EFFECT OF INSECTICIDE APPLICATION TIMING ON POLLINATOR DIVERSITY IN MUSTARD FIELD BORDERED BY ATTRACTANT CROP

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DHAKA-1207

JUNE, 2021

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REGISTRATION NO: 14-06066

A Thesis
Submitted to the Faculty of Agriculture,
Sher-e-Bangla Agricultural University, Dhaka,
in partial fulfilment of the requirements
for the degree of

MASTER OF SCIENCE (MS) IN

ENTOMOLOGY

SEMESTER: JANUARY-JUNE, 2021

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CERTIFICATE

This is to certify that thesis entitled, **“Effect of insecticide application timing on pollinator diversity in mustard field bordered by attractant crop”** 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** in **Entomology,** embodies the result of a piece of Bonafede research work carried out by **SADIA AFRIN SHEFA,** Registration **No. 14-06066** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Date:
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DEDICATED TO
MY
BELOVED PARENTS

ABDUR RAHMAN
&
SHEULI RAHMAN

ACKNOWLEDGEMENTS

All praises are due to the "Almighty Allah" Who kindly enabled the author to complete the research work and the thesis leading to Master of Science.

*The author would like to express her heartiest respect, her deep sense of gratitude and sincere, profound appreciation to her supervisor, **Prof. Dr. Mohammed Sakhawat Hossain,** Department of Entomology, Sher-e-Bangla Agricultural University, Dhaka for his sincere guidance, scholastic supervision, constructive criticism and constant inspiration throughout the course and in preparation of the manuscript of the thesis.*

*The author would like to express her heartiest respect and profound appreciation to her Co-supervisor, **Prof. Dr. Md. Mizanur Rahman;** Department of Entomology, Sher-e-Bangla Agricultural University, Dhaka for his utmost cooperation and constructive suggestions to conduct the research work as well as preparation of the thesis.*

The author expresses her sincere respect to honorable chairman and all the teachers of Department of Entomology, Sher-e-Bangla Agricultural University, Dhaka for providing the facilities to conduct the experiment and for their valuable advice and sympathetic consideration in connection with the study.

Mere diction is not enough to express her profound gratitude and deepest appreciation to her father, mother, brothers, sisters, and relatives for their ever-ending prayer, encouragement, sacrifice and dedicated efforts to educate me to this level.

JUNE, 2021

SAU, Dhaka **The Author**

EFFECT OF INSECTICIDE APPLICATION TIMING ON POLLINATOR DIVERSITY IN MUSTARD FIELD BORDERED BY ATTRACTANT CROP

ABSTRACT

By

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The experiment was conducted in the experimental field of Sher-e-Bangla Agricultural University, Dhaka-1207, during November 2019 to February 2020 to find out the optimum time of insecticide application in mustard field to reduce mortality rate of the natural pollinating agents of mustard. The experimental design was a split-plot design with three replicates and two factors Factor A: Main factor; Mustard crop bordered by other crops T_1 - Mustard bordered by Dill (Shova), T_2 - Mustard bordered by Linseed (Neela), T_3 - Mustard bordered by Coriander (BARI Dhonia-1), T_4 - Mustard bordered by Niger and T_5 - Mustard without border crop (Control); Factor B: Second factor; insecticide: Actara 25WG@ (0.3gm/L; at the 15 days intervals) application timing where S_1- Spray insecticide at 8.30 am, S_2- Spray insecticide at 11.30 am, S_3- Spray insecticide at 2.30 pm and S_4- Spray insecticide at 5.00 pm. Effect of insecticide application time of pollinators was calculated and it was revealed that mustard plants with border crops were greatly influenced by the presence of different pollinators which increase crop yield. Diversity of insect pollinators was observed and among others presence of honey bee was observed as most frequently visited pollinators (33,24). The Shannon-Weaver diversity index in mustard field bordered with Niger for species was 1.87, 16.4 % community dominance observed species in mustard field bordered with Niger. Total seed weight/plant of mustard significantly influenced by different pollination conditions with the influence of bordered crops. The highest seed weight/plant (1123 g) was recorded from treatment T_4 - Mustard bordered by Niger. The lowest seed weight/plant (922.70 g) was recorded from treatment T_5 – Mustard without border crop (Control). The optimum time of insect pollinator foraging was observed at 11.30 am and the least number of insect pollinator observed at 5.00 pm, insecticide application should be at 5.00 pm to avoid minimum pollinator drifting. Application of any insecticides at 5.00 pm affect lowest to the insect pollinators population.

