control plots ((P<0.05), as shown in Fig. 3. There was no significant difference in the length of the pod ($F_{1,78}$=1.981, P= 0.163) between control and treatment plot (Fig. 3).

Table 1. Insect pollinator diversity, their relative abundance and foraging sources

Insect name	Order	Family	Foraging source	Relative abundance (%)
Apis cerana Fabricius, 1793	Hymenoptera	Apidae	PN	24.35
Apis mellifera Linnaeus, 1758	Hymenoptera	Apidae	PN	11.28
Bombus sp.	Hymenoptera	Bombidae	PN	0.51
Coccinella undecimpunctuta Linnaeus, 1758	Coleoptera	Coccinellidae	C	7.69
Coccinella septempunctata Linnaeus,1758	Coleoptera	Coccinellidae	C	9.07
Episyrphus balteatus (De Geer, 1776)	Diptera	Syrphidae	PN	12.51
Eristalis sp.	Diptera	Syrphidae	PN	19.33
Musca domestica Linnaeus,1758	Diptera	Muscidae	PN	2.41
Aglais cashmiriensis Kollar,1848	Lepidoptera	Nymphalidae	PN	1.64
Pieris canidia Sparrman,1768	Lepidoptera	Pieridae	N	1.84
Vanessa cardui Linnaeus,1758	Lepidoptera	Nymphalidae	N	2
Junonia lemonias Linnaeus,1758	Lepidoptera	Nymphalidae	N	1.69
Lampides boeticus Linnaeus1767	Lepidoptera	Lycaenidae	N	1.23
Neptis hylas Linnaeus, 1758	Lepidoptera	Nymphalidae	C	1.33
Eurema hecabe Linnaeus,1758	Lepidoptera	Pieridae	C	1.69
Eurydema sp.	Heteroptera.	Pentatomidae	C	1.38

