ROLE OF HONEYBEE AS A POLLINATOR IN CONTRIBUTING YIELD OF MUSTARD

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ROLE OF HONEYBEE AS A POLLINATOR IN CONTRIBUTING YIELD OF MUSTARD

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CERTIFICATE

This is to certify that the thesis entitled, **“ROLE OF HONEYBEE AS A POLLINATOR IN CONTRIBUTING YIELD OF MUSTARD”** submitted to the faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **Master of Science (MS) in Entomology,** embodies the result of a piece of bona fide research work carried out by **Md. Iqbal Hossain**, Registration No. 07-02372, under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.

I further certify that any help or sources of information, as has been availed of during the course of investigation have been duly acknowledged.

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DEDICATION

DEDICATED

TO

MY DEAR PARENTS

AND RESPECTED

SUPERVISOR

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ABBREVIATION AND GLOSSARY

Abbreviation	**Acronyms**
%	Percentage
@	At the rate of
AEZ	Agro-Ecological Zone
Agric.	Agriculture
Agril.	Agricultural
Appl.	Applied
Biol.	Biology
cm	Centi-meter
CV	Coefficient of Variation
DAS	Days after Sowing
Environ.	Environmental
et al	et alii, And others
Res.	Research
SAU	Sher-e-Bangla Agricultural University
Sci.	Science
Soc.	Society
t/ha	Ton per hectare
viz	*videlicet* (L.), Namely

ROLE OF HONEYBEE AS A POLLINATOR IN CONTRIBUTING YIELD OF MUSTARD

ABSTRACT

The experiment was conducted at the research farm of Sher-e-Bangla Agricultural University, Dhaka-1207 during the period from November, 2016 to February, 2017. The experiment was consisted of three (3) different strategies of pollination of mustard flowers *viz*., T_1= Open field (Control), T_2= Netting with honey bee and T_3= Netting without honey bee. BARI sharisha-8 (*Brassica Juncea* L.) was used as planting material. Randomized Complete Block Design (RCBD) was selected to lay out the present experiment with 7 replications. Result demonstrated that honey bee was the most abundant hymenopterans in the mustard field as a pollinator. The growth, yield and yield contributing traits were significantly influenced by different strategies of pollination by honey bee. The maximum (3.50 g) 1000-seed weight was recorded from treatment T_2 followed by T_1 (3.10 g) and the lowest 1000-seed weight (2.68 g) was recorded from T_3. The highest seed yield (2.45 t ha^{-1}) was exhibited from treatment T_2 followed by T_1 (2.30 t ha^{-1}) whereas the lowest seed yield (1.67 t ha^{-1}) was recorded from T_3. Finally; it can be concluded that, netting with honey bee was the best one for maximum pollination for obtaining better yield of mustard.

CHAPTER I

INTRODUCTION

Mustard (*Brassica juncea* L.*)* is the third most important edible oilseed crops of the world after soybean and oil palm and the oldest cultivated amphidiploids which is a dicotyledonous angiosperm plant belongs to Brassicaceae or Cruciferaceae family which is mostly pollinated by insects (Bhowmik *et al*., 2014). Edible oil plays a very important role in human nutrition. As a high-energy component of food, edible oils are important for meeting the calorie requirements. Each gram of oil/fat supplies 9 kilocalories of energy, whereas each gram of carbohydrate/protein furnishes about 4 kilocalories of energy (Stryer, 1980). Fats and oils are also the sources of essential fatty acids. The main essential fatty acids of vegetable oils are linoleic and linolenic acids. Fats and oils are used to synthesize phospholipid, which are important components of active tissues *viz*., brain, nerve, and liver of human beings and other animals. These crops are grown under a wide range of agro-climatic conditions. Indian mustard is the most important member of this group, accounting for more than 70% of the area under rapeseed-mustard, followed by toria, yellow sarson and brown sarson (Bhowmik *et al*., 2014). The oil content varies from 37 to 49%. *Brassica juncea* (Indian mustard). Rapeseed-Mustard is a principal oilseed crop, which plays a significant role in the national economy of Bangladesh. But seed yield/ha is very low compared to other rapeseed growing countries of the world, but the productivity of this crop is very low. The area for mustard production in Bangladesh 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 (Alam *et al*., 2014).

The low average yield of mustard is due to cultivation of traditional varieties, non-availability of seeds of high yielding varieties and delayed sowing (Alam *et al*., 2014). 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). Sushil *et al*. (2013) said that, low seed yield due to inadequate pollination is often faced as a major problem of *Brassica* seed production. Inadequate pollination is caused by several factors and the most important of which includes the lack of adequate number and diversity of pollinators.

Thus, there is a need to ensure pollination by conserving the pollinators and attracting them towards the crop land. Pollination by insects is inevitable for *Brassica*, since they are generally incompatible (Sihag, 2001) and the pollen is heavier and sticky, which is unable to be easily wind borne. The blooming phase of any plant is the most crucial period as the diversity of insects, both occasional and regular is higher than any other phase of that plant species (Bhalchandra *et al*., 2014; Rasheed *et al*., 2015).

Besides contributing to the preservation of natural ecosystems, bee pollination is one of the main alternatives for the improvement of crop productivity (Westcott and Nelson, 2001; D'Avila and Marchini, 2005). Thus, honey bees, *Apis mellifera* L. are considered the most important flower visitors (Delaplane and Mayer, 2000) of most efficient *B. napus* L. (Free, 1993; Sabbahi *et al*., 2005). The yield of rapeseed and canola can be increased by several percent through pollination by insects (Manning and Wallis 2005; Sabbahi *et al*., 2005, 2006).

However, the productivity of mustard through the cross pollination by the role of important insect vectors especially hymenopterans honey bee is not well investigated to us. So, keeping the fact in view the present study was undertaken to-

Study the effect of managed bee on the yield and yield contributing characters of mustard.

CHAPTER II

REVIEW OF LITERATURE

Mustard is an important oil seed crop in Bangladesh. The production is not as good as developed country due to its sub-optimal production strategies, climatic fluctuation and mode of pollination. Among these factors the mode of pollination by insects and major group of insect are not well investigated to us. So, to prepare a well plan for the present study on the role of honey bee on the yield performance of mustard different secondary sources of information were reviewed in this chapter.

Role of honey bees and other pollinating insects on the production of mustard/canola and other oil seeds crops

Painkra and Shrivastava (2015) undertaken an experiment on the effect of pollination by indian honey bee, *Apis cerana indica* on yield attributing characters and oil content of Niger. Significantly higher number of capitulum setting plant^{-1} was obtained in treatment with control (36.19 capitulum plant^{-1}). However, the lowest capitulum plant^{-1} was found with total closed (29.08 capitulum plant^{-1}). The maximum weight per capitulum was observed in treatment with control (0.244 g capitulum^{-1}) but the minimum capitulum^{-1} weight was found in treatment with total closed (0.094g capitulum^{-1}). The highest seed yield plant^{-1} was recorded in treatment with control (2.33 g plant^{-1}) and the lowest seed yield plant^{-1} was obtained in treatment with total closed (0.76 g plant^{-1}). But significantly highest healthy seed was noticed in treatment with control (95.60 per cent). However, the lowest healthy seeds were found in treatment with total closed (3.17 per cent).

Pashte and Said (2015) carried out a study on the importance of honey bees on the pollination of profitable crops. Honey bees are most important pollinators around the world and are major pollinators in tropical ecosystems. Honey bee visits the flowers to obtain their food and in return pollinate them. Bee pollination as a new agricultural production strategy has huge possibilities. Profitable crops like onion, sunflower, Apple and cucurbitaceous crops are specifically reliant upon or are benefited by honey bee pollination. Apart from the beekeeping products bee pollination benefits society by increasing food security and improving live hoods.

Pudasaini *et al*. (2015) conducted an experiment to determine the effect of pollination on seed quality of rapeseed in Nepal. The experiment was designed in Randomized Complete Block with four replications and five treatments. The rapeseed plots were caged with mosquito nets at 10% flowering except natural pollination. Two-framed colonies of *Apis mellifera* L. and *Apis cerana* F. were introduced separately for pollination and control plot caged without pollinators. The highest germination percent was observed on *Apis cerana* F. pollinated plot seeds (90.50% germination) followed by *Apis mellifera* L. pollinated plots (87.25 %) and lowest on control plots (42.00% germination) seeds. Similarly, seed test weight of *Apis cerana* F. pollinated plots (3.22 gm/1000 seed) and *Apis mellifera* L. pollinated plots (2.93 gm/1000 seed) were and lowest on control plots (2.26 gm/1000 seed) recorded. Likewise, oil content was recorded highest on pollinated by *Apis cerana* F. (36.1%) followed by pollinated by *Apis mellifera* L. (35.4%) and lowest on control plots (32.8%). This study clearly indicated pollination increases the seed quality of rapeseed and therefore, management of honeybee is necessary for producing higher quality of rapeseed under Chitwan condition.

