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RESEARCH ARTICLE

DIVERSITY OF INSECT POLLINATORS AND THEIR EFFECT ON THE CROP YIELD OF *BRASSICA JUNCEA* L., NPJ-93, FROM SOUTHERN WEST BENGAL

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ABSTRACT

Study was carried out from the end of 2011 till mid of 2013, from South Bengal region, to explore the diversity of insect pollinators of *Brassica juncea* and their influence on the seed yield and quality. Insect flower visitors / pollinators were recorded at time periods starting from 07.00 to 17.00 hrs. 19 insect species belonging to 13 families under 4 orders *viz.* Hymenoptera, Coleoptera, Diptera and Lepidoptera, were recorded at the day time, among which 4 species (*Apis dorsata, A. cerena, A. mellifera and Vespa sp.*) were both pollen and nectar collectors, 14 species were only nectar collectors and rest of them were just, visitors. Peak activity of the insects was mainly observed from 11 am till 2 pm. Qualitative and quantitative effects of pollination on the percent fruit set; number of seeds per siliqua and mean weight of 100 seeds were compared in controlled and open pollinated plants and were reported to be significantly ($P<0.01$) higher in open pollinated *viz.*, 6.41, 52.80 and 240, respectively. Further, pollination increases the germinability of the resulting seeds to 36 ± 3 %. Lower seed yield was assumed to be probably due to the availability of less number of viable pollen.

INTRODUCTION

Agricultural production forms one of the most important economic sectors (FAOSTATS, 2013) where the quality of most crop species is increased by pollination (Klein *et al*, 2007; Gallai *et al*, 2009) which is a highly important, but also seriously endangered (Potts *et al*, 2010) ecosystem service. More than 75% of the 115 leading crop species worldwide dependent on or at least benefit from animal pollination, where as wind and self pollination are sufficient for only 28 crop species (Klein *et al*, 2007). Thereby, animal pollination contributes to an estimated 35% of global crop production (Klein *et al*, 2007).

Rapeseed and mustard are the third most important edible oilseed crops of the world after soybean and oil palm, mostly pollinated by insects (Ahlawat, 2012). These crops are grown under a wide range of agro-climatic conditions. Indian mustard is the most important member of the group, accounting for more than 70% of the area under rapeseed-mustard, followed by toria, yellow sarson and brown sarson (Ahlawat, 2012). The oil content varies from 37 to 49%. *Brassica juncea* (Indian mustard) is the oldest cultivated amphidiploids (Ahlawat, 2012), which is a dicotyledonous angiosperm plant belongs to Brassicaceae or Cruciferaceae family. In India, it is grown in 23 states and union territories. According to the information given by Department of Agriculture and Co-operation, Ministry of Agriculture, India, of the total production (5.08 m tonnes) of the country, Rajasthan (1.943 m tonnes), Uttar Pradesh (0.845 m tonnes) and Haryana (0.796 m tonnes) accounts for over 71% of production. Whereas, West Bengal comes to the forth position and accounts for only 6.629% (0.337 m tones) of production (Ahlawat, 2012). The area for mustard production in West Bengal has been on increase but the productivity has been diminishing hence the indigenous oil production of the state could not match the growing demand of population. Although such decline could be attributed to pests, diseases damage, poor soil fertility or water stress, but there is evidence that insufficient pollination can also significantly minimize the crop yield (Free, 1999). Pollination is an important process in maintaining healthy and bio diverse ecosystems. Insects constitute one among the primary groups of pollinating agents, as the association between insects and flowers are well established. Insect pollination is important to the reproduction and persistence of many wild plants (Ollerton *et al*, 2011). Various insect groups, which are of prime significance in pollination of different agricultural, horticultural and medicinal herbal crops mainly belong to the orders Hymenoptera, Diptera, Coleoptera, Lepidoptera, Thysanoptera, Hemiptera and Neuroptera (Free, 1993; Kearns *et al*, 1998 ; Mitra and Parui, 2002; Mitra *et al*, 2008).