CONTENTS

LIST OF TABLES

LIST OF FIGURES		
Figure	**TITLE**	**PAGE NO.**

LIST OF PLATES		
Plate	**TITLE**	**PAGE NO.**

LIST OF APPENDICES

LIST OF ABBREVIATION AND ACRONYMS

AEZ	=	Agro-Ecological Zone
BARI	=	Bangladesh Agricultural Research Institute
BBS	=	Bangladesh Bureau of Statistics
FAO	=	Food and Agriculture Organization
et al.	=	And others
DAS	=	Days after Sowing
Mg	=	Milligram
kg	=	Kilogram
SAU	=	Sher-e-Bangla Agricultural University
SRDI	=	Soil Resources Development Institute
g	=	Gram
cm	=	Centimeter
wt	=	Weight
LSD	=	Least Significant Difference
^{0}C	=	Degree Celsius
NS	=	Not significant
Max	=	Maximum
Min	=	Minimum
%	=	Percent
NPK	=	Nitrogen, Phosphorus and Potassium
CV%	=	Percentage of Coefficient of Variance

CHAPTER 1

INTRODUCTION

Mustard is one of the important *oleiferous* crops and constitutes a major source of edible oil for human consumption and cake for animals. Mustard plant belongs to the genus *Brassica* under the family Cruciferae. In Bangladesh, mainly three mustard species are cultivated viz, *Brassica campestris, Brassica juncea* and *Brassica napus*. This crop is well adapted to almost all agro-climatic zones and grows in Rabi season. It is one of the leading oil seed crops in the world as well as in Bangladesh. It is used as a condiment, salad, green manure and fodder crop, and as a leaf and stem vegetable in the various mustard growing countries of the World (FAO, 2004). The major oilseed crops grown in Bangladesh are mustard, sesame, groundnut, black cumin and linseed. The minor oil crops are Niger, soybean, sunflower, safflower and castor. The major contribution of oil comes from mustard (64.58%) followed by sesame (7.01%) and groundnut (invisible oil 6.85%) (BBS 2020). At present, oilseed crops are grown in 478947.36 hectares which is 5.46% of the cultivable land producing 9,72,000 tons of oilseeds annually (BBS 2020). While in case of spices, the area under the spices cultivation is 0.4 million hectares with annual production of 2.5 million metric tons and the annual demand of spices seeds is 3.0 million metric tons. Spices cover almost 2.6 percent of total cropped area in Bangladesh (BBS 2020). As a result, Bangladesh needs to invest to import edible oils from other countries for mitigating the demand for additional population and changing of dietary habits and nutritional awareness for total population. This statement indicates that production of mustard crop urgently needs to be increased in Bangladesh.

However, increasing of mustard cultivation area is difficult due to several reasons. Among them, climate change and insect pest infestation are the major obstacles to produce mustard crop. There are many insect pests of mustard crop like mustard aphid, sawfly and mustard leaf eating caterpillar. Among them, mustard aphid, *Lipaphis erysimi* (Kalt.) (Homoptera*: Aphididae*) is the most destructive one (Das, 2002). Mustard aphid is the most serious and destructive pest and limiting factors for successful cultivation of mustard in South Asia. Both nymphs and adults of the mustard aphid infest the leaves, inflorescences and immature pods resulting poor pod setting and yield reduction, as a result the plant show stunted growth, flowers wither and pod formation is hindered. They also induce growth of fungus that causes dirty and black pods and leaves. Mustard aphid causes 35.4% to 96% yield loss, 30.9% seed weight loss and 2.75% oil loss.

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. Sushil *et al.,* 2013 said that, low seed yield due to inadequate pollination is often faced as a major problem of Brassica seed production. Poor pollination is one of the major problems of low yield production and optimum pollination is one of the important factors in increasing the production and productivity of crop yield and essential for the propagation of a multitude of plant species. 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 (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 (D'Avila and Marchini, 2005).

All field need pollinators and bees as well as insect pollinators are among the best. Without them, there would be limited flowers and even fewer fruits and vegetables. Different types of pollinators are found in mustard field. Among many insect pollinators honey bee (*Apis mellifera*) was found as the main insect pollinator during mustard flowering season. Cross pollination of entomophiles crops by honeybees is considered as one of the effective and cheapest method for triggering the crop yield both qualitatively and quantitatively (Mohapatra *et al*., 2014). Honey bees, like other insects, are reasonably sensitive to a range of chemical insecticides (Hardstone and Scott, 2010). Insecticide use, apart from loss of natural vegetation cover (Winfree *et al*., 2009), has been cited as one of the major drivers of the recent decline in pollinator populations (Whitehorn *et al*., 2012).

Trees, shrubs and herbaceous plants can provide food and nesting habitat for pollinators. An abundance of different flower shapes, sizes, and colors will appeal to a variety of pollinators. Grouping plants together in sunny locations helps pollinators find and feed on desirable flowers while expending less energy in the search for plants. By observing flowers in the garden and taking note of any flower visitors, gardeners can learn which plants are most attractive to pollinators. Research suggests native plants are four times more attractive to native bees than exotic flowers. They are also usually well adapted to the growing conditions and can thrive with minimum attention. In gardens, heirloom varieties of herbs and perennials can also provide good foraging. Different flower shapes and colors attract different pollinators. For example, Dill, Linseed, Coriander, Niger. Some cultivars and hybrids don't offer the pollen and nectar rewards that so-called "straight species" do, since the quality and quantity of nectar and pollen are sometimes lost during breeding. Plants bred with "double" flower petals are often inaccessible to pollinators. Gardeners can include less refined plants along with plant cultivars to offer broad pollinator appeal.