Bartomeus *et al*. (2014) reported that insect pollination enhanced average crop yield between 18 and 71% depending on the crop. Yield quality was also enhanced in most crops. For instance, oilseed rape had higher oil and lower chlorophyll contents when adequately pollinated, the proportion of empty seeds decreased in buckwheat, and strawberries' commercial grade improved; however, we did not find higher nitrogen content in open pollinated field beans. Complex landscapes had a higher overall species richness of wild pollinators across crops, but visitation rates were only higher in complex landscapes for some crops. On the contrary, the overall yield was consistently enhanced by higher visitation rates, but not by higher pollinator richness.

Chambo *et al.* (2014) performed an experiment to evaluate two self-fertile hybrid commercial rapeseed genotypes for yield components and physiological quality using three pollination tests and spanning two sowing dates. The treatments consisted of combinations of two rapeseed genotypes (Hyola 61 and Hyola 433), three pollination tests (uncovered area, covered area without insects and covered area containing a single colony of Africanized *Apis mellifera* honeybees) and two sowing dates (May 25th, 2011 and June 25th, 2011). The presence of Africanized honeybees during flowering time increased the productivity of the rapeseed. Losses in the productivity of the hybrids caused by weather conditions unfavorable for rapeseed development were mitigated through cross-pollination performed by the Africanized honeybees. Weather conditions may limit the foraging activity of Africanized honeybees, causing decreased cross-pollination by potential pollinators, especially the Africanized *A. mellifera* honeybee.

The rapeseed hybrids respond differently depending on the sowing date, and the short-cycle Hyola 433 hybrid is the most suitable hybrid for sowing under less favorable weather conditions.

Gebremedhn and Tadesse (2014) conducted a field study to evaluate the effect of the honeybee pollination on seed yield and yield parameters of *G. abyssinica*. The flowers of niger open and liberate pollen early in the morning, the style emerges about midday and the plant is thus basically self-sterile. Hence *G. abyssinica* is a cross pollinated crop with cross pollination percentage ranging from 0 to 100 percent. The study had three treatments; these were crops caged with honeybee, caged without honeybee and open pollinated. The highest seed yield/ha was found in crops caged with honeybees (16.7 quintal) followed by open pollinated crops (13.3 quintal), while crops excluded from insects had the lowest yield (9.6 quintal). So the study discovers that honeybees and other insect pollination had a significant effect on seed yield of *G. abyssinica*. Therefore, it is recommended to keep sufficient number of honeybee colonies in the vicinity of *G. abyssinica* fields during its flowering period to increase the pollination efficiency and thereby enhance seed productivity.

Goswami and Khan (2014) carried out a study to evaluate the diversity and abundance of different insect visitors on mustard (*Brassica juncea*) at Pantnagar. A total of 19 insect visitors belonging to order Hymenoptera (15) and Diptera (4) were found to visit the mustard blossoms at Pantnagar. The abundance (percentage of insect/m^2/2min.) of Hymenopterans was maximum followed by the Dipterans and others. In Hymenopterans, the honeybees (*Apis* bees) were observed maximum followed by non *Apis* bees and the scolid wasp. Insect pollinations increased the number of pods and percent pod set.

Mohapatra and Sontakke (2014) conducted an experiment to investigate the effect of honey bee pollination on yield parameters of mustard (*Brassica campestris*) under the coastal agroclimatic region of Odisha, India. There were 3 treatments: T_1-caged plot with one bee hive of *Apis cerana indica*, T_2-open pollination plot (without nylon cover) and T_3-caged plot without bee colony (pollination exclusion). In T_1 and T_3, nylon net mesh measuring 5× 4 × 5 m of 2-mm mesh size was erected at 10 to 15% flowering of the crop. In the bee-pollinated treatment (T_l), one bee hive of *A. cerana indica* with 4-5 frames of honey bees having two entrances was kept half inside and half outside the nylon net cover. It was ensured that no part of the net touched the branch and prevented the entry of any insect visitor. During peak flowering, the foraging activity of *A. cerana indica* was studied from both caged and open pollinated plots at different diurnal clock hours. Similarly, the foraging activity of other bee pollinators in open pollination plot was studied at different diurnal clock hours during peak flowering. Various biometric parameters, *i.e.*, healthy pods per plant, thousand-seed weight and seed yield, as well as seed oil content, were determined. The Other major bee pollinators visiting mustard flowers in open pollination plot consisted of *Apis dorsata*, *A. florea* and *Trigona iridipennis*.

Sanas *et al*. (2014) conducted a study to determine the role of honeybees in quantitative yield parameters of mustard (*Brassica juncea* L.). The mustard variety 'Varuna' was grown following all the recommended agronomic practices. The plots were kept unsprayed throughout the crop season. The colonies of honeybee (*Apis cerana indica* F.) were placed in hives before the initiation of flowering.

The present study contained 3 pollination treatments, *viz*., Plants kept open to all pollinators (T_1), Plants caged with *Apis* hives (T_2) and plants caged without access to any pollinators (T^3). The difference in siliquae per plant, seeds per siliqua, thousand seed weight, seed yield per plant and per plot were found significant ($p<0.05$) and highest values were obtained from open pollination, followed by plants caged with honey bee hives and plants caged without access to any pollinators. The introduction of honeybees in agricultural crops plays a dynamic role in pollination which in turn resulted in higher production.

Shakeel and Inayatullah (2013) studied an experiment on the impact of insect pollinators on the yield of two canola varieties (‘Ganyou’ in which pollen viability and germination are adversely affected by soil salinity, and ‘Oscar’ that is more tolerant of soil salinity) in Peshawar, Pakistan. Yields were similar between the two cultivars. Significant differences were observed between pollinated and covered plants for three yield parameters (i.e., total yield, number of seeds per sliquae, and weight of 100 seeds). Average yields were 189.36 ± 1.7 pods/plant in the pollinated plots and 142.26 ± 2.4 pods/plant in the covered plots. There was an average of 15.06 ± 0.9 seeds/siliqua in pollinated plots and 11.06 ± 0.8 seeds/siliqua in covered plots. The weight of 100 seeds was 0.556 ± 0.02 g in pollinated plots and 0.376 ± 0.01 g in covered plots.

Sushil *et al*. (2013) carried out an experiment on the impact of planned honeybee pollination on the seed production of three Brassica vegetables, *Brassica oleacea* var. italica, *B. rapa* pekinensis and *B. oleracea* var. gongylodes and the pollination behaviour of *Apis mellifera* was studied under Indian Himalayan conditions.

Among the three crops tested, more number of bees were found visiting broccoli crop under net house condition (6.05 bees/plant) followed by kohlrabi (5.35 bees/plants) and Chinese cabbage (5.05 bees/plant). Bees spent more time in Chinese cabbage flower (6.92 sec) while it was 6.50 sec in broccoli and 5.54 sec in kohlrabi. Bees in the open conditions were found to spend less time in a flower as compared to the net house conditions. Honeybees played an important role in enhancing the seed production of all the crops under study. Planned honeybee pollination was found to inflict maximum impact on the seed production of broccoli with an increase in seed yield of 29.2 per cent. The net profit was also more in case of broccoli, which was calculated to be 1324.60$ per ha in honeybee pollinated broccoli crop when compared to the natural pollinated crop.

Mahfouz *et al*. (2012) conducted a study to determine the insect pollinator orders visiting sesame, fluctuation percent of Hymenopterous fauna during flowering period, foraging activity of the pollinating insects belonging to Hymenoptera, Coleoptera, Lepidoptera and Diptera orders and foraging activity of *Apis mellifera*, *Anthidium* sp. and *Xylocopa* sp. at four time periods *i.e*., 9-11 am, 11-1 pm, 1-3 pm and 3-5 pm. Results revealed that insect percentage of Hymenoptera order was high followed by Lepidoptera, Diptera and Coleoptera. The highest activity of Hymenopterous fauna was in fourth week of flowering period and decreased gradually in the last weeks. Total number of pollinators was highest at 9-11 am followed by that at 11-1 pm, 1-3 pm and 3-5 pm. Among the bees, the number of *Apis mellifera* was the maximum followed by *Xylocopa* sp. And lastly *Anthidium* sp. at all time periods. It was also evident that temperature, wind and relative humidity also affect the percentage of insects visiting sesame flowers.

