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Original research article

Diversity and Abundance of Insect Pollinators Affecting Seed Production in Mustard (*Brassica napus* L.)

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Abstract The studies revealed that honeybees *Apis dorsata*, *A. mellifera*, *A. cerana* and *A. florea* were the dominant flower visitors and comprised of 83.20% of the total flower visiting insects. Their activities increased with temperature and sunshine and decreased with relative humidity, wind speed, rainfall and evaporation. Foraging population of honeybees were significantly and positively correlated with maximum temperature and minimum temperature and negatively with relative humidity in the morning. The other important insects frequenting mustard flowers were *Andrena* spp. *Danaus plexippus*, *Pieris brassicae*, *Musca* sp. and *Syrphus* sp; the latter group of insects mostly collected nectar and frequented at interrupted hours and were not considered as dependable pollinators. The data revealed that hand pollination resulted in significantly higher siliquae per plant than those excluded from insect visits. The caged plants on an average had 448.94 siliquae per plant whereas those left for open pollination resulted in 769.05 siliquae per plant. Open pollination was found to be superior to both the treatments as the number of siliquae produced was much higher 944.54. Similarly, Seeds/20 Siliquae, 1000 seed weight, Seed yield per plant, Seed yield per plot and Seed germination (%) was significantly higher in bee pollinated plots compared to open pollination and self pollination. Open pollination and hand pollination resulted in 118 and 76 per cent enhanced seed yield in mustard as compared to control.

Keywords Insect pollinators, *Apis dorsata*, *A. florea*, *A. mellifera*, *A. cerana*, Foraging behavior, Mustard, Seed production

INTRODUCTION

Oilseed crops have been the backbone of agricultural economy of India from time immemorial and play a vital role in agricultural industries and trade. On the oilseed map of the world, India occupies a prominent position, both in regard to acreage and production. Oilseeds form the second largest agricultural commodity after cereals in India, sharing 14 per cent of the country's gross cropped area and accounting for nearly 5 per cent of the gross national product and 10% of the value of all agricultural products. Today these crops are cultivated on about 16.5 million hectares, with total production of 10 million tons which constitutes approximately one-tenth of the total cultivated area in India. About 14 million persons are engaged in the production of oilseeds and another one million in their processing (Hegde, 2000). Despite of good agronomic practices the level of productivity of oilseeds in India is far below than the average productivity in the world. The low productivity of oil seeds/oil crops can be attributed to various factors such as heavy infestation of pests and diseases and the lack of proper pollination (Rao *et al*., 1981; Free, 1993; Abrol, 2007, 2008, 2009).

Amongst various oilseed crops, rapeseed and mustard are the important crops which are dependent upon or benefited by insect pollination. Approximately, 95% of species of Crucifers require cross-pollination, although

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some cauliflower varieties are self-fruitful. In male sterile oilseed rape (*Brassica napus*), yields of the species were increased by honey bee pollination (Westcott and Nelson, 2001). Introduction of hives of *A. mellifera* in controlled pollination helps to increase the production of crops such as rapeseed (*B. napus*) (Sabbahi *et al*., 2006), since this plant is characterized by the production of abundant pollen and good quality nectar at relatively high concentrations of sugar, in flowers with a color and structure which are attractive to insects, particularly bees (Smith, 2002; Sabbahi *et al*., 2006).

Most of the oilseed crops are cross pollinated and adequate pollination is vital for any significant seed production. An increase in seed yield as a result of insect pollination has been reported in mustard (Mohammed, 1935; Latif *et al*., 1960). Latif *et al*. (1960) found that *Apis cerana* colonies near sarson and toria fields increased the seed yield by 60%. Similar increase in field has been reported in oil seed rape (Langride and Goodman, 1975; Kisselhagen, 1977) and *Brassica campestris* var. Jambuck (Kubisova *et al*., 1980).