Farmers usually spray chemical insecticides many times during the crop season to control insect pests. This leads to environmental pollution with a consequence of increased health hazard to the growers and consumers. This insecticide has tremendous effects on environment, biodiversity, human and animal health. Moreover, it also leads to the development of resistance to target pests with also a negative effect on natural enemies (Tiwari *et al.* 2005) and other beneficials, and causes disruption of biodiversity. From the above points of view the proposed study was taken to fulfill the following objectives;

1. To know the number of pollinators in mustard crop and neighboring attractant crop before insecticide application.
2. To know the number of pollinators in mustard crop and neighboring attractant crop after insecticide application.
3. To find out the optimum time of insecticide application in mustard yield to reduce mortality rate of the natural pollinating agent.
4. To study the effect of pollinator with the presence of neighboring attractant crop on yield of mustard.

CHAPTER 2

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 optimum time of insecticide application based on the time of pollination by insects and major group of insects are not well investigated to us. So, to prepare a well plan for the present study on the role of honey bee as mustard pollinator and optimum time of insecticide application from different secondary sources of information were reviewed in this chapter.

2.1 General review on mustard pollinators

2.1.1. Taxonomy of Honey bee

Taxonomic position of Honey bee:

Phylum: Arthropod

Class: Insecta

Order: Hymenoptera

Family: Apidae

Genus: *Apis*

Species: *Apis mellifera*

Honey bees represent just a small fraction of the approximately 20,000- 30,000 known species of bees. Several other bees produce and store some kind of honey, but only members of the genus *Apis* are true honey bees (Kleinjans, *et al.*, 2012).

Foraging of Honey bees

Honeybee, *A. mellifera* L. was reared in around Bangladesh Agricultural University campus in Mymensingh to study its life cycle, behavior, pollen gathering activity, honey production and its effect on yield of mustard. There was no relationship between sunrises, sunset, first out from the box and last entrance into the box. But positive relationship was found with day temperature to first out and last entrance. The highest number of bees collected pollen in the 3rd week of March. Maximum pollen gathering activities were found at 12.00 to 1.00 p.m. The highest amount of honey production was 4.00 kg per box in mustard and there was positive correlation between percent pollen gathering activity and honey production. The highest number of queen cell was found in the month of March. The results showed that honey bee pollination had significant effect on increase in all the plant parameters and yield (Islam, *et al*., 2015). Yucel and Duman (2005) found that honey bee workers visited onion flowers from 8.15 to 16.30 h and the peak foraging was between 11.00 to 12.00 h.

2.1.2. Taxonomy of Syrphid fly

Taxonomic position of Syrphid fly:

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Diptera

Section: Aschiza

Superfamily: Syrphoidea

Family: Syrphidae

The family Syrphidae, which syrphids belong to is arguably the most recognized group of fly pollinators. While the name flower-flies implies that these dipterans are seen often on flowers, their alternative name “hoverflies” refers to their ability to hover in midflight. Syrphids are ubiquitous and consist of more than 6,000 species worldwide. They can be found in all regions of the world except Antarctica. Syrphids are particularly abundant in habitats of high altitude and latitude, and are important pollinators in forest ecosystems. As adults, syrphids visit generalist flowers, actively foraging on nectar and pollen.

Syrphid fly as pollinator

Though syrphids interact directly with pollen by either actively or passively collecting it, only recently, syrphids (and other flies) have been recognized as important pollinators. Indeed, in a series of very recent global analyses, it was estimated that the contribution of syrphids to pollination could be equaled to that of bees, at least in some cases. In contrast to bees, syrphids are more mobile and capable of traveling longer distances, likely dispersing pollen greater distances. Further, many syrphid species are migratory, and these migration events contribute to extreme pollen dispersal, which in Britain has been quantified as involving a number of individuals not much different to that of British managed honeybees at peak population size. Even though it has been long thought that syrphids are mostly "incidental" pollinators, recent studies demonstrate that most individuals are consistent in their floral choices, which tends to suggest that they are not only abundant but also efficient pollinators. Supporting this, some experiments indicate that even though syrphids may carry less pollen grains than bumblebees or bees, flowers visited exclusively by syrphids are better pollinated than those visited exclusively by bumblebees. In addition to mostly visiting a larger variety of flowers than honeybees, syrphids are generally more abundant in natural and agricultural habitats than wild bees. Moreover, syrphids may fill niches that are not covered by larger pollinators. For example, large bumble bees tend to visit large flowers; and the flower complex of some plants such as *Solidago virgaurea* is small and thus can be effectively pollinated by small syrphid flies (Cerruti *et al.,* 2020).

2.1.3 Taxonomy of Wasp

Taxonomic position of Wasp:

Class: Insecta

  Subclass: Pterygota

    Superorder: Holometabola

      Order: Hymenoptera

        Suborder: Apocrita

Infraorder: Aculeata

Superfamily: Vespoidea

Family: Vespidae

Genus: *Polistes*

Species: *Polistes fuscatus*

Wasp as pollinator

Wasps are an important part of the flower-visiting guild and often frequent flowers in search of nectar and/or insect prey. Some wasps are considered generalist pollinators, and passively transfer pollen while feeding on nectar from various plants. While doing so, they often overlap with other pollinators, such as bees, flies or butterflies. However, because they generally lack abundant body hairs and do not feed on pollen, they are considered less efficient pollinators than their bee relatives. Further, some behave more frequently as nectar thieves than as true pollinators, especially when they pierce the base of flowers to access the nectar without contacting the plant's reproductive organs. This said, despite not having the reputation of bees, wasps can and do effectively contribute to pollination. In some plant systems and environments, they can become the most efficient pollinator, surpassing bees. For example, in a study involving pollinators and the plant *Schinus terebinthifolius*, some social wasp pollinators were more abundant and species-rich than bee visitors. Another study found that in some environments, the western yellowjacket (*Vespula pensylvanica*) was a more effective pollinator than the honey bee (*Apis mellifera*). In that investigation, it was observed that pollen of the plant *Scrophularia californica* was more efficiently transferred by *Vespula* wasps and *Bombus* bees (bumblebees) than by honey bees, which visited the plant but did not pollinate. In this same study, the median number of pollen grains delivered per individual floral visitor also varied among the groups (*Apis* = 4, *Bombus* = 9, and *Vespula* = 34). As a result, this study demonstrated that even though honey bees seemed to be the most abundant floral visitor, the western yellowjacket was the most effective pollinator. Though wasps are sometimes the best pollinator of some generalist flowers, they are typically recognized as specialist pollinators. Specialist unlike generalist pollinators, are very selective in their floral choices, and frequent flowers of one or a very few plant species. In instances where this type of specialization has evolved, rewards involved are either special (e.g., brood site) or inexistent in that the wasp is lured and exploited by the plant. In either case, the plant reproduction relies exclusively on these specialized visitors (Cerruti *et al.,* 2020).

2.1.4 Taxonomy of Butterfly

A butterfly is an insect of the Order Lepidoptera that belongs to either the superfamily Papilionoidea or the superfamily Hesperioidea ("the skippers").

Taxonomic position of Butterfly:

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Lepidoptera
Family: Pieridae
Genus: *Pieris*
Species: *P. brassicae*

Butterfly as pollinator

Butterflies like to perch on larger flower heads when they hunt nectar, collecting pollen on their legs and body as they search for food. The legs and the butterfly's proboscis are longer and farther away from the flower's pollen so less pollen collects on its body parts than it does on bees, but still, they are very effective pollinators (Cerruti *et al.*, 2020).

2.2 Role of pollinating insects on the production of mustard

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/m2 /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: T1-caged plot with one bee hive of *Apis cerana indica*, T2-open pollination plot (without nylon cover) and T3- caged plot without bee colony (pollination exclusion). In T1 and T3, 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 (Tl), 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, contained 3 pollination treatments, viz., Plants kept open to all pollinators (T1), Plants caged with Apis hives (T2) and plants caged without access to any pollinators (T3). The difference in siliquae per plant, seeds per siliqua, thousand seed weight, seed yield per plant and per plot were found significant 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.

Sanas *et al.,* (2014) also found that A. mellifera increased the number of seed per pod (23.27%) in mustard under Konkan condition of Maharastra. There are many studies showings the pollinator ‘s role and findings are confirmative with Prasad *et al.* (1989) they reported in *B. juncea*, open pollination gave the maximum yield (13.4 q/ha) followed by plots caged with one *A. cerana* honeybee colony (11.3 q/ha), whereas plots caged without bees (exclusion of pollinators) gave the lowest seed yield (10 q/ha). Chand and Singh (1995), reported that the mustard plots caged without any pollinator had lowest seed yield (966 kg/ha). Whereas, the free access to all the pollinator showed the maximum yield (1620 kg/ha) followed by plots caged with honeybees (1160 kg/ha). Sanas *et al.*, (2014), also reported that mustard plot gave maximum seed yield (963.45 Kg/ha) pollinated by free access to all pollinators and lowest yield (602.52 Kg/ha) in pollination without insect. Whereas plots gave yield (763.75 Kg/ha) pollinated by honeybees (*A. cerana indica* Fab).

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, a greater 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 (T1), partial exclusion (T2) and free pollination (T0) 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 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.

In addition to Hymenoptera pollinators, a number of important Diptera pollinators have been recorded on Brassica crops. Perhaps the most important Diptera pollinators of Brassica are hover flies (Syrphidae) (Conner & Rush 1995). Syrphids certainly do consume pollen (Conner & Rush 1995), but they also transfer a large amount of pollen on their body to other flowers (Herrera 1987). When Syrphid pollinators seek nectar, their heads become powdered with pollen and they can act as excellent agents of pollen dispersal (Westerbergh & Saura 1994). In some instances, though, Rush et al. (1995) noted that Syrphids may only carry large pollen loads from the first visitation to a flower, because the body of the insect becomes saturated in the following flower visits. Generally speaking, then, hover flies are often considered to be fewer effective pollinators than bees, but they nevertheless play an important role in cross-pollination (Hoyle *et al.* 2007). Another important characteristic of hoverflies is that they have a seasonal behavior that allows them to contribute differentially to gene flow at different times during the flowering cycle (Langridge & Goodman 1975). Hoyle *et al.* (2007) reported that Syrphids visited fewer plants in succession than bumblebees, but tended to visit a few adjacent plants and then suddenly fly several meters away and resume small-scale flower visitation once again, thus increasing cross pollination and gene flow (Westerbergh & Saura 1994).