Rajasri *et al*. (2012) conducted a field study to find out the role of honeybees on pollination, seed setting and seed quality of hybrid sunflower. The foraging behavior of natural bee visitants was studied on the parental lines of sunflower hybrid NDSH1 during the flowering period. Most predominant bees observed are Rock bees, *Apis dorsata*, European bee, *Apis mellifera*, Indian bee, *Apis cerana indica* and Stingless bees, *Trigona irridipenis*. Bee visitants are more on R line compared to A line. The seed setting percentage and seed yield were significantly increased when the honeybees were supplemented to the open pollination. The yields were drastically reduced when the crop was covered with insect proof net. In addition, increased seedling vigour, germination%, field emergence, oil content and quality of seed was observed with the deployment of honey bees coupled with supplemental hand pollination.

Te and Ebadah (2011) carried out an experiment on the evaluation of seasonal fluctuation of insect pollinators and the efficiency of honeybees for black cumin plants *Nigella sativa* pollination were during the flowering periods stage. Four Orders of visitor insects were captured by the insect sweep net technique on black cumin plants. These Orders were Hemiptera, Coleoptera, Diptera and Hymenoptera. Orders Diptera and Hymenoptera were ranked as the most abundant species. Daily peak activity was detected at 12 noon and 2 pm in both experimental seasons. Honeybee that visits the black cumin plants leads to the increase of the number of seeds set and then yield production. Thus strategies to promote pollination by honeybee may be helpful in enhancing seed yield in *N. sativa* and other cultivated species.

Duran *et al*. (2010) carried out an experiment to evaluate the role of bees on the yield potential of hybrid rape seed due to that recent introduction of hybrid varieties raises the question if bees (*Apis mellifera* L.) contribute as pollinator agents in developing the full yield potential of rapeseed (*Brassica napus* L.). In order to evaluate the yield achieved by *B. napus* cv. Artus pollinated by *A. mellifera* testing was carried out. This consisted in isolating or excluding rapeseed plants from pollinators with exclusion cages. Treatments applied were total exclusion (T_1), partial exclusion (T_2) and free pollination (T_0) with a density of 6.5 hives ha^{-1}, in order to determine the following yield components traits: grains per silique, siliques per plant, 1000-grain weight and yield. The experimental design used was randomized complete blocks with three treatments and three replicates. Results obtained show that the parameter least affected by bee intervention was the grains per silique variable. In contrast, siliques per plant and 1000 grain weight parameters presented significant differences, contributing to a yield greater than 5 t ha^{-1}; which represented a figure 50.34% higher than in the treatment without bees. It may be concluded that the inclusion of bees in crops is fully justified as a production tool.

Tara and Sharma (2010) pointed out the qualitative and quantitative effects of pollination on fruit set; number of seeds per siliqua and mean weight of 100 seeds were compared in controlled and open pollinated plants of sarson. Percent fruit set, number of seeds per siliqua and mean seed weight of 100 seeds were significantly ($P<0.01$) higher in open pollinated *viz*., 8.09, 9.37 and 141.86 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 *Apis dorsata* followed by *Apis mellifera* and *Apis cerana* were observed to be the most common pollinating species.

Munawar *et al*. (2009) conducted an experiment in order to quantify the response of honeybee on canola seed yield, Islamabad, in complete randomized block design with two treatments (*i.e*., i. Plants caged with honeybees; ii. Plants caged without honeybees) with four replications each. Number of pods set, pods weight, seed count in hundred pods and yield per plant were measured in twenty randomly harvested plants. The results showed significant increase in all the plant parameters caged with bees as compared to the plants without bees (control). Number of pods and number of seeds pod-1 with pollination were 815 and 20 while without pollination was 349 and 15. The seed weight and yield (gm) with pollination was 26 and 7.6 (gm) and without pollination was 9.3 and 1.51 (gm), respectively. It is concluded from the experiment that honeybee visitation to the canola flowers is important for pollination and increasing seed yield.

Oz *et al*. (2009) carried out a study to determine the efficiency of pollination with honeybee (*Apis mellifera*) on sunflower hybrid seed production under different types of pollination in Turkey. Three pollination types (1) in cages with honeybees, (2) hand pollination (in cages) and (3) in cages without honeybees were used in crosses between two male and three female parents in all possible combinations. The experiment factors; pollination types, male parents and female parents were designed in a split-split plot of randomized complete block. Seed set ratios were 98-99% for pollinations in cages with honeybees or by hand as artificial whereas this ratios was reduced to a level of 4-5% by pollination in cages without honeybees in the both experimental years.

Pollination in cages without honeybees produced 93.94% less number of filled seeds per head compared with pollinations by hand and in cages with honeybees. In addition, pollinations in cages with honeybees and by hand resulted in higher 100-seed weight compared with pollination in cages without honeybees. Pollination in cages with honeybees and by hand increased seed yield per head by about 20.6 and 22.6%, respectively, compared with pollination in cages without honeybees. Results indicated that the use of honeybees for sunflower hybrid seed production improved seed set ratio, 100-seed weight, number of filled seed per head and seed yield per head.

Oz *et al*. (2008) conducted an experiment to determine the effects of pollination using honeybees on seed yield and quality characteristics of the rapeseed 'Pulsar' winter variety of canola (*Brassica napus* ssp. oleifera) was grown and small colonies of honeybee (*Apis mellifera* anatoliaca) were placed in mini hives with or without pollen trap. The present study contained 4 pollination treatments, *viz*., T_1, an open field; T_2, a cage without bees, T_3, a cage with bees in a regular mini hive and T_4 a cage with bees mini hive with pollen trap. Seed yield was 3205, 1823, 2360 and 2357 kg/ha for the treatments T_1, T_2, T_3 and T_4, respectively.
The differences in pods/plant, seeds/pod and seed yield/plant were found significant ($p<0.05$) and highest values were obtained from open field, followed by a cage with bees in a regular mini hives and a cage with bees in a bottom pollen trap hive. Seed weight, branches/plant, harvest index, seed protein and oil content were insignificantly influenced by treatments. Honeybee pollination increased the seed yield significantly but not protein and oil percentage in seeds.

Koltowski (2005) carried out an experiment to investigate the response of 7 cultivars of winter rapeseed (Kana, Lirajet, Liropa, Marita, Polo, Silvia, and Skrzeszowicki) to a lack of pollinating insects. Analyses were conducted on plant samples from plots freely available to the pollinating insects and from plots kept in the blooming period under a gauze cover made of plastic mesh with a mesh size of 2x2 mm. The cultivars examined did not demonstrate any specific reactions to the lack of pollination by insects and their responses were found to be similar. Under very similar conditions of growth and development of plants in both experimental variants, (with a similar number of plants per area size unit, with similar heights and comparable numbers of branches and blooming yield) it was found that the plants freely visited by the pollinating insects, compared to those kept under gauze cover, were setting a similar number of fruits from 100 flowers and a higher number of seeds per pod (by 22% on average), which at a similar, yet slightly lower weight of 1000-seeds, resulted in an average yield increase of 10%.

Sabbahi *et al*. (2005) studied an experiment on the evaluation of role pollination on seed production of canola (*Brassica napus* L.). It is achieved with the assistance of various pollen vectors, but particularly by the honey bee, *Apis mellifera* L. Although the importance of pollination has been shown for the production of seed crops, the need to introduce bee hives in canola fields during flowering to increase oil seed yield has not yet been proven. With the purpose of showing this, hives of *A. mellifera* were grouped and placed in various canola fields (nine fields; three blocks with three treatments; 0, 1.5, and 3 hives per hectare). A cage was used to exclude pollinators and bee visitations were observed in each field.

After the harvest, yield analyses were done in relation to the bee density gradient created, by using pod set, number of seeds per plant, and weight of 1000-seeds. Results showed an improvement in seed yield of 46% in the presence of three honey bee hives per hectare, compared with the absence of hives. The introduction of honey bees contributed to production and consequently, these pollinators represented a beneficial and important pollen vector for the optimal yield of canola.

Williams *et al*. (1987) conducted an study to investigate the effect of insect pollination on plant development and seed yield of winter oil-seed rape, cultivar Jet Neuf, by comparing plots caged with a honeybee colony, plots caged to exclude insects and plots uncaged and 'open-pollinated' by naturally occurring insects. Plants in the bee-pollinated plots finished flowering earlier, showed more advanced pod growth, and were shorter than those in the plots without bees. Pods from the plots with honeybees contained more seed post-flowering than those from plots without honeybees but the proportion of them that grew into mature seeds for harvest was determined by water availability during seed growth.

So, from the review study it may said that, the hymenopterans bees and honey bees has the best capacity to pollinate the oil seed crops. That is why under present study different modes of honey bee pollination was made on the yield contributing traits of mustard.

CHAPTER III

MATERIALS AND METHODS

A brief description of site of the research plot, traits of soil and climate, planting material, treatments, layout and design of experiment, intercultural operations, harvesting, and data collection of mustard were described in this chapter. These are described below:

3.1 Site of research

Research farm of Sher-e-Bangla Agricultural University, Dhaka-1207 was used to conduct the present study during the period from November 2016 to February, 2017. The experimental field was located at 90º 33 E longitude and 23º71 N latitude at a height of 8m above the sea level. The land of research area was Medium high topography.