So far, honeybees alone are considered as significant pollinators on *Brassica* crop, however a number of other insects also visit on this crop during flowering period as reported by various workers from different parts of the country (Kakar, 1981; Thakur *et al*., 1982; Bhalla *et al*., 1983; Mishra *et al*., 1988; Prasad *et al*., 1989; Priti and Gupta, 1992; Kumar *et al*., 1994; Singh *et al*., 1994; Sinha *et al*., 1994; Chaudhary, 2001; Singh *et al*., 2004; Abrol, 2010). Floral morphology is well known to affect the efficiency of pollen removal and deposition during pollinator visits (Campbell, 1989; Murcia, 1990; Young and Stanton, 1990; Nishihiro *et al*., 2000; Yang *et al*., 2002; Kudo, 2003) and to have the potential to increase or decrease seed production in both self-fertilizing and out crossing plants.

Earlier studies on the effects of pollinator insects to seed set of Cruciferae had been reported. In male sterile oilseed rape (*B. napus*), yields of the species were increased by honey bee pollination (Westcott and Nelson, 2001; Steffan-Dewenter, 2003). Similarly, in sarson (*B. campestris*), insects pollination increase the seed yield, caused formation of well-shaped, larger grain, and more viable seed (Khan and Chaudhry, 1995).

Atmowidi *et al*. (2007) analysed the diversity of pollinator insects and its effect to seed set of mustard (*Brassica rapa*) planted in agricultural ecosystem in West Java. At least 19 species of insects pollinated the mustard, and three species, i.e. *Apis cerana*, *Ceratina* sp., and *Apis dorsata* showed a high abundance. The higher abundance and species richness of pollinators occurred at 08.30−10.30 am and the diversity was related to the number of flowering plants. Insect pollinations increased the number of pods, seeds per pod, seed weights per plant, and seed germination. Pollinators provide key ecosystem services to both natural and agro-ecosystems.

Despite of good agronomic practices the level of productivity of oilseeds in India is far below than the average productivity in the world. The low productivity of oil seeds/oil crops can be attributed to various factors such as heavy infestation of pests and diseases. Out of the various factors, one of the most important factors for low production of oil seed crop is due to the failure of seed setting attributable to the lack of proper pollination (Rao *et al*., 1980; Free, 1993; Abrol, 2007, 2008, 2009). Pollination is one of the most important natural factors enhancing crop production. Among the various pollinating agents, insect pollinators play a predominant role in increasing the yield of oil seed crops. Almost one third of total cropped area under oil seeds has been reported to be entomophilous (Mishra *et al*., 1988). Insect pollinators not only enhance the yield of the crop but also contribute uniform and early setting. Bisht *et al*. (1980) found that flowers of rapeseed visited by *Apis* species had higher pod set, more number of seeds per pod and weight of seed was also higher than those deprived of pollinator visit. Mishra *et al*. (1988) found that in *Brassica campestris* percent pod setting, number of pods per plant and proportion of healthy seeds was significantly higher in open pollinated flowers than in net caged and muslin bagged ones. Similarly, average weight of seeds and oil content was higher in open pollinated flowers. *A. cerana* was the most common pollinating species. The other pollinators observed included *A. mellifera*, syrphid flies etc.

Tara and Sharma (2010) studied the role of honeybees on yield parameters of *Brassica campestris* var. *sarson* and found that percent fruit set, number of seeds per siliqua and mean seed weight of 100 seeds were significantly higher in open pollinated than in controlled ones. Moreover, seeds of open pollinated plants were larger in size and viable than controlled ones. The crop was visited by many insect pollinators but *A. dorsata* followed by *A. mellifera* and *A. cerana* were observed to be the most

common pollinating species.

Among different oilseed crops, canola *Brassica napus* L. belonging to the Brassicaceae (Cruciferae) family, also known as the mustard family, is a popular oilseed cash crop. Many aspects of its production technology are needed to be exposed. The average yield of canola is less than the potential of the existing cultivars due to many factors which affect the yield, but insect pests are the most important one. *Brassica napus* is an important source of edible oil cultivated in many countries throughout the globe. Mustard is cultivated in around 6 million hectares of land in India. The projected demand for oilseeds in India alone is around 34 million tons by 2020, of which about 14 million tons (41%) is to be met by mustard alone (Yadava and Singh, 1999).