Diptera and Hymenoptera are regarded as the most important pollinators of Brassica species (Easthan & Sweet 2002). Previous studies have shown that there is strong species-specific variation in pollinator foraging behavior in *Brassica rapa* crops. For instance, *A. mellifera* is thought to be the most efficient pollinator in *Brassica rapa* crops because they carry pollen not only on their legs but also on the body setae (Eastham & Sweet 2002). The behavior of *Apis mellifera* tends to increase cross-pollination rates, because bees have a tendency to move between clusters of flowers on different plants, rather than staying in one cluster on a single plant (Langridge & Goodman 1975).

2.3 Role of chemical insecticide on pollination

Insecticides may also influence foraging behavior. Yang *et al.* (2008) reported effects of sublethal doses of imidacloprid on the foraging behavior of honey bees which manifested as a delay in their visit to the feeding site. The delay depended on the imidacloprid concentration. Schneider *et al.* (2012) found a significant reduction in foraging activity as well as longer foraging flights at doses of two neonicotinoid insecticides; 0.5 mg/bee or more for clothianidin and 1.5 mg/bee or more for imidacloprid during the first 3 h after treatment. In contrast, the presence of residues in the nectar and pollen of oilseed rape and maize due to seed treatment with thiamethoxam was reported to represent a low risk to honey bees (Pilling *et al.*, 2013). More investigations on these factors are urgently required especially since neonictinoids are so widely used.

Insecticides have been cited as one of the major drivers of pollinator loss. However, little is known about insecticide impacts on natural populations of native honey bee species. This study looked into the effect of insecticides with respect to oxidative stress in the laboratory and in field populations of two native Indian honey bee species (*Apis dorsata* and *A. cerana*) by examining a combination of biomarkers, e.g., superoxide dismutase, catalase and xanthine oxidase. A significant upregulation of all three biomarkers was observed in both treated individuals in laboratory experiments and field populations sampled from a insecticide use gradient. This study reports, for the first time, an increase in expression of xanthine oxidase in an invertebrate system (honey bees) exposed to insecticides (Chakrabarti, *et al.*, 2015).

However, in many cases, colonies are put in place near nectar and pollen rewarding places – and where food is abundant individuals tend to stay in a favorite site – and after collecting enough food will return to the colony. This common pattern will lead to dominant pollination patterns that occur within a range of a few 100 meters or even less (e.g., when colonies are placed in a flowering orchard). The whole issue of the impact of long-range flights on pollination over larger distances depends on too many factors to draw one general conclusion. In an extreme case scenario, two isolated but attractive fields at a large distance with a bee colony in between can be visited by the same individual bees at the same day, taking maximum pollen loads with them. In such a case, significant pollen transport could occur at a distance of two times 10 km (Kleinjans, *et.al.*, 2012).

Oilseed rape is one of the most preferred crops for honey bees and possibly for other pollinating insects as well. At the time of mass flowering, it attracts pollinating insects from over large distances. Due to the importance of this crop and suitability for experimental studies, several investigations have been done on the foraging activity and pollen transports from oilseed rape and similar cruciferous crops and weeds. Most recent studies of Rader, *et al* (2011) and Chifflet *et al.* (2011) have shown that these crops attract bees easily from distances of at least 500 m to 1,000 m.

2.4 Role of pollinator attractant crops

With regard to out-colony factors, the availability of suitable plant resources has a great impact on foraging activity, and forager bees have a preference for some resources over others. Moreover, Fulop and Menzel (2000) found that the reward volume (e.g., sucrose solution or nectar) has an impact on foraging activity and that bees can perceive the amount of reward from the feeding source.

CHAPTER 3

MATERIALS AND METHODS

The present experiment was conducted in the experimental field of Sher-e-Bangla Agricultural University, Dhaka-1207, during November 2019 to February 2020 to find out the optimum time of insecticide application in mustard field to reduce mortality rate of the natural pollinating agents of mustard. The details of different experimental materials and methodologies followed during the course of the investigation are described under the following sub-headings.

3.1 Location and duration of the experimental site

The research work was conducted in the experimental field of Sher-e-Bangla Agricultural University, Dhaka-1207 (Plate 1) during the Rabi season of 2019-20. (From November 2019 to February 2020).

3.2 Soil of the experimental site

The soil of the experimental field belongs to the Tejgaon series under the Agro ecological Zone, Madhupur Tract (AEZ- 28) and the general soil type is Shallow Red Brown Terrace Soils. It was medium high land, fertile, well drained, fairly leveled and slightly acidic with pH varying from 5.8 to 6.5, CEC 25-28 (Haider *et al.*, 1991).

3.3 Climate

The experimental area has sub-tropical climate characterized by heavy rainfall during May to September and scantly rainfall during rest of the year. The experiment was carried out during rabi season of 2019-2020. Temperature during the cropping period ranged from 13.32˚ to 24.12˚ C.

Plate 1. The experimental field layout in the research field of SAU, Dhaka

3.4 Preparation of the field

The plot selected for the experiment was opened by power tiller driven rotovator, afterwards the land was ploughed and cross-ploughed followed by laddering to obtain a good tilth. The corners of the field were spaded, weeds and stubbles were removed and the large clods were broken into smaller pieces to obtain a desirable tilth of soil for sowing of seeds. The target land was leveled and the experimental plot was divided into 54 ((5+4) ×3×2) equal plots with a plot size of 2.0 m x 1.5 m and plot to plot distance 0.5 m; block to block distance 1.0 meter.