3.2 Soil

The pH of the research plot was 5.67 and soil texture was silty clay in nature which contains 24% sand, 48% silt and 28% clay. The physio-chemical properties of the soil are presented in Appendix IV. The research area was belongs to the Madhupur Tract Agro Ecological Zone (AEZ-28) as shown in Appendix I.

3.3 Climate

Sub-tropical climate is characterized by the research areas with three distinct seasons, *kharif-1* (April-June), *kharif-2* (July-September) and *robi*/winter (October-March). Due to lower rainfall this weather is the favorable for crop production.

The average maximum temperature during the period of experiment was 29.19°C and the average minimum temperature was 9.16°C. Details of the meteorological data related to the temperature, relative humidity and rainfalls during the period of the experiment was collected from the Bangladesh Meteorological Department, Dhaka and presented in Appendix II.

3.4 Planting materials used for experiment

BARI sharisha-8 (*Brassica Juncea* L.) was used as planting material. It is herbaceous growing to a height of 0.9 to 1.10 meters with tap root system. The leaf is sessile in nature. The number of primary branches per plant was 4 to 5. Fruit is two chambered. Twenty five to thirty seeds were present per siliqua. The color of flower is yellow and the seed is blackish in color. Total life span is 90-95 days and it has the potentiality to give 2.0-2.5 t ha^{-1} seed per hectare at well managed condition (BARI, 2017).

3.5 Experimental Design and layout

The experiment was laid out in a Randomized Complete Block Design with 7 replications. The unit plot size was 2.5 m ×2.0 m having 1.0 m space between the replications and 0.75 m between the plots. Each plot contains two rows having 40 cm distance between the row and 10 cm between plants. The design and layout of the experimental plot was presented in Appendix III.

3.6 Collection of seeds:

BARI sharisha-8 was collected from Regional Oil Seed Research Centre, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur-1701.

3.7 Preparation of land and rate of fertilization

A power tiller was used to open the plots selected for the research in the last week of October 2016 and exposed to the sun for a week, after which the land was harrowed, ploughed and cross-ploughed four times followed by laddering to obtain a good tilt conditions of soil with removal of weeds and stubbles. During land preparation 8 t/ha decomposed cow dung were mixed. Urea, TSP, MoP, Gypsum, Zinc sulphate and Boric acid were applied as the source of Nitrogen (N), Phosphorus (P_2O_5), Potassium (K_2O), S, Zn and Boron (B).

Name of fertilizer/manures	Dose of application (kg/ha)
Decomposed cowdung	8000
Urea	250
TSP	180
MoP	100
Gypsum	150
Zinc sulphate	7
Boric acid	10

Source: BARI Krishi Projukti Hat Boi, 2012.

3.8 Procedures of fertilizer application

Cowdung was applied at one week before of seed sowing in the final land. Except urea, all the fertilizers were applied during final land preparation. Urea was applied in two installments: first half was applied as basal dose during final land preparation and second half was applied after 40 days of sowing. The split urea was applied as side dressing of standing crops followed by light irrigation.

3.9 Processing and treatment of seed for sowing

After collecting the seeds of BARI sharisha-8 from the regional oil seed research centre, Bangladesh Agricultural Research Institute, Gazipur. Germination test was done before sowing. The rate of germination was found more than 94%. The seeds were treated with Provex 200 WP @ 2 g per kg seed to protect seedlings against foot and root rot diseases and from later *Alternaria* blight.

3.10 Seeds sowing

At November 7, 2016 seeds are sown continuously in 2-2.5 cm deep furrows made by hand iron tine maintaining desired row spacing. Before seed placement in rows light water was applied in furrows for easy emergence of seeds. After placement of seeds were covered with soil by hand. Within ten days of sowing the germination was satisfactory.

3.11 Treatments of the experiment:

The experiment was consisted on three treatments are as follows:

T_1 = Open field (Control)

T_2= Netting with honey bee

T_3= Netting without honey bee

Two frame nucleus boxes were used as rearing for honey bees and sugar candy with a stick was supplied as supplemental food for honey bee.

3.12 Intercultural operations

3.12.1 Operations like thinning, weeding were done as and when necessary for proper growth and development of the crop. Thinning was done during first weeding at 25 DAS keeping a distance of 10 cm in between plants. .

3.12.2 Pest Management

For reducing the dimension of Grey leaf blight of mustard disease, Rovral 50 WP @ 0.5 g/L of water were applied at an interval of one week.

3.12.3 Watering and drainage

Two watering were done throughout the growing season. The first watering was given at 7 days after emergence followed by watering 15 days after the first water application and mulching was also done by breaking the soil crust after irrigation properly. Stagnant water was effectively drained out at the time of heavy rains.

3.13 Harvesting

At physiological maturity stage the crop was harvested. Harvesting was done when 80-85% of leaf becomes brownish to yellow in color (2 February, 2017). The harvested plants were brought to the threshing floor and dried in the sun. The seed and stover were than separated, cleaned and dried in the sun for 5 consecutive days for achieving proper moisture content of seed. The seed yield per hectare was calculated from the yield of seed per plot with each respective treatment.

3.14 Sample collection and data recording:

To take and calculate the data on different traits five plants were selected randomly from each plot. A brief description of procedures of data collection from different parameters presented as per bellow:-

The percent proportion of different insects were counted by considering the major insect orders. Then, all the counted insect found in crop land sorted as per as their respective orders.

i. Plant height (cm)

Five plants were randomly selected from each plot and then the height of plant was measured by using a measuring tape in centimeter unit and then means were calculated.

ii. Number of primary branches/plant

The branching main stem is called primary branch. Five plants were randomly selected from each plot and then total number of primary branches per plant of those five plants was counted lefting the main stems and then means were measured.

iii. Pod formation at 45, 55 and 65 DAS

Five plants were randomly selected from each plot and then the number of pod formation was taken at different days after sowing and then means were taken.

iv. Number of siliqua/plant

Total number of siliqua borne on the five sample plants of each unit plot was counted and the means was taken as number of siliqua per plant.

v. Number of seeds/siliqua

Total number of seeds found in all the siliqua on the five sample plants of each unit plot was counted and the means was taken as number of seeds per siliqua.

vi. 1000-seed weight (g)

Thousand seeds were taken from each plot and weighted with the help of electronic balance and then the mean was taken in gram unit.

vii. Seed yield (t/ha)

Weighted the total amount of seed produced per plot with the help of electric balance and then the yield of seed per plot was converted to per hectare in tons unit.

viii. Seed yield increase over control (%)

The role of honey bee was significantly differed the yield of mustard in the field. So, to see the exact percentage of yield increase of seed over control treatment functionally the percent was brought:-

Mean value of treated plot–Mean value of untreated plot/Mean value of untreated plot× 100.

ix. Correlation co-efficient (r)

By using the MS Excel spread sheet the correlation between number of siliqua/plant, number of seeds/siliqua and seed yield were calculated to show the importance of honey bee pollination of mustard flowers.

3.15 Statistical Analysis:

After collection of all the data, those were analyzed following the ANOVA techniques and using the MSTAT-C computer package program. The means were separated by using Least Significant Difference (LSD) at 5% level of probability (Gomez and Gomez, 1984).

CHAPTER IV

RESULT AND DISCUSSION

The present study was aimed to observe the role of honeybee as a pollinator in contributing yield of mustard. In this chapter; figures, tables and appendices have been used to present, discuss and compare the findings obtained from the present study. The honey bee had the significant roles on pollination behavior of mustard and its yield contributing characters. So, the ANOVA (analysis of variance) of data in aspects of all the visual and measurable characteristics have been presented in Appendix (V-VI). The all possible reveals and interpretations were given under the following headings:

4.1 Percentage of major insect

Insects of different orders' visited experimental plots during experimentation period (Figure 1). Among different insect families the maximum (87%) hymenopterans insect visited the mustard field followed by Lepidoptera (6%), coleopterans (4%), Dipterous (2%) and the minimum visitors were Homopterous (1%). Mahfouz *et al*. (2012) found the similar pattern of insects foraging for pollination of sesame. They found that, hymenopterans were the most forgeable insects order in rapeseed plant.