Note: PN= Pollen and nectar, N=Nectar and C=Causal visitor

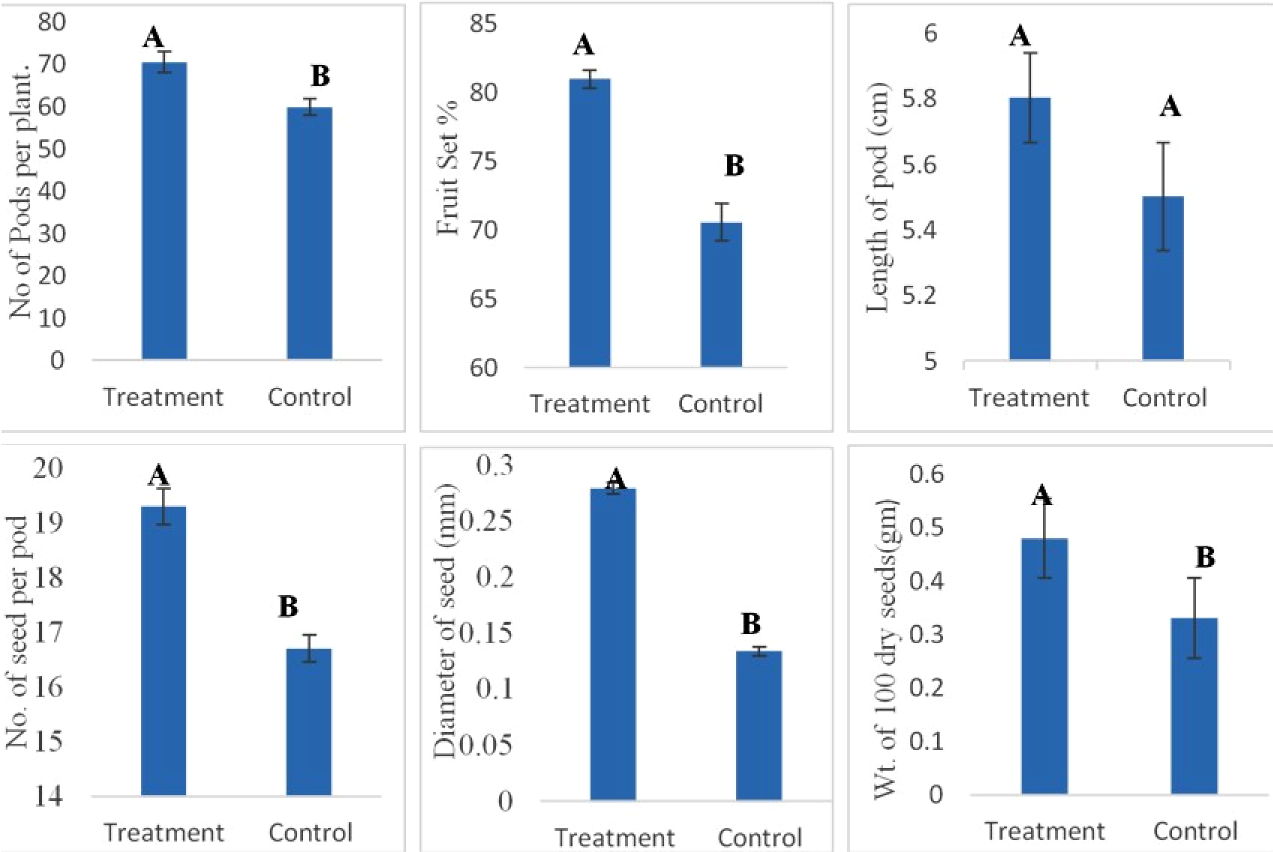


Note: I = Mean + S.D

Fig. 2. Foraging activities of different orders of pollinator insects

DISCUSSION

Insect pollination has a crucial impact on the qualitative and quantitative yield attribute on cruciferous crops (Sihaj, 2017). Different insect groups are responsible for pollinating the mustard crop. This study revealed that pollinator insects of mustard comprised of 16 different species under five separate orders. Among them, Hymenoptera (36 %) was the most abundant insect order visiting mustard flowers followed by Diptera (34 %), Coleoptera (17 %), Lepidoptera (12 %) and Heteroptera (1 %). Similar results on insect visitors were reported by Dhakal (2003) on the rapeseed field in which five orders of insect were recorded, and Hymenoptera was the most abundant. Devi *et al*. (2017) also recorded a total of 88 insects belonging to 63 genera under 31 families and nine orders visiting mustard flowers, among which, Hymenoptera was the most abundant. But low species number in the present study may be due to the fact that research was carried out for one season only compared to two years research of Devi *et al*. (2017). The results of the present investigation also follow similar trends as reported by early workers. Kunjwal *et al.* (2014) observed a total of 30 species belonging to four orders Hymenoptera, Diptera, Lepidoptera, and Coleoptera visiting mustard, *B. juncea* flowers. Among them, Hymenoptera were the primary insect pollinators. Nine families of insect pollinators were recorded, and among them, family Apidae was the most abundant, followed by Syrphidae.



Note: Bars with the same letter are not significantly different at the 5% significance level

Fig. 3. Mean+S.E. comparison of different crop yielding parameters between treatment and control plot

These findings are similar to Pudasaini *et al.* (2015) which recorded seven families of Hymenoptera with Apidae as a dominant family. *A. cerana* was the most abundant pollinator insect in this study which is similar to the findings of Mishra and Gupta (1988). Pudasaini *et al*. (2015) and Kunjwal *et al*. (2014) argued that among many insect flower visitors *A. mellifera* was the most common pollinating species of mustard. Higher abundance of *A. cerana* in this study was due to the presence of *A. cerana* colonies rearing by farmers near the study area. Butterfly species such as *Aglais caschmirensis, Pieris canidia, Vanessa cardui, Junonia lemonias, Lampiides boeticus, Neptis hylas* and *Eurema hecabe* were less abundantly present. These findings are similar to those reported by Bhowmik *et al*. (2014) and Pudasaini *et al.* (2015). Butterflies were mostly limited to floral visitors, both in abundance and diversity (Ali *et al*., 2011). Hoverflies species like *Eristalis* sp. and *Episyrphus balteatus* were also present in relatively high abundance. Surprisingly, *Coccinella undecimpunctuta* and *C. septempunctata* was reported to be quite high, and it was probably because of their predatory action on the aphids that are commonly found on mustard.