3.5 Application of fertilizers

Recommended doses of N, P, Zn and B (30 kg N from urea, 30 kg P from TSP and 2 kg Zn from ZnO respectively) were applied. The whole amount of TSP and ZnO half of the urea fertilizer were applied as basal dose during final land preparation. The remaining half of urea was top dressed after 20-22 days of germination.

3.6 Design of the experiment and layout

The experiment was laid out in a Split-plot design with three (3) replications. The total numbers of plots were 54 ((5+4) ×3×2) equal plots for 9 treatments, each measuring 2.5 m × 1.5 m (3.75 m^2). The adjacent block and neighboring plots were separated by 1.0 m and 0.5 m, respectively.

3.7 Treatments

The experimental design is a split-plot design with three (3) replicates and two factors

Factor A: Main factor; Mustard crop bordered by other crops

T_1 - Mustard bordered by Dill (Shova)

T_2 - Mustard bordered by Linseed (Neela)

T_3 - Mustard bordered by Coriander (BARI Dhonia-1)

T_4 - Mustard bordered by Niger

T_5- Mustard without border crop (Control)

Factor B: Second factor; Insecticide application timing: Actara 25WG@ (0.3gm/L; at the 15 days intervals)

S_1- Spray insecticide at 8.30 am (number of insect pollinators before/after application)

S_2- Spray insecticide at 11.30 am (number of insect pollinators before/after application)

S_3- Spray insecticide at 2.30 pm (number of insect pollinators before/after application)

S_4- Spray insecticide at 5.00 pm (number of insect pollinators before/after application)

3.8 Detail procedure of the study

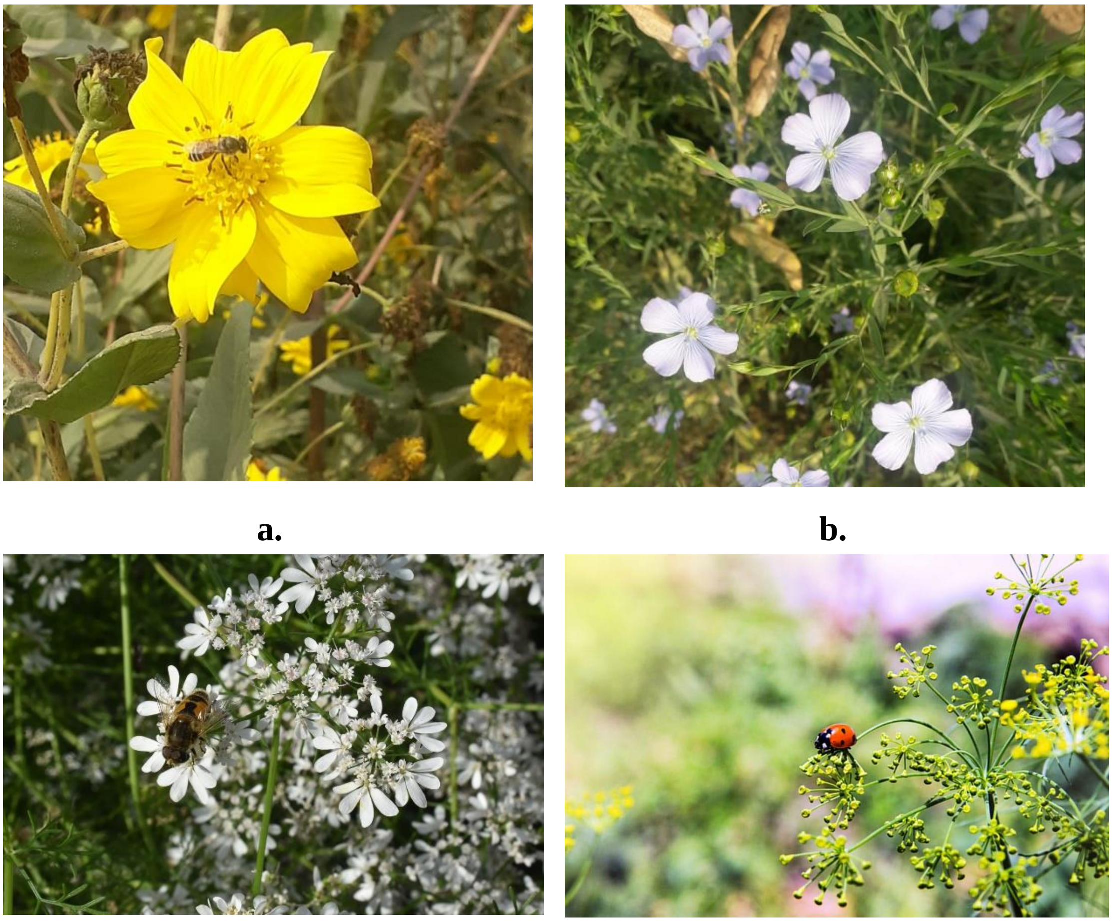
The detail procedure considering the materials used and methodology followed in the study were furnished in below:

3.8.1 Materials

The mustard variety BARI shorisha-14 was cultivated in the designed field to investigate the present study according to the objectives mentioned earlier.