Oilseed rape is very attractive to honey bees, bumblebees and solitary bees due to mass-flowering crop and abundance of nectar and pollen. It provides a food resource of considerable value in sustaining bee populations in agro ecosystems at a time when bees are in decline. The studies were conducted to determine pollinators of mustard, *Brassica napus* L. at various crop stages and to evaluate the impact of pollinators on crop production.

MATERIALS AND METHODS

1. Study site

The present investigation was carried out in the experimental farm of Division of Entomology, Sher-e-Kashmir university of Agricultural Sciences & Technology main campus Chatha, Jammu located 15 kms from Jammu city (32°402 N latitude and 74°822 E longitude) with an altitude of 293 meters above mean sea level in the sub-tropical foothill lands of Shivalik in Jammu and Kashmir during Rabi-2014-15. The climate of the area is typically subtropical. The study was made on Mustard (*Brassica napus* L.) Variety DGS-1 raised in the 2nd fortnight of October. The spacing adopted was 45 × 10 cm. All the recommended agronomic practices were followed for raising the crop.

2. Sampling of pollinating insects

1) Recording of parameters

The study was conducted to determine the abundance and diversity of insects frequenting radish flowers, their foraging ecology and impact on seed production. Observations on insect pollinators commenced during 2nd week of March. Number of insect pollinators of each species visiting mustard were recorded at 10 per cent flowering till its complete cessation. For this purpose, hourly observations on insect counts were made at the beginning of each hour right from commencement to the cessation of activities. Observations were made at weekly intervals from 8.00 to 1800 hours right from the commencement of the flowering till their complete cessation. Five plots of 1×1 m^2 were marked in the crop and insects recorded by visual counting method from each side of the plot for one minute at hourly intervals in the beginning of each hour (Abrol, 2006). The mean of these observations constituted reading for each hour. Simultaneously, air temperature and relative humidity were recorded with a dry and wet bulb thermometer kept in shade. Sunshine, windfall, windspeed, rainfall and evaporation were obtained from observatory of Metrological observatory of Agronomy Department located near the experimental field. Besides, the insect visitors of mustard were also collected using hand net and preserved in Absolute alcohol for further identification.

3. Impact on quality and quantity of mustard seed production

The role of insect pollination on quality and quantity of fruit production was evaluated using following techniques. 1) Hand pollination: Emasculation of pollen from anthers to stigma of receptive flowers, 2) Self-pollination: For this purpose, counted number of plants were enclosed in pollination cages 5 × 3 meters, and 3) Open pollination: Counted number of plants were left for open pollination. On maturity the harvested from each treatment are compared for quantity and physico-chemical characteristics which included Siliqua per plant, Seeds/20 Siliquae, 1000 seed weight, Seed yield per plant and Seed germination (%). Yield and yield attributing characters were determined using standard procedures and finally the yield is expressed in kg/ha.

4. Statistical analysis

The recorded data were analyzed for their variation between different treatments using Statistical Package for

the Social Science (SPSS) and O.P Stat.

RESULTS

Mustard (*Brassica napus* L.) is one of the important oil seed crops grown in India. The studies were conducted to determine the associated pollinators, their role in crop production. The result obtained is presented under the following heads.

1. Diversity of insect pollinators on mustard flowers

The data presented in Table 1 shows that mustard flowers attracted wide variety of insects belonging to 4 orders, 7 families, 9 genera and 14 species. Of all these insects, honeybees *Apis dorsata*, *A. mellifera*, *A. cerana,* and *A. florea* were the dominant flower visitors and comprised of 83.20% of the total flower visiting insects. Their abundance was in the order: *A. mellifera* > *A. dorsata* > *A. cerana* > *A. florea*. The other important insects frequenting mustard flowers were *Andrena* spp., *Danaus plexippus*, *Pieris brassicae*, *Musca* sp., and *Syrphus* sp.; the latter group of insects mostly collected nectar and frequented at interrupted hours and were not considered as dependable pollinators. The detailed investigations were, therefore, carried out on honeybees which frequented mustard flowers in large numbers throughout the day and were anatomically suited for pollen collection.