Apart from its use in spice and oil extraction, it is used in the manufacture of greases. The oil cake is used as feed and manure. Green stem and leaves are a good source of green fodder for cattle. The oil cakes contain 'sinirgin', that causes palatability problem due to its bitter taste, and glucosinolate that limits use of oil cake as protein supplement. The leaves of young plants are used as green vegetables as they supply sulphur and minerals in the diet. In the tanning industry, mustard oil is used for softening leather (Ahlawat, 2012).

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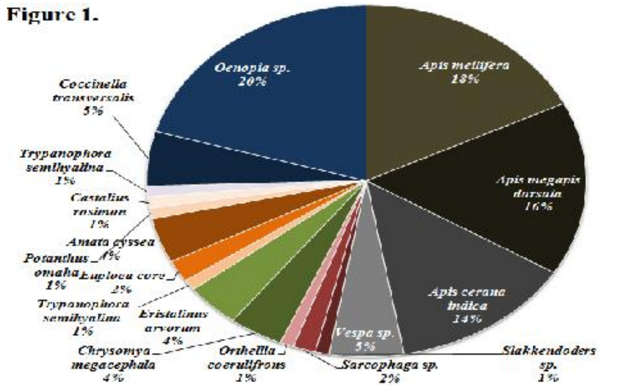


Figure 1 Relative abundance of various species visiting *Brassica juncea.*

The inflorescence is an elongated corymbose raceme, borne terminally on the main stem and branches, carrying bright yellow flower (Langer *et al*, 1991; Pua and Douglas 2004). The position of the anthers and the stigma make the flower unsuitable for self pollination (Roubik, 1989). Each flower has 6 stamens; 4 with long and 2 with short filaments. The pistil is compound, which is separated by a false septum, thus providing 2 chambers (Langer *et al*, 1991; Pua and Douglas 2004). Moreover, pollination in mustard is carried out by both wind and insects but mainly by honey bees, which visits the flower for nectar and pollen (Roubik, 1989; Free, 1999). The bowl shaped flower of mustard is a suitable place for the landing of insect pollinators, especially honey bees (Roubik, 1989).

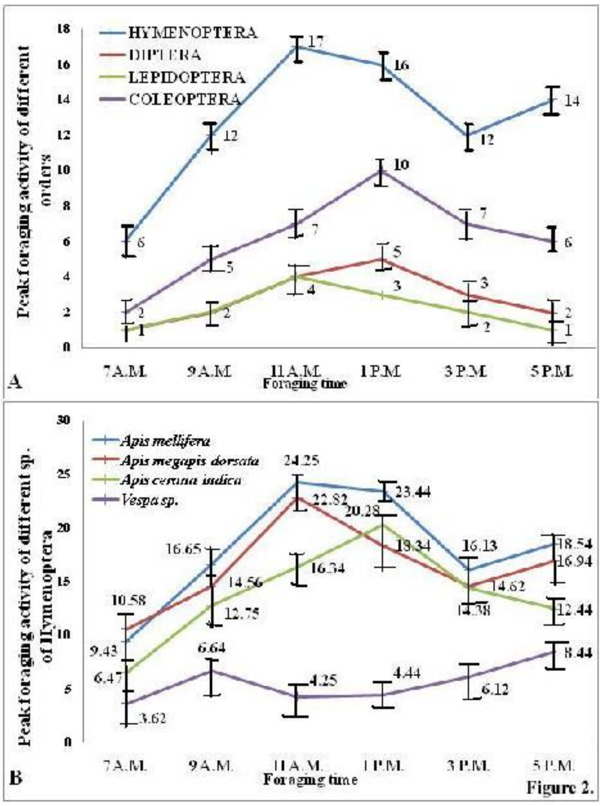


Figure 2 (A) Peak foraging activity of different orders of pollinators/ visitors. (B) Peak foraging activity of different species under order hymenoptera.