3.8.2 Seed sowing

Seeds of the BARI shorisha-14 variety of mustard collected from BADC were sown in the selected field on 17th November 2019 in lines following the recommended row to row distance of 30 cm. After germination the seedlings (Plate 2) were sprinkled with water.



a. b. c. d.

Plate 2. Presence of pollinators influenced by border crops in the experimental plot. (a. Niger, b. Linseed, c. Coriander and d. Dill)

3.8.3 Intercultural operation

The weeds found in the mustard field were cleaned and removed manually. The thinning of the mustard seedlings was also done as required during the growing season and care was taken to maintain uniform plant population per plot. Three times flood irrigation was given in the field at vegetative stage.

3.8.4 Application of the treatments

The selected treatments comprising different insecticides with their assigned doses were started to apply in the respective plots when the aphids were first appeared in the mustard field. The first appearance or incidence of pollinators was determined by visit and daily direct visual observation of mustard plants. Therefore, considering the first appearance of the pollinators in the field, treatment applications were started at 30 days after sowing (DAS) of the mustard seeds with 15 days interval.

3.9 Data collection and calculation

Ten plants per plot were selected randomly and tagged for data collection. Data collection was started at 30 DAS. All data were collected before and after the application of insecticide application. After the completion of data collection, insecticides were sprayed as schedule.

The data was collected on the following parameters after each application: (details)

Effect of insecticide application timing on pollinator diversity

1. Diversity of insect pollinators
2. Abundance of pollinators influenced with pollinator attractant crops
3. Time of foraging of insect pollinators
4. Number of insect pollinators before and after insecticide application/plot

Collection, identification and determination of pollinator diversity of insect pollinators

Free-living insects were collected from the open plot during blooming stage. Insect collection was done by using a 30 cm diameter sweep net having 1.5 mm mesh and attached with 2 m long rod. Sweeping was done before and after insecticide application. The collected insects were brought to the Entomology Laboratory for identification and counting. They were killed by storage in a freezer for a few hours, mounted on points, dried and morphotyped. Insects were identified to genus or species level following morphological characters and compared to the museum specimens. On the basis of the collection dates, the pollinator species were separated and their abundance (number $sweeps^{-1}$) was recorded.

Some of the collected specimens were identified up to species level and the rest specimens were identified up to genus level using morphological technique. For morphological identification, mounted specimens were imaged with an Entovision Imaging System. The specimens were preserved at the Insect Museum of the Department of Entomology at Sher-e-Bangla Agricultural University. Microsoft

Office Excel 2019 was used for statistical analysis. Biodiversity of the community of research plots was calculated using the Shannon-Weaver

Diversity index H'= $-\sum(\frac{ni}{N} \times log2\left(\frac{ni}{N}\right))$

where, n_i /N is the proportion of each super family within the community, N expresses the total number of super families within the community.

Yield and yield contributing attributes of mustard cultivated with pollinator attractant crops

1. Total number of pod/plant

Total number of pod was counted from each replication from randomly selected ten plants. then average number of pod/plant was counted. Average number of seed per plant was also counted and total seed weight was measured.

2. Length of pod

Length of pod was counted from each replication from randomly selected ten plants in cm scale. then average length of pod/plant was counted. Average length of seed per plant was also was measured.

3. Diameter of pod

Diameter of pod was counted from each replication from randomly selected ten plants in cm scale. then average diameter of pod/plant was counted. Average diameter of seed per plant was also was measured.

4. Total pod dry weight/plant (%)

Total pod dry weight per plant was counted from each plot from randomly selected ten plants.

5. Total seed weight/plant (g)

Total seed weight per plant (g) was counted from each plot from randomly selected ten plants.

3.10 Harvesting, threshing and cleaning

Mustard was harvested at the maturity (94 days of sowing) was done manually from each plot. Harvested crops of each plot was bundled separately, properly tagged and brought to shade. Care was taken for harvesting, threshing and also cleaning of mustard. The seeds were cleaned and finally the weight was recorded and converted into per hectare yield. Mustard cultivated with pollinator attractant crops of each plot was threshed separately, cleaned, sun dried and weighed.

3.11 Data analysis

The data collected on different parameters were analyzed using MSTAT-C computer software package and means for each parameter were separated by DMRT to determine the significant variations among treatment means at 5% level of significance.

CHAPTER 4

RESULTS AND DISCUSSION

An experiment was conducted in the experimental field of Sher-e-Bangla Agricultural University, Dhaka-1207, during November 2019 to February 2020 to find out the optimum time of insecticide application in mustard field to reduce mortality rate of the natural pollinating agents of mustard. The experimental design is a split-plot design with 3 replicates and two factors Factor A: Main factor; Mustard crop bordered by other crops T_1 – Mustard bordered by Dill (Shova), T_2 – Mustard bordered by Linseed (Neela), T_3 – Mustard bordered by Coriander (BARI Dhonia-1), T_4 – Mustard bordered by Niger and T_5 – Mustard without border crop (Control); Factor B: Second factor; Insecticide Actara 25WG@ (0.3gm/L; at the 15 days intervals) application timing where S_1- Spray insecticide at 8.30 am, S_2- Spray insecticide at 11.30 am, S_3- Spray insecticide at 2.30 pm and S_4- Spray insecticide at 5.00 pm. The results of the present study have been discussed and possible interpretations are furnished and presented in this chapter under the following sub headings.