2. Seasonal abundance of pollinators on mustard flowers

The data presented in Table 2 shows the seasonal abundance pattern of honey bees *A. dorsata*, *A. mellifera*, *A. cerana*, *A. florea,* and other flower visitors in relation to abiotic factors such as maximum temperature, minimum temperature, morning and evening relative humidity, wind speed, sunshine, rainfall and evaporation. The observations were made at biweekly intervals during the entire flowering periods for five different days commencing from 0800 to 1800 hours at hourly interval. The data recorded during different hours was pooled to obtain observation for each day. The data in general revealed that activity of honey bees increased with temperature and sunshine and decreased with relative humidity, wind speed, rainfall and evaporation. However, the species differences in the population dynamics of bees were evident as of all the honey bees *A. mellifera* was most abundant followed by *A. dorsata* > *A. cerana* > *A.*

Table 1. Insect visitors and their percentage proportion on mustard flowers during February–March 2015

Order	Family	Species	Percentage composition	Total
Hymenoptera	Apidae	*Apis mellifera*	31.20	83.20
		A. dorsata	24.20	
		A. cerana	22.40	
		A. florea	5.40	
	Andrenidae	*Andrena leaena*	1.80	1.80
		A. ilerda	1.06	1.06
Lepidoptera	Danaidae	*Danaus plexippus*	2.00	2.0
	Pieridae	*Pieris rapae*	1.80	1.80
Diptera	Muscidae	*Musca* spp.	3.05	3.05
	Syrphidae	*Eristalis* spp.	1.40	6.00
		Metasyrphus corollae	1.60	
		Episyrphus balteatus	1.20	
Coleoptera	Coccinellidae	*Coccinella septumpunctata*	1.00	2.80
		C. sexmaculata	1.80	

florea. The influence on the population dynamics of bees as maximum population of all the bee species were observed after 12 days of flowering.

3. Correlation coefficient (r) between bee activity and weather parameters

Analysis of data (Table 3) revealed that foraging population of *A. florea* was highly significantly and positively correlated with maximum temperature and minimum temperature and negatively with relative humidity in the morning and rainfall but was no significant with relative humidity in the evening and wind speed. However, the foraging population of *A. florea* correlated significantly and positively with sunshine hours. Similarly, population of *A. dorsata* was significantly and positively correlated with maximum and minimum temperature and sunshine hours and negatively with relative humidity and rainfall. It was no significant with evening relative humidity and wind speed. In case of *A. cerana*, the population was highly significant and positively correlated with maximum temperature, minimum temperature where as non significant with wind speed, sunshine hours and relative humidity in the evening but was negatively correlated and highly significant with rainfall and relative humidity in the morning. Same trend was observed for *A. mellifera* and other pollinators. This clearly reveals that all the four species of honeybees and other pollinators varied in their response to climatic conditions prevailing at a unit time.

4. Impact of pollination treatments on seed yield of mustard

The data presented in Table 4 revealed that hand pollination resulted in significantly higher siliquae per plant than those excluded from insect visits. The caged plants on an average had 477.12 siliquae per plant whereas those left for open pollination resulted in 737.86 siliquae per plant. Open pollination was found to be superior to both the treatments as the number of siliquae produced was much higher 737.86. Similarly, Seeds/20 Siliquae, 1000 seed weight, Seed yield per plant, Seed yield per plot and Seed germination (%) was significantly higher in bee pollinated plots compared to open pollination and self pollination. Open pollination and hand pollination

Table 2. Seasonal abundance of different pollinators on mustard in relation to weather parameters

Days of observation (DAS)	No. of pollinators					Weather parameters							
	A. florea	*A. dorsata*	*A. cerana*	*A. mellifera*	Others	Max T	Min T	RH1	RH2	WS	SS	RF	EV
4	0.67	6.71	5.67	8.59	4.67	23.0	4.0	77	37	2.2	6.9	0.0	1.6
8	1.31	7.33	6.43	9.65	5.25	11.4	6.2	94	90	1.8	0.0	0.0	0.0
12	2.19	8.21	6.71	11.27	5.81	9.8	6.4	97	89	1.6	0.0	0.0	0.4
16	2.44	8.79	7.51	12.13	6.21	16.6	9.6	100	60	4.2	0.8	5.4	0.0
20	2.27	8.55	7.33	11.79	5.60	19.4	4.0	93	49	0.6	7.9	0.0	1.4