It has been observed and reported that diverse form of insect species of different orders, playing important role as pollinators or visitors in the mustard. So far, except few (Hari *et al*, 1994; Roy *et al*, 2014), no data is available regarding the pollinators from South Bengal region; hence the present study will definitely help to identify the insect pollinators, their diversity and abundance from this locality that varies with temperature and photoperiod. The present work further shows the pollination efficiency and ecological services played by the pollinators in increasing the seed yield of the crop (both qualitatively and quantitatively) thereby increasing the germinability of the resulting seeds to 36 ± 3%. For determining the cause of high seed yield, pollen viability test was also done.

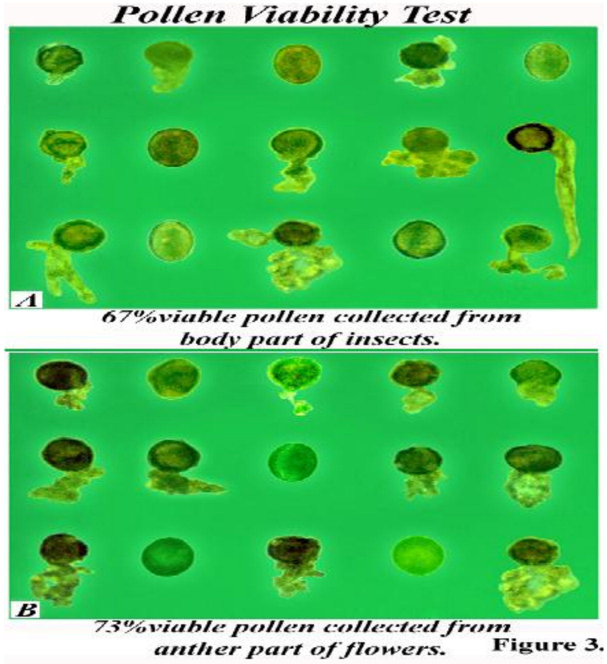


Figure 3 Comparative study of *In vitro* analysis of pollen viability in, (A) pollen collected from various body parts of *Apis mellifera*, (B) pollen collected from the anthers of the open pollinated flowers.

MATERIALS AND METHODS

The study area

The study was conducted at Agricultural land from Gandamara (Lat-22.854; Log-88.721), of District North 24 Parganas from South Bengal region from 5th of November 2011 to 20th of March, 2013. The study was conducted in three plots measuring each of 54m^2 and each plot was further divided into almost equal 6 quadrates. Pollinating insects at different time periods, *viz* 7-8 am, 9-10 am, 10-11 am, 11-12 am, 12-1 pm, 2-3 pm and 4-5 pm were observed and collected. Record of daily mean temperature (18^{o}C-25^{o}C) and relative humidity (50%-65%) was recorded from the local meteorological laboratory.
The recommended fertilizer doses required for the irrigation of the crop variety, NPJ-93, was N= 95 kg/ha, P=40 kg/ha and K=40 kg/ha. Seed rate of 6.5 kg/ha and spacing of 30x10 cm was recommended for the particular variety of crop.

The methods for collection, killing, preservation, setting and pinning of the insects were adopted from the manual of Zoological Survey of India (Jonathan and Kulkarni, 1986). The collected specimens were identified from Zoological Survey of India, Kolkata,West Bengal. The population of the above mentioned crop plants were observed and initial hypothesis built on these observations suggested very little or even no change with regard to the pollination population in each of the quadrate of the particular plant species. As such, sampling was conducted at the discretion of the investigator. The diversity index of different orders of the insect species was calculated using most widely used Shannon-Weiner diversity index method (Belavadi and Ganeshaiah, 2013).