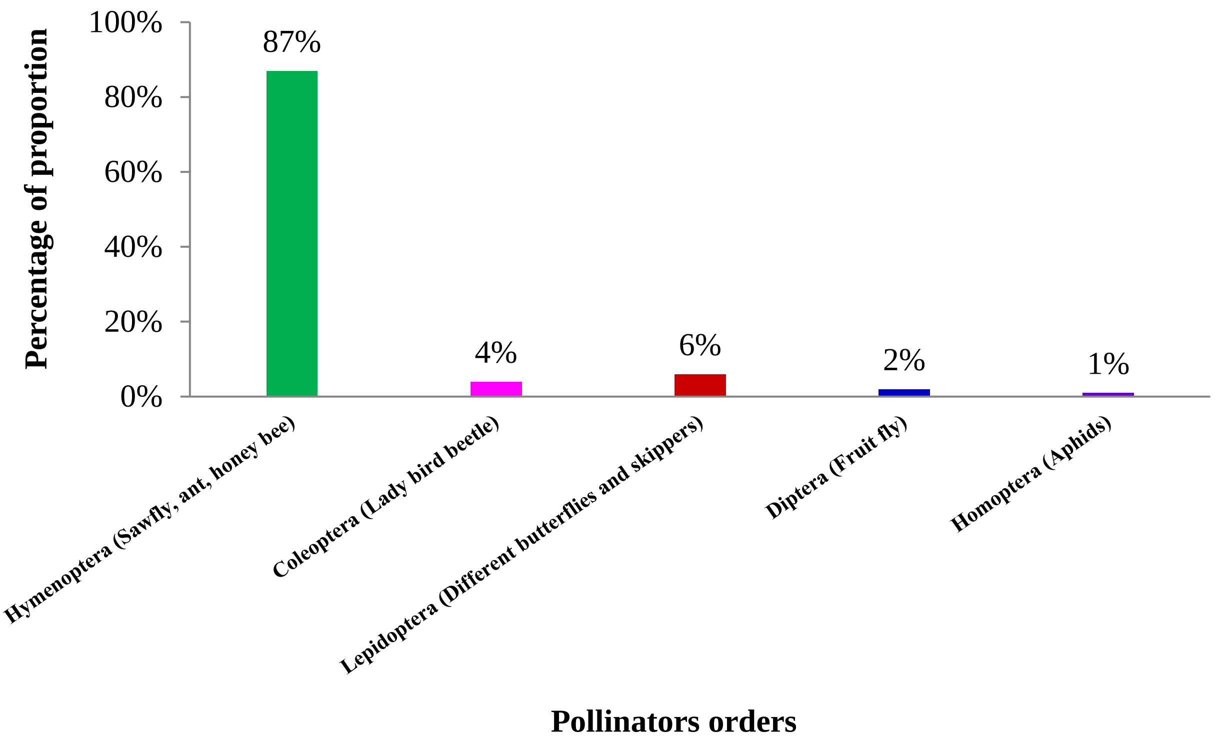

Figure 1. Percentage proportion of the major insect orders visiting mustard during flowering period

4.2 Plant height

The plant height of mustard was not significantly differed due to different pollination condition (Figure 2). Numerically the tallest plant (96.80 cm) was recorded from netting with honey bee (T_2) and the shortest plant (94.85 cm) was recorded from netting without honey bee (T_3). The honey bee has no any effects of plant height due to it is a varietal trait. So, under present study no significant differences among pollination modes were found.

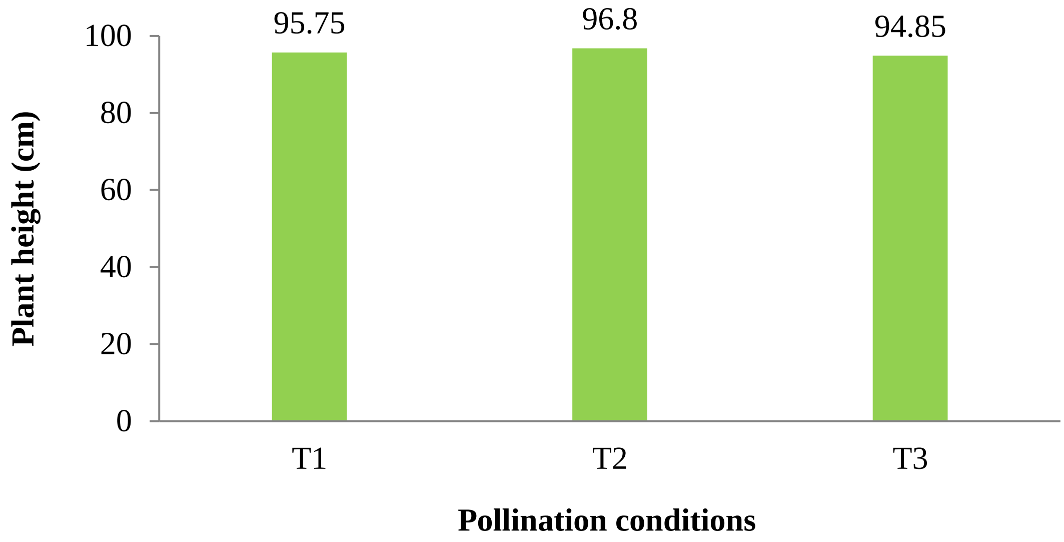

Figure 2. Effect of different pollination conditions on the plant height of mustard (LSD $_{(0.05)}$ = NS)

T_1= Open field (Control), T_2= Netting with honey bee and T_3= Netting without honey bee

4.3 Number of primary branches plant^{-1} (no.)

The number of primary branches plant^{-1} of mustard significantly affected by different pollination condition (Figure 3). The results of the experiment revealed that, the maximum number of primary branches plant^{-1} (4.52) was recorded when the plots netting with honey bee (T_2) followed by T_1 and the minimum number of primary branches plant^{-1} (3.34) was recorded from when the plots netting without honey bee (T_3).

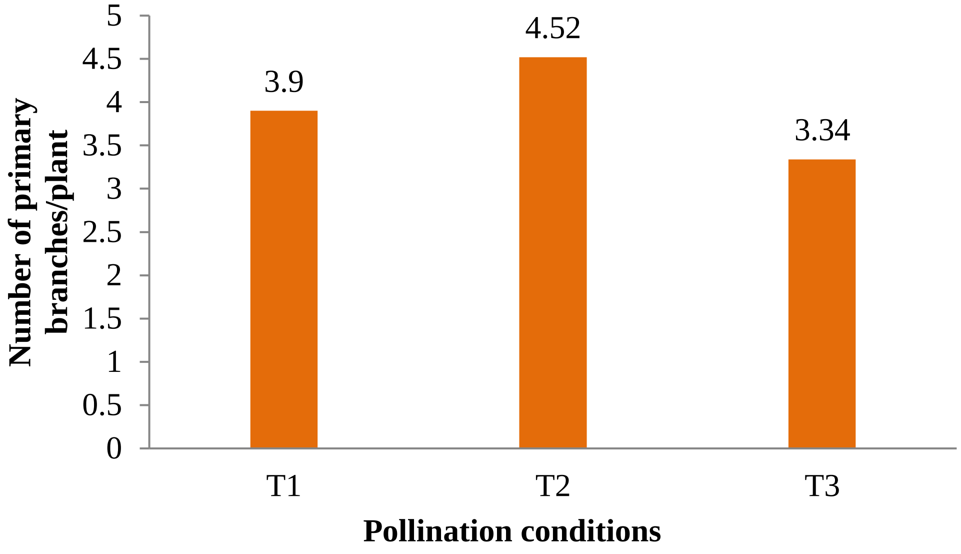

Figure 3. Effect of different pollination conditions on the number of primary branches plant^{-1} of mustard (LSD $_{(0.05)}$ = 0.54)

T_1= Open field (Control), T_2= Netting with honey bee and T_3= Netting without honey bee

4.4 Number of siliquae setting plant^{-1} (no.)

The number of siliquae setting plant^{-1} of mustard was significant due to different pollination condition at 45, 55 and 65 days after sowing (DAS) (Table 1). The maximum number of siliquae setting plant^{-1} (124.30, 133.30, 137.00 at 45, 55 and 65 DAS, respectively) were recorded from treatment T_2 followed by T_1 at 45, 55 and 65 DAS and the minimum number of siliquae setting plant^{-1} (92.25, 98.25 and 100.83 at 45, 55 and 65 DAS, respectively) was produced by T_3 treatment. Kumar and Singh (2003) found that the number of filled seeds per capitulum (728.2) seed set (75.5%) and 1000-seed weight (55.9 g) was highest with hand + insect pollination, than open pollination and the crop netted without bees in sunflowers.