DAS - Days after flowering, Max T - maximum temperature, Min T - minimum temperature, RH1 - relative humidity morning, RH2 - relative humidity evening, WS - wind speed, SS - sunshine hours, RF - rainfall, EV - evaporation.

Table 3. Correlation coefficient matrix between bee pollinators and weather parameters

S. No.	Name of the pollinator	Correlation coefficient (r) with						
		Max T	Min T	RH1	RH2	WS	SS	RF
1.	*A. florea*	0.265**	0.485**	−0.755**	0.155	0.192NS	0.231*	−0.606**
2.	*A. dorsata*	0.246*	0.580**	−0.880**	0.160	0.185NS	0.204*	−0.504**
3.	*A. cerana*	0.257**	0.569**	−0.788**	0.041	0.178NS	0.151NS	−0.645**
4.	*A. mellifera*	0.342**	0.528**	−0.867**	0.228*	0.124NS	0.249*	−0.504**
5.	Others	0.416**	0.785**	−0.867**	0.300**	0.132NS	0.196*	−0.707**

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

Table 4. Effect of pollination on yield parameters of mustard

Treatments	Siliqua per plant	Seeds/ 20 Siliquae	1000 seed weight	Seed yield per plant	Seed germination (%)	Folds increase in seed yield over control	Percentage increase in seed yield over control
Hand pollination	642.38	110.68	11.75	43.31	92.40	1.34	74.00
Open polination	737.86	120.60	12.68	53.63	94.20	1.54	64.00
Self pollination	477.12	87.40	10.96	24.63	81.39	–	–
Mean	619.12	106.22	11.79	40.52	89.33		
Sem ±	2.40	0.88	0.76	0.83	1.76		
CD	9.43	3.47	1.38	3.27	6.93		
CV	1.23	1.44	2.32	3.12	3.42		

resulted in 64.00 and 74 per cent enhanced seed yield in mustard as compared to control.

DISCUSSION

1. Diversity of insect pollinators on mustard flowers

The studies revealed that mustard flowers attracted a wide variety of insects. Of all the insects, honeybees *A. dorsata*, *A. mellifera*, *A. cerana,* and *A. florea* were the dominant flower visitors and comprised of 83.20% of the total flower visiting insects. Their abundance was in the order: *A. mellifera* > *A. dorsata* > *A. cerana* > *A. florea*. The other important insects frequenting mustard flowers were *Andrena* spp., *Danaus plexippus*, *Pieris brassicae*, *Musca* sp., and *Syrphus* sp. In earlier studies also, honeybees have been reported as significant pollinators on *Brassica* crop, however a number of other insects also visit on this crop during flowering period as reported by various workers from different parts of the country (Kakar, 1981; Thakur *et al*., 1982; Bhalla *et al*., 1983; Mishra *et al*., 1988; Campbell, 1989; Prasad *et al*., 1989; Murcia, 1990; Young and Stanton, 1990; Priti and Gupta, 1992; Kumar *et al*., 1994; Sinha *et al*., 1994; Nishihiro *et al*., 2000; Chaudhary, 2001; Yang *et al*., 2002; Kudo, 2003; Singh *et al*., 2004).

2. Seasonal abundance of pollinators on mustard flowers

The data on seasonal abundance revealed that activity of honey bees increased with temperature and sunshine and decreased with relative humidity, wind speed, rainfall and evaporation. However, the species differences in the population dynamics of bees were evident as of all the honey bees *A. mellifera* was most abundant followed by *A. dorsata* > *A. cerana* > *A. florea*. Atmowidi *et al*. (2007) analysed the diversity of pollinator insects and its effect to seed set of mustard (*Brassica rapa*) planted in agricultural ecosystem in West java. At least 19 species of insects pollinated the mustard, and three species, i.e. *A. cerana*, *Ceratina* sp., and *A. dorsata* showed a high abundance. The higher abundance and species richness of pollinators occurred at 08.30–10.30 am and the diversity was related to the number of flowering plants.