Among the recorded 16 species, seven species were found foraging on both pollen and nectar of mustard flowers. Of them, hymenopteran species were the most common, followed by dipterans. Four species were found foraging on nectar which mostly included butterflies, and the rest of the five species were recorded as casual visitors of the mustard flowers. These findings are in line with Soliman *et al*. (2015) who reported all hymenopteran visitors as both pollen and nectar foragers. In contrast, all dipterans and lepidopterans were nectar foragers and only accidentally transferred pollen, and coleopterans were casual visitors of canola flowers and did not participate in nectar or pollen foraging.

All the insect groups were found active throughout the day, but their peak foraging activity timing was different for different groups. The peak foraging activities of the members of Hymenoptera and Diptera were observed between 12 hr to 14 hr with 12.88 individuals/1m^2/min and 13 individuals/1m^2/min, respectively. Butterflies were less active in the morning, and their activities reached a peak at 14 hr with 2.55 butterflies/1m^2/min. Foraging activities of coleopterans remained reasonably constant throughout the day, whereas heteropteran species foraging became peak at 14 hr to 16 hr. These findings are similar to Bhowmik *et al*. (2014) who observed the peak foraging activity of the members of Hymenoptera, Coleoptera, Lepidoptera and Diptera at 12 hr, 13 hr, 12 hr and 14 hr respectively. But, Roy *et al*. (2014) found the peak foraging activities of the members of Hymenoptera, Coleoptera, Diptera and Hemiptera at 14 hr and that of Lepidoptera during 12 hr. Honeybee activities were recorded maximum between 12 hr to 14 hr as it provides more floral rewards in terms of pollen which is regarded as source of protein.

A significant difference was observed in the number of pods between treatment and control plots. The effect of insect pollination increases pod number up to 17.84 % in the treatment plot. The present findings are in line with the results of Kumari *et al*. (2013) who reported that the maximum number of pods per plant in *B. juncea* in open-pollinated plots which were significantly higher than that in *A. mellifera* pollinated plots and the lowest were observed in pollinators' exclusion. There was a significant difference in the fruit set percentage between treatment and control plots with 80.94 % and 70.55 % respectively, with an increase of 14.72 % fruit set between control and treatment plots. The results of the present investigation are in conformity with the earlier recorded observations of Tara and Sharma (2010) on *B. campestris* which revealed that the seed set was less (79.96 %) in a controlled experiment as compared to open-pollinated flowers (88.05 %).

There was no significant difference in the length of pods between treatment and control plots. It was also determined that the length of the pod increased by 5.4 %, but insect pollination has no role in it. There was a significant difference in number of seeds per pod, the diameter of seeds and weight of 100 dry seeds between treatment and control plot. These findings are similar to Devi *et al*. (2017) who revealed that the seeds per pod and 1000 seed weight were significantly higher in open pollination (15.49 and 15.59 seeds per pod) followed by hand pollination (14.25 and 14.18 seeds per pod) during 2015 and 2016, respectively. Significantly less seed 12.16 and 12.14 seeds per pod were recorded in pollinators' exclusion over the two years of study. The mean thousand weight of mustard seed was significantly more in open modes of pollination (3.11 and 3.12 g) followed by in hand pollination (2.95 and 2.98 g) during 2015 and 2016. The lowest mean thousand seed weight (2.36 g) was recorded in pollinator's exclusion over the two years of study (Devi *et al.* 2017). The results of the present investigation also corroborate the observations made by Singh and Singh (1992) who reported that insect-pollinated plots produced three times heavier seed then self-pollinated plants in *B. campestris*. The present findings also supported the results of Kamel *et al.* (2015) who observed that the weight of 1000 seeds was higher in open-pollinated plants (3.13 g) than those of caged plants (2.4 g) in *B. napus*.

CONCLUSION

It was found that the yield and quality of mustard increased significantly by pollination of honeybees and other insect pollinators. It may be stated that a decline in the species diversity could pose a severe threat on crop pollination and seed production. Though it was a preliminary attempt to make a report of insect pollinators of mustard crops from Kusma, Parbat, it will undoubtedly help the future workers as a baseline data of pollinators in the area. Hence, pollinator's friendly cultivation practices should be practiced for the conservation and management of insect pollinators for higher production and productivity of mustard.

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