Table 1. Effect of different pollination conditions on the number of siliquae setting plant^{-1} of mustard at different days after sowing

Treatments	No. of siliquae setting plant^{-1} at different days after sowing		
	45 DAS	55 DAS	65 DAS
T_1	108.5 b	115.57 b	119.0 b
T_2	124.3 a	133.3 a	137.0 a
T_3	92.25 c	98.25 c	100.83 c
LSD $_{(0.05)}$	14.32	16.33	17.41
CV (%)	8.59	9.10	9.48

T_1= Open field (Control), T_2= Netting with honey bee and T_3= Netting without honey bee

4.5 Number of siliquae plant^{-1} (no.)

The number of siliquae plant^{-1} of mustard varied significantly due to different pollination condition (Figure 4). The result revealed that, the highest number of siliquae plant^{-1} (122.00) was found from treatment T_2 followed by T_1 and the lowest number of siliquae plant^{-1} (89.00) was found from T_3.

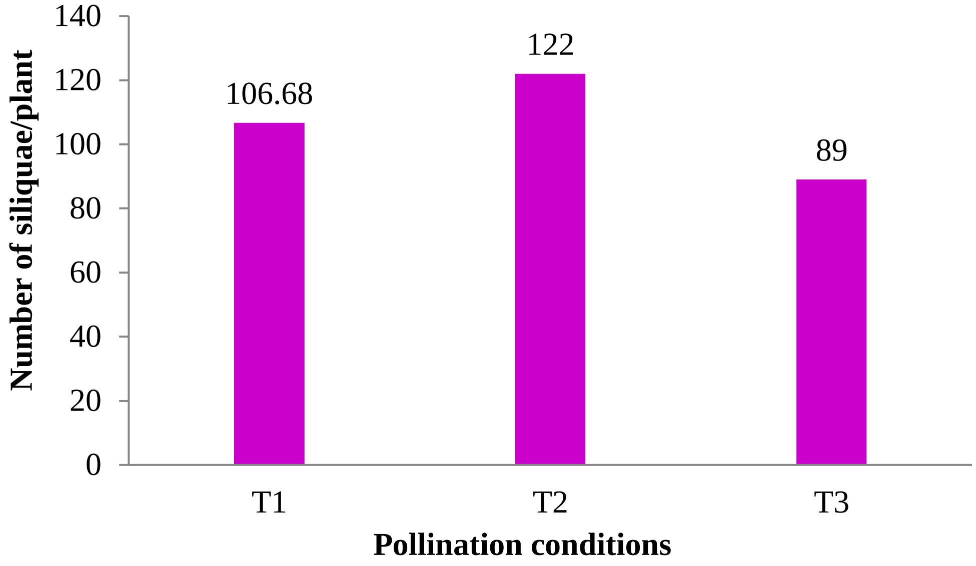

Figure 4. Effect of different pollination conditions on the number of siliquae plant^{-1} of mustard (LSD $_{(0.05)}$ = 15.15)

T_1= Open field (Control), T_2= Netting with honey bee and T_3= Netting without honey bee

4.6 Number of seeds siliquas^{-1}

The number of seeds siliqua^{-1} of mustard significantly varied due to different pollination condition (Figure 5). The result revealed that, the highest number of seeds siliqua^{-1} (28.50) was recorded from treatment T_2 and the lowest number of seeds siliqua^{-1} (15.75) was recorded from T_3. The present findings was in agreement with Free and Nutall (1968) who observed that *B. juncea* plants caged with bees produced 25 per cent more seed than plants caged without bees. Kumar and Singh (2003) found that the number of filled seeds per capitulum was highest with hand + insect pollination, than open pollination and the crop netted without bees in sunflowers.

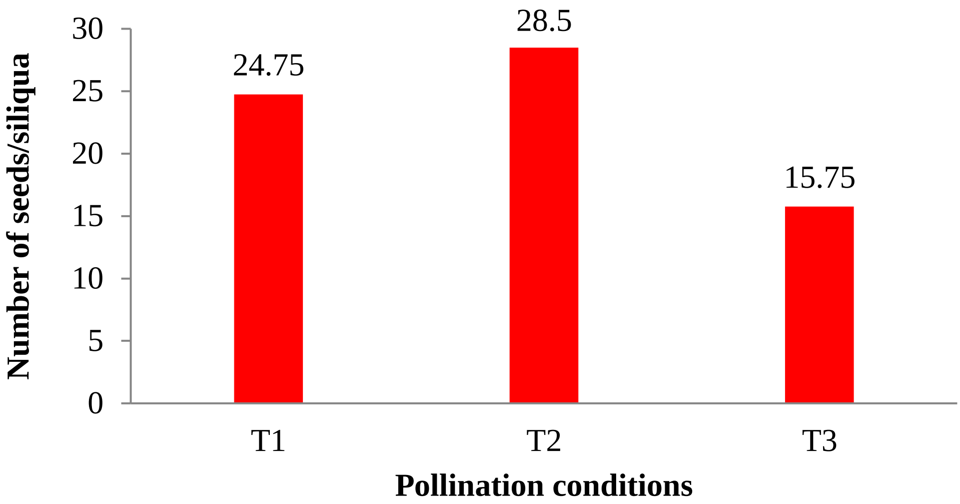

Figure 5. Effect of different pollination conditions on the number of seeds siliqua^{-1} of mustard (LSD $_{(0.05)}$ = 2.31)

T_1= Open field (Control), T_2= Netting with honey bee and T_3= Netting without honey bee

4.7 1000-seed weight (g)

Different pollination condition significantly affected the 1000-seed weight of mustard (Figure 6). The highest 1000-seed weight (3.50 g) was recorded from treatment T_2 followed by T_1 and the lowest 1000-seed weight (2.68 g) was recorded from T_3. The present result is in partial agreement with Munawar *et al*. (2009) who reported that the test weight of 1000-seed of canola, *Brassica napus* was with highest seed weight plants caged with honeybees (26.00g). The lowest was in plants caged without honeybees (9.30g).

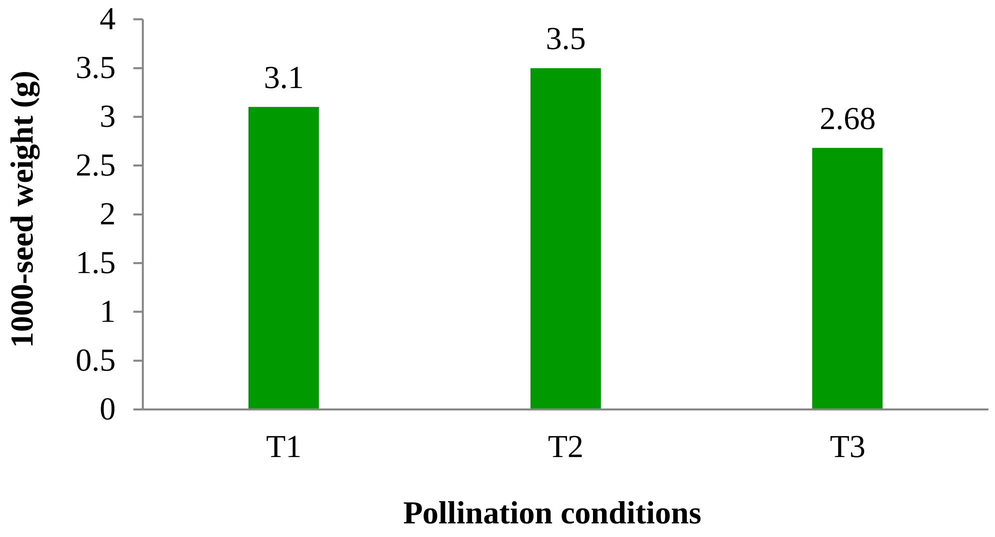

Figure 6. Effect of different pollination conditions on the 1000-seed weight of mustard (LSD $_{(0.05)}$ = 0.35)

T_1= Open field (Control), T_2= Netting with honey bee and T_3= Netting without honey bee

4.8 Seed yield (t ha^{-1})

Seed yield of mustard significantly influenced by different pollination conditions (Figure 7). The highest seed yield (2.45 t ha^{-1}) was recorded from treatment T_2 followed by T_1 and the lowest seed yield (1.67 t ha^{-1}) was recorded from T_3. Gaddanakeri *et al*. (2008) who also recorded the higher seed yield of sunflower (849 kg/ha) in intercropping system of sunflower + niger and lowest was in sole crop of sunflower (747 kg/ha) indicating the role of pollinators in both cross pollinated crops. The main reason of the higher seed yield per hectare may be the higher weight of seed per plant and higher 1000-seed weight may also be the reasons of highest seed yield. Khan and Chaudhary (1988) emphasized upon the view that insect pollination led to the formation of well-shaped larger grains and more viable seeds than self-pollinated plants. Oz *et al*. (2009) who also reported that increased production of sunflower seed in area with introduction of honey bees of related to areas without bees.