Pollination efficiency index

Pollination efficiency index for the most frequent and abundant insect using by Vithanage (1999). This is usually the product of the average flower to insect ratio per visitor species in an hour (the number of flowers visited and the number of individuals visiting those flowers in an hour) and the average

Table 1 List of insect pollinators/ visitors of *Brassica juncea* L. NPJ-93

Name Of Species	Family	Order	Forage Source*	Shannon-Wiener Diversity IndexH' = -ģ Pi(LnPi)
Slakkendoders sp.	Sciomyzidae	Diptera	N	= 1.4096
Sarcophaga sp.	Sarcophagidae		N	
Orthellia coerulifrons	Muscidae		N	
Chrysomya megacephala	Calliphoridae		N	
Eristalinus arvorum	Syrphidae		N	
Episyrphus balteatus			N	
Stomorhina discolor			N	
Apis megapis dorsata	Apidae	Hymenoptera	PN	= 1.4905
Apis cerana indica			PN	
Apis mellifera			PN	
Vespa sp.	Vespidae		PN	
Coccinella transversalis	Coccinellidae	Coleoptera	C	NOT CALCULATED
Oenopia sp.			C	
Trypanophora semihyalina	Zyganidae	Lepidoptera	N	= 1.2295
Euploea core	Nymphalidae		N	
Amata cyssea	Arctiidae		N	
Potanthus omaha	Hesperiidae		N	
Tagiades japetus			N	
Castalius rosimon	Lycaenidae		N	

**Note- N: Nectar foragers, PN: Pollen and Nectar foragers, C: Casual foragers*

Table 2 The abundance percentage of the various insect orders visiting *Brassica juncea* at different time period.

Number per 9m^2 per 10 minutes at day time*

Order	Name Of Species	7 A.M.	9 A.M.	11 A.M.	1 A.M.	3 A.M.	5 A.M.	Average	Abundance Percentage
Hymenoptera	*Apis Mellifera*	9.43	16.65	24.25	23.44	16.13	18.54	18.07	33.62
	Apis Megapis Dorsata	10.58	14.56	22.82	18.34	14.62	16.94	16.31	30.34
	Apis Cerana Indica	6.47	12.75	16.34	20.28	14.38	12.44	13.77	25.62
	Vespa Sp.	3.62	6.64	4.25	4.44	6.12	8.44	5.585	10.38
Hymenoptera	-	6.89	12.65	16.91	16.62	12.81	14.09	13.43	52.74
Diptera		1.20	1.80	4.24	4.86	3.10	2.64	2.97	11.66
Lepidoptera		0.86	2.2	3.9	3.48	2.24	2.8	2.58	10.13
Coleoptera		2.24	5.64	7.10	9.8	7.84	6.28	6.48	25.45

**average of 25 observations*

Table 3 Mean number of pollen grains counted from the body hair of *Apis sp.* found in *Brassica juncea*

Insect Species	Brassica Pollen	Foreign Pollen	Flower: Insect Ratio Per Hour	Pollination Efficiency Index*
Apis mellifera	1635	37	184:51	5898
Apis megapis dorsata	1282	69	184:43	5485
Apis cerana indica	938	32	184:32	5393

** calculated using procedures developed by Vithanage (1999).*

number of the Brassica pollen grains counted on the body of the visitor species.

Biometric analysis

The number of flowers in a plant sample and the number of plants per 1 m^2 enabled a calculation of the number of flowers in the specified sowing area. In further biometric analyses each sample was examined for the height of plants, the number of branches per plant, and the numbers of pods and seeds per plant. Each sample was weighed and the weight of 100 seeds (both for controlled and open pollinated flowers) was calculated. The obtained results were statistically elaborated using an ANOVA. The significance of differences was determined.