3. Correlation coefficient (r) between bee activity and weather parameters

Analysis of data revealed that foraging population of *A. florea* was highly significantly and positively correlated with maximum temperature and minimum temperature and negatively with relative humidity in the morning and rainfall but was nonsignificant with relative humidity in the evening and wind speed. Similarly, population of *A. dorsata*, *A. cerana,* and *A. mellifera* was significantly and positively correlated with maximum and minimum temperature and sunshine hours and negatively with relative humidity and rainfall.

In general, each bee pollinator has specific ecological threshold for foraging activity which differ inter and intra specifically depending upon the level of adaptation of a given species in an environment (Burill and Dietz, 1981; Abrol and Kapil, 1986). The bee activity increased with temperature but was not affected by relative humidity and vapour pressure. Nunez (1977) found that in case

of *A. mellifera*, morning activity was related to nectar flow and in the evening it was correlated with the photoperiod. Iwama (1977) found that the interaction between temperature and light intensity was responsible for the flight activity of *Tetragonisca angustica*. Abrol and Kapil (1986) found that light intensity and solar radiations were important factors controlling flight activity of *Megachile lanata*.

4. Impact of pollination treatments on seed yield of mustard

The data revealed that hand pollination resulted in significantly higher siliquae per plant than those excluded from insect visits. Open pollination and hand pollination resulted in 118 and 76 per cent enhanced seed yield in radish as compared to control.

An increase in seed yield as a result of insect pollination has been reported in mustard (Mohammed, 1935; Latif *et al*., 1960). Latif *et al*. (1960) found that *A. cerana* colonies near sarson and toria fields increased the seed yield by 60%. Similar increase in field has been reported in oil seed rape (Langride and Goodman, 1975; Kisselhagen, 1977) and *Brassica campestris* var. Jambuck (Kubisova *et al*., 1980).

Bisht *et al*. (1980) found that flowers of rapeseed visited by *Apis* species had higher pod set, more number of seeds per pod and weight of seed was also higher than those deprived of pollinator visit. Mishra *et al*. (1988) found that in *Brassica campestris* per cent pod setting, number of pods per plant and proportion of healthy seeds was significantly higher in open pollinated flowers than in net caged and muslin bagged ones. Similarly, average weight of seeds and oil content was higher in open pollinated flowers. *A. cerana* was the most common pollinating species. The other pollinators observed included *A. mellifera*, syrphid flies etc.

Tara and Sharma (2010) studied the role of honeybees on yield parameters of *Brassica campestris* var. *sarson* and found that percent fruit set, number of seeds per siliqua and mean seed weight of 100 seeds were significantly higher in open pollinated than in controlled ones. Moreover, seeds of open pollinated plants were larger in size and viable than controlled ones. The crop was visited by many insect pollinators but *A. dorsata* followed by *A. mellifera* and *A. cerana* were observed to be the most common pollinating species.

CONCLUSIONS

Most of the crucifers require cross-pollination, although some cauliflower varieties are self-fruitful. Mustard are self-incompatible plants that require cross-pollination, making pollinators crucial. The availability of sufficient pollinators available under natural conditions may vary in different locations. Such studies on pollinator diversity and abundance are helpful to supplement the areas with managed colonies of honeybee for obtaining optimum yields in terms of seed quantity, weight, and harvest, conserving and enhancing native pollinators - especially the bees- can promote the mustard productivity. Furthermore, findings of current study can be widespread over large geographical range as *A. dorsata* and many species of bees are distributed widely across India, Pakistan, Oman, Sri Lanka, Iran, Afghanistan, Indonesia, Philippines, and southern China. Further studies should focus other hundreds of native pollinators for their pollination potential coupled with basic investigations on their life cycle and biology e.g. nesting biology and host plant range.

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