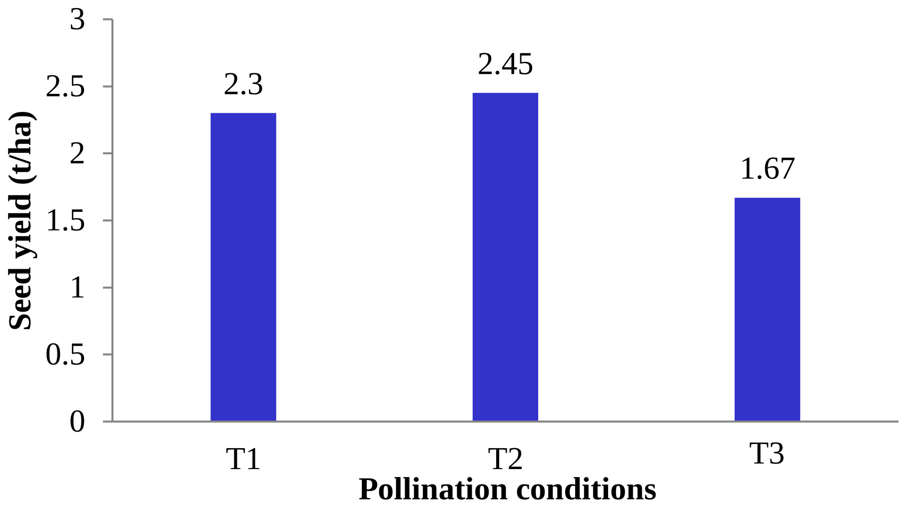

Figure 7. Effect of different pollination conditions on the seed yield of mustard (LSD $_{(0.05)}$ = 0.14)

T_1= Open field (Control), T_2= Netting with honey bee and T_3= Netting without honey bee

4.9 Seed yield increase over control (%)

Netting with honeybee (T_2) produced (6.52%) higher seed yield over control (T_1) and T_3 exhibited the negative response (-27.39%) on seed yield increase over control (Table 2).

Table 2. Effect of different pollination conditions on the seed increased over control

Treatments	Seed yield increase over control (%)
T_1	------
T_2	6.52
T_3	-27.39

T_1= Open field (Control), T_2= Netting with honey bee and T_3= Netting without honey bee

4.10 Correlation coefficient (r)

Data from harvested plant samples were used to calculate the correlation co-efficient (r). Different pollination conditions influenced the different traits of mustard and so, the correlation co-efficient was calculated among some yield and yield contributing traits. A linear relation (r=0.95) was exhibited between the number of siliquae plant^{-1} and seed yield (t ha^{-1}) of mustard (Fig. 8). In figure-9, a linear relation (r=0.99) was found between number of seeds siliqua^{-1} and seed yield (t ha^{-1}) of mustard. From the correlation study it may be said that, there was about 95 to 99 % of seed yield of mustard was dependent on different yield contributing traits. Correlation among various characters indicated that all these characters had significant contribution to seed yield and yield would be increased by improving these yield attributes.

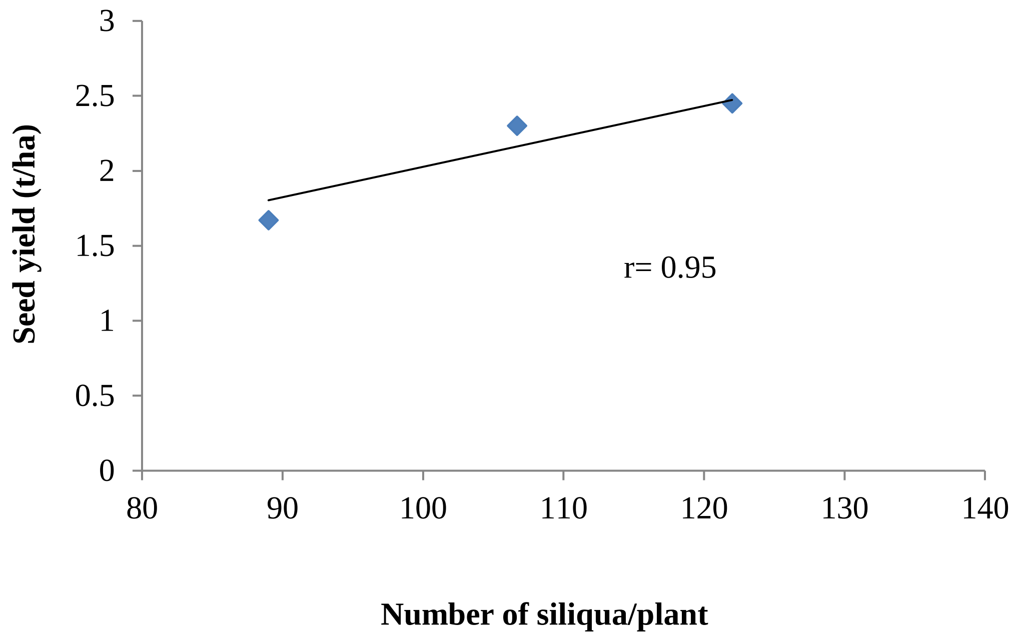

Figure 8. Relationship between number of siliquae plant^{-1} and seed yield (t ha^{-1}) of mustard

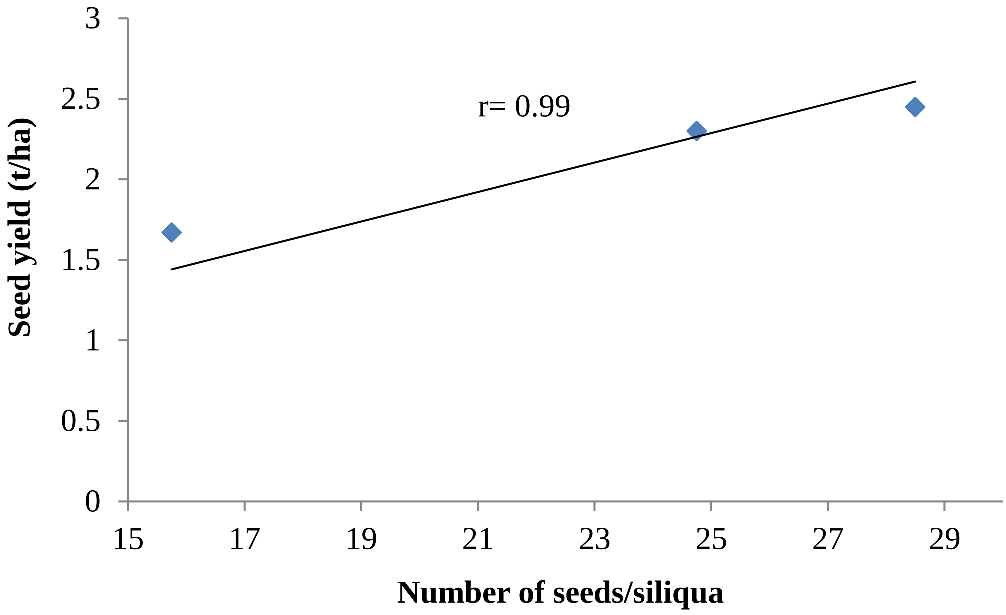

Figure 9. Relationship between number of seeds siliqua^{-1} and seed yield (t ha^{-1}) of mustard

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted at the research farm of Sher-e-Bangla Agricultural University, Dhaka-1207 during the period from November, 2016 to February, 2017 in *rabi* season. The experimental field was located at 90º 33 E longitude and 23º71 N latitude at a height of 8m above the sea level. The experimental site belongs to the agro-ecological zone of “Madhupur Tract”, AEZ-28. A sub-tropical climatic zone was prevailed around experimental site, most provably characterized by winter during the months from November 01, 2016 to February, 2017 (*rabi* season). Silty clay in texture was the main traits of top soil. The soil was also characterized by pH-5.57.

The experiment was consisted of three (3) different strategies of pollination of mustard flowers *viz*., T_1= Open field (Control), T_2= Netting with honey bee and T_3= Netting without honey bee. BARI sharisha-8 (*Brassica Juncea* L.) was used as planting material. Randomized Complete Block Design (RCBD) was selected to lay out the present experiment with 7 replications. The allocated plots were fertilized by recommended doses of fertilizers. All the intercultural operations and plant protection measures were taken as per when needed. Data on different growth yield and yield contributing characters were collected and analyzed by using MSTAT-C computer package and means were compared by Least Significant Difference (LSD) at 5% level of probability.

Results demonstrated that, honey bee has the important role on the pollination of mustard flowers for better yield. Results also revealed that, Among different insect families the maximum (87%) hymenopterans insect visited the mustard field followed by Lepidoptera (6%), coleopterans (4%), Dipterous (2%) and the minimum visitors were Homopterous (1%). Numerically the tallest plant (96.80 cm) was recorded from netting with honey bee (T_2) and the shortest plant (94.85 cm) was recorded from netting without honey bee (T_3).