Analysis of qualitative and quantitative parameters in crop yield

Flowering started from the last week of December 2012. Plants with unopened floral buds were enclosed in insect mesh nets for self and wind pollination only (preventing insect pollination) and open flower buds left for self pollination, pollination by wind and insects. Six quadrate area was randomly selected in the field area of 54 m^2 having 300 plants/ 9m^2 for each of the experimental design as under-

1. Affect of pollination on fruit set: {Number of fruits (siliqua) / Number of buds} x 100.
2. Affect of pollination on number of seed per siliqua: The number of seeds per siliqua was counted before harvesting period.

3. Affect of pollinating insects on fruit quality: It was studied by collecting the ripe seeds. It was assessed in terms of increase in the weight of seed, measured with the help of micro electric balance. For this, 100 seeds were collected for each experimental design and mean weight of 10 samples with hundred seeds was found. The data so found was analyzed statistically.

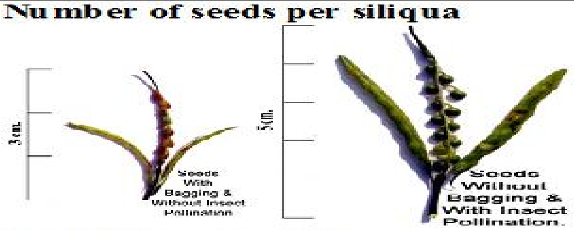
In vitro pollen viability analysis

A minimum of five anthers (just after dehiscence) were transferred on to a cavity slide with a drop of distill water and crushed well with a spatula to get all pollen out to the water. The content was then transferred to a glass vial and then it was made up to 5 ml by adding more distill water. Further, the pollen grains were also collected from the body hair of honey bee (*Apis mellifera* was chosen) simply by dipping the insect in distil water in a glass vial. In vitro pollen viability was assessed by growing the pollen grains in an artificial medium containing sucrose to record the percent of them germinating (Belavadi and Ganeshaiah, 2013). A known number of pollen grains present in an optimum concentration of sucrose solution (about 15 pollen grains taken at a time) were placed on a cavity slide. Percent of sucrose solution required for germination needs to be determined in advance. This was done by using 2, 5, 8, 10, 15, 20, 25, 30, 40 and 50% sucrose solution. The test was repeated several times to find out the ideal concentration to be used. The cavity slides containing pollen grains in the sucrose solution was placed in a petriplate with a wet filter paper to maintain the humidity. Observations were recorded on the number of pollen grains with pollen tubes after 1, 2, 4, 6, and 8 hours respectively. The images were taken with the help of *Primo Vert*, 40 x inverted microscope, Carl Zeiss.

Assessment of seed germination

Seed germination was assessed by placing the two types of seeds, (one obtained from controlled pollinated flower and the other type from open pollinated flower), in a plastic petridish with double layers of Whatman filter paper. On the sowing date, the filter paper was saturated with double distill water and then kept moist for 2 days. On the second day the germination was scored as successful with the appearance of two cotyledons of the seedling.

Table 4: Qualitative and quantitative effect of open pollination of plants of *Brassica juncea*.

S/N	Parameter	Control	Open pollinated	% Increase
1	Fruit set (%)	76.92	83.33	6.41
2	Number of seeds per siliqua 	8.9	13.6	52.80
3	Length(cm) of siliqua 	3.6	5.3	47.22
4	Weight (gm) of 100 dry seeds 	0.15	0.51	240
5	Diameter of seeds (mm) 	0.12	0.29	141.66
6	Colour and Shape of seeds	Light Brown & shrink	Deep Brown & Round	-
7	Germinability of seeds 	15 per 25 seeds	24 per 25 seeds	36

RESULTS AND DISCUSSIONS

Diversity, abundance, foraging activity and pollination efficiency index of insect pollinators / visitors in Brassica juncea

'Pusa vijay', NPJ-93, variety of *Brassica juncea* was found to visit by 19 different insect species under four orders (Table 1). Among the four orders, Lepidoptera and Diptera shared maximum number of species (six and seven species respectively), followed by Hymenoptera (four species) and Coleoptera (two species).