4.1 Effect of insecticide application timing on insect diversity and pollinator abundance

4.1.1 Diversity of insect pollinators

A total number of six families and seven species was identified. Diversity of insect pollinators was observed and among others presence of honey bee was observed as most frequently visited pollinators (29,20). butterfly was the second most visible insect pollinator (18) and the lowest presence (7) was observed for the pollinator of *Pieris rapae* from the family Pieridae. The Shannon-Weaver diversity index in mustard bordered with linseed field for species was 1.865, with the dominance was 0.165. There were 16.5% community dominance for 7 observed species in mustard field bordered with dill (Table 1).

Table 1: Total insect diversity in mustard field bordered with Dill (T_1).

Family	Species	Abundance (No. of insects/m^2	x	x^2	-x ln(x)	Frequency of visits
Apidae	*Apis cerana F.*	20	16.40%	0.027	0.296	*
	Apis mellifera L.	29	23.80%	0.057	0.342	***
Scollidae	*Vespula vulgaris*	16	13.10%	0.017	0.266	*
Syrphidae	*Syrphus corollae*	10	8.20%	0.007	0.205	*
Pieridae	*Pieris rapae*	7	5.70%	0.003	0.164	*
Pieridae	*P. brassicae*	18	14.80%	0.022	0.282	*
Formicidae	*Formica sp.*	22	18.00%	0.033	0.309	**
Total	122					
Dominance	0.165					
Shannon Entropy	1.865					
Community dominance	16.5%					

*=Less frequent visitors **=Frequent visitor ***=Most frequent visitors

A total number of six families and seven species was identified. Diversity of insect pollinators was observed and among others presence of honey bee was observed as most frequently visited pollinators (30,21). butterfly was the second most visible insect pollinator (18) and the lowest presence (8) was observed for the pollinator of *Pieris rapae* from the family Pieridae. The Shannon-Weaver diversity index in mustard bordered with linseed field for species was 1.867, with the dominance was 0.165. There were 16.5% community dominance for 7 observed species in mustard bordered with linseed field (Table 2).

Table 2: Total insect diversity in mustard field bordered with Linseed (T_2).

Family	Species	Abundance (No. of insects/m^2	x	x^2	-x ln(x)	Frequency of visits
Apidae	*Apis cerana F.*	21	16.50%	0.027	0.298	**
	Apis mellifera L.	30	23.60%	0.056	0.341	***
Scollidae	*Vespula vulgaris*	16	12.60%	0.016	0.261	*
Syrphidae	*Syrphus corollae*	10	7.90%	0.006	0.2	*
Pieridae	*Pieris rapae*	8	6.30%	0.004	0.174	*
Pieridae	*P. brassicae*	18	14.20%	0.02	0.277	**
Formicidae	*Formica sp.*	24	18.90%	0.036	0.315	**
Total	127					
Dominance	0.165					
Shannon Entropy	1.867					
Community dominance	16.5%					

*=Less frequent visitors **=Frequent visitor ***=Most frequent visitors

A total number of six families and seven species was identified. Diversity of insect pollinators was observed and among others presence of honey bee was observed as most frequently visited pollinators (31,22). butterfly was the second most visible insect pollinator (19) and the lowest presence (8) was observed for the pollinator of *Pieris rapae* from the family Pieridae. The Shannon-Weaver diversity index in mustard bordered with coriander field for species was 1.862, with the dominance was 0.166. There were 16.6% community dominance for 7 observed species in mustard bordered with coriander field (Table 3).

Table 3: Total insect diversity in mustard field bordered with Coriander (T_3).

Family	Species	Abundance (No. of insects/m^2	x	x^2	-x ln(x)	Frequency of visits
Apidae	*Apis cerana F.*	22	16.70%	0.028	0.299	**
	Apis mellifera L.	31	23.50%	0.055	0.34	***
Scollidae	*Vespula vulgaris*	17	12.90%	0.017	0.264	*
Syrphidae	*Syrphus corollae*	10	7.60%	0.006	0.195	*
Pieridae	*Pieris rapae*	8	6.10%	0.004	0.17	*
Pieridae	*P. brassicae*	19	14.40%	0.021	0.279	**
Formicidae	*Formica sp.*	25	18.90%	0.036	0.315	**
Total	132					
Dominance	0.166					
Shannon Entropy	1.862					
Community dominance	16.6%					

*=Less frequent visitors **=Frequent visitor ***=Most frequent visitors

A total number of six families and seven species was identified. Diversity of insect pollinators was observed and among others presence of honey bee was observed as most frequently visited pollinators (33,24). butterfly was the second most visible insect pollinator (21) and the lowest presence (9) was observed for the pollinator of *Pieris rapae* from the family Pieridae. The Shannon-Weaver diversity index in mustard bordered with Niger field for species was 1.87, with the dominance was 0.164. There were 16.4 % community dominance for 7 observed species in mustard bordered with Niger field (Table 4).

Table 4: Total insect diversity in mustard field bordered with Niger (T_4).

Family	Species	Abundance (No. of insects/m^2	x	x^2	-x ln(x)	Frequency of visits
Apidae	*Apis cerana F.*