The maximum number of primary branches plant^{-1} (4.52) was recorded when the plots netting with honey bee (T_2) followed by T_1 and the minimum number of primary branches plant^{-1} (3.34) was recorded from when the plots netting without honey bee (T_3). The maximum number of siliquae setting plant^{-1} (124.30, 133.30, 137.00 at 45, 55 and 65 DAS, respectively) were recorded from treatment T_2 followed by T_1 at 45, 55 and 65 DAS and the minimum number of siliquae setting plant^{-1} (92.25, 98.25 and 100.83 at 45, 55 and 65 DAS, respectively) was produced by T_3 treatment. The highest number of siliquae plant^{-1} (122.00) was found from treatment T_2 followed by T_1 and the lowest number of siliquae plant^{-1} (89.00) was found from T_3. The highest number of seeds siliqua^{-1} (28.50) was recorded from treatment T_2 and the lowest number of seeds siliqua^{-1} (15.75) was recorded from T_3. The highest 1000-seed weight (3.50 g) was recorded from treatment T_2 followed by T_1 and the lowest 1000-seed weight (2.68 g) was recorded from T_3. The highest seed yield (2.45 t ha^{-1}) was recorded from treatment T_2 followed by T_1 and the lowest seed yield (1.67 t ha^{-1}) was recorded from T_3. Correlation among various characters indicated that all these characters had significant contribution to seed yield.

Conclusion

Based on the results of the present study the following conclusion could be drawn:

1. Honey bee is the most abundant hymenopterans in the mustard field as a pollinator.
2. The growth, yield and yield contributing traits were significantly influenced by different strategies of pollination by honey bee.
3. Netting with honey bee was the best one for better yield of mustard.

Recommendation

1. For seed production of mustard, netting with honey bee after sowing may be best suited for obtaining maximum seed yield.

2. The results got from the present study should be confirmed by conducting similar type of experiments in different Agro-ecological Zones (AEZs) of Bangladesh with the combination of other variety at different conditions.

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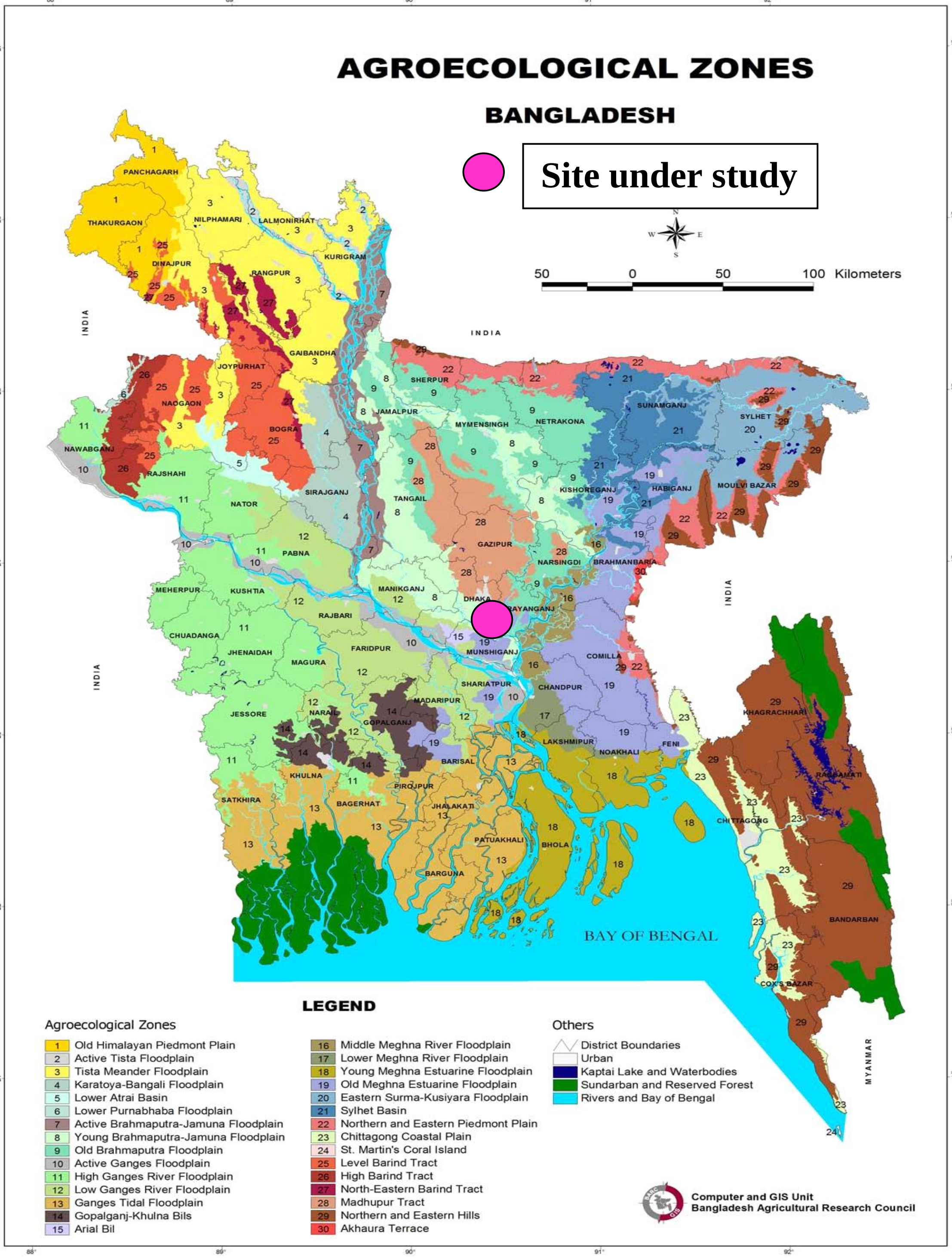
APPENDICES

Appendix I: Map showing the site used for research



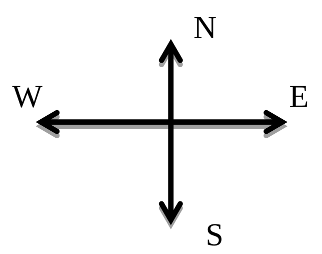
Appendix II. Monthly meteorological information during the period from November, 2016 to February, 2017

Year	Month	Air temperature (^{0}C)		Relative humidity (%)	Total rainfall (mm)
		Maximum	Minimum		
2016-2017	November	29.19	12.23	57.28	49
	December	26.00	9.16	70.43	1
	January	24.92	9.87	72.79	Trace
	February	25.85	14.54	78.42	Trace

Source: Metrological Centre (Climate Division), Agargaon, Dhaka.

Appendix III. Layout of research plot

2.5 m T_1	2.0 m T_3	T_2	T_1	T_3	T_2	T_1
T_2	T_1	T_3	T_2	T_2	T_3	T_2
T_3	T_2	T_1	T_3	T_1	T_1	T_3
R_1	R_2	R_3	R_4	R_5	R_6	R_7



Legends :

Line to line distance : 40 cm

Plant to plant distance : 10 cm

Unit plot size : 2.5 m × 2.0 m

Appendix IV. The physico-chemical properties of the soil of research plot (0-15 cm depth)

Soil parameters	Observed values
pH	5.57
Total N (%)	0.0679
Phosphorus	21.25 µg/g soil
Sulphur	24.29 µg/g soil
Boron	0.52 µg/g soil
Copper	3.38 µg/g soil
Zinc	2.98 µg/g soil

Source: Soil Resources Development Institute (SRDI), Khamarbari, Dhaka.

Appendix V. Mean square values of the data for plant height, number of primary branches/plant and siliquae setting per plant of mustard

Source of variation	df	Plant height	Number of primary branches /plant	Siliquae setting per plant at different days after sowing		
				45 DAS	55 DAS	65 DAS
Replication	6	78.073	0.201	84.850	19.600	44.400
Treatments	2	38.802 NS	0.287*	634.825**	608.575**	675.925**
Error	12	74.676	0.122	86.392	112.308	127.692

** = Significant at 1 % level of probability, * = Significant at 5 % level of probability and NS = non-significant

Appendix VI. Mean square values of the data for number of siliquae/plant, number of seeds/siliqua, 1000-seed weight and seed yield of mustard

Source of variation	df	Number of siliquae/plant	Number of seeds/siliqua	1000-seed weight	Seed yield
Replication	6	35.783	0.333	0.008	0.094
Treatments	2	718.700**	107.250**	0.223*	0.368**
Error	12	96.700	2.250	0.051	0.008

** = Significant at 1 % level of probability, * = Significant at 5 % level of probability

Plate 1. Plot with honeybee box covered with net

Plate 2. Pollination of BARI sharisha-8 flowers by honeybee

Plate 3. Plants with siliqua of BARI sharisha-8

Plate 4. Seeds of BARI sharisha-8