Among 19 species, 15 species were found as active and frequent visitors of mustard flowers. Of them, Hymenopteran species were reported to be common with significantly active throughout the day, followed by Coleoptera, Diptera and Lepidoptera. Abundance of Hymenoptera was observed to be maximum (~ 52.74 %) followed by Coleoptera (25.45 %), Diptera (11.66 %) and Lepidoptera (10.13%) (Fig.1). Out of the three honey bee species the abundance of *Apis mellifera* was maximum (18 %) followed by *A. dorsata* (16 %) and *A. cerana indica* (14 %) where as with *Vespa sp.* the abundance percentage was reported to be only 5 %. The relative abundance percentages of rest of the species can be compared from Fig.1. Surprisingly, abundance percentage of *Oenopia sp.* under Order Coleoptera was reported to be quiet high (20 %) and it was probably because of their predatory action on the aphids that are commonly found on the *Brassica sp.* Further, all the four species of Hymenopteran visitors were observed as both pollen and nectar foragers, where as all Lepidopteran and Dipteran species were reported to be nectar foragers (Table1) and only accidentally transfer the pollens. The rest two species under order Coleoptera were found as casual visitors of the flowers and were not participating in nectar or pollen collection. It is evident from Fig. 2 that, throughout the day all the insect groups were found active but their peak foraging activity time were different. The peak foraging activity of the members of Hymenoptera, Coleoptera, Lepidoptera and Diptera was observed to be at 12 p.m., 1 p.m., 12 p.m. and 2 p.m. respectively. Moreover, the highest foraging activity of *A. mellifera* was 8.48 bees/ $9m^2$/10 min, *A. dorsata* with 7.46 bees/$9m^2$/10 min and *A. cerana* with 3.18 bees/$9m^2$/10 min was reported (Fig. 2). Shannon-Weiner diversity index H’ was calculated and found to be of 1.49 for order Hymenoptera, 1.40 for the species of order Diptera and 1.22 for the species from order Lepidoptera (Table 1). Diversity index was not calculated for the species from order Coleoptera. Higher diversity index indicated a rich and even distribution of the species under order Hymenoptera.

Pollination efficiency index for the species under family Apidae and Vespidae under order Hymenoptera was determined (because of their both pollen and nectar foraging habit) using procedures developed by Vithanage (1990) (Table 3). By counting the mean number of pollen grains from the body hair of the hymenopterans insects, it was recorded that pollination efficiency index was maximum in *Apis mellifera* followed by *A. dorsata* and least with *A. cerana* and *Vespa sp*. (Table 3).

Considering the above parameters like forager abundance, foraging rate and number of pollen grains sticking to the body of the insects, it was established that though insect species from order Lepidoptera and Diptera may help in pollination process, but it was *Apis mellifera* that was found to be the most effective pollinators of *Brassica sp.* in this study.

Pollen viability

The quality of pollen is assessed on the basis of viability and vigour. Viability refers to the ability of the pollen to deliver formation of functional pollen tube or functional sperm cells to the embryo sac following compatible pollination (Shivanna and Johri, 1989). Pollen viability depends on various factors like temperature, relative humidity, stigma receptivity and biochemical changes on the membrane of the pollen (Shivanna and Mohan Ram, 1993). Assessment of pollen viability on the basis of its function is a cumbersome, time consuming and not always feasible method (Heslop-Harrison *et al,* 1984). Therefore one of the shortest methods is the *in vitro* germination test (Belavadi and Ganeshaiah, 2013). Pollen grains were collected (*vide supra*) just after the period of dehiscence from 10 a.m. - 11 a.m. from open pollinated flowers. Daily period of pollen presentation based on observations of number of dehisced stamens at hourly intervals in *Brassica sp*. has been reported to be from 7 a.m. - 5 p.m. and the peak period is from 7 a.m.-10 a.m. The percentage of day’s pollen presentation during peak period is 63 % (Percival, 1955). Simultaneously pollen was also collected from the body hair of honey bee (*Apis mellifera*). The stigma morphology of *B. juncea* is reported to be protogynous i.e. stigma exertion is prior to an thesis (Chandrashekar *et al,* 2013). Studies on stigma receptivity and pollen viability revealed significant variability and found to be at its peak up to 3 days after an thesis and reduced drastically thereafter. The ideal concentration of sucrose solution required for the growth of pollen tubes in this case was found to be between 18-20%. Pollen grains collected from both the sources were assessed for the growth of pollen tube in the desired sucrose solution after 2, 4, 6 and 8 hrs of observations respectively. It was observed to be 67 % viability in case of pollen collected from the hymenopteran body parts whereas pollen viability was about 73% when collected from anthers of open pollinated flower after the period of dehiscence, that further showed that pollen grains collected by the honey bees are also viable like the pollen grains collected from the anthers of the open pollinated flowers (Fig 3).

Effect of pollinators on the seed and crop yield of Brassica juncea

It was reported by many naturalists that cross pollination

It was reported by many naturalists that cross pollination causes early seed set and higher yield (Free, 1993; Mishra, 1997). Utilization of pollinators especially honey bees is considered as one of the cheapest eco friendly approach in maximizing the yield of cross pollinated crops (Free, 1970; Pateel and Sattagi, 2007). Many investigations have consistently confirmed that yields can be increased to an extent of 50-60 % in fruits and plantation crops, 45-50 % in sunflower, sesamum and niger, 100-150 % in cucurbitaceaous crops through good management of pollinators (Melnichenko and Khalifman, 1960). Seed yield data so obtained is presented in Table 4, which revealed that fruit set was 76.92 % in controlled experiment, while it was 88.33 % in open pollinated flowers, which showed an increase of 6.41 % in open pollinated flowers as compared to the controlled one. Similarly the mean number of siliqua/ seed was 8.9 and 13.6 while mean seed weight of 100 seeds was 0.15 gm and 0.51 gm respectively, in controlled and open pollinated experimental designs. These figures show an increase of 52.80 % seeds/ siliqua and 240 % mean weight of 100 seeds in open pollinated flowers than in controlled one. It was also determined that the length of siliqua and the diameter of seed increased by 47.22 % and 141.66 %, respectively, in the open pollinated flower than controlled one (Table 4). These results are in conformity with the already recorded observations of Chand and Sing (1995) on *Brassica juncea*, Mishra *et al*.(1998) on *B. campestris*, Khan and Chaudhury (1998) regarding the well shaped larger grains and more viable seeds in insect pollinated crops, Sing *et al*, (2004), Siddique *et al*, (2009) and Tara and Sharma (2010).

Germinability of the seeds

Germinability of both the types of seeds; one obtained from controlled pollinated flowers and the other type from open pollinated flowers, were also studies. It was observed that in case of controlled pollinated flowers where insect pollinators were excluded showed late germination and only 60 ± 5.6 (mean ± S.D. germinability) % seeds were germinated, where as in open pollinated flowers almost 96 ± 3.2 (mean ± S.D. germinability) % seeds germinated that further revealed that cross pollination by insects increases the germinability of the resulting seeds or in brief, it may be stated that in presence of pollinating insects, it is capable of setting more pods, more number of seeds and high seed weight which ultimately resulted in considerable increase in seed yield and increase in germinability of the resulting seeds (Table 4).

CONCLUSION

In conclusion, it may be stated that decline in the species diversity could pose a serious threat on the crop plant pollination and seed production. Though it is a preliminary attempt to make a report of insect pollinators for the mustard crops from South Bengal, it will certainly help the future workers as a baseline data of pollinators and pollination of the crops in this area. Hence, conservation of these species diversity is an absolute necessary for the crop plant pollination and seed production in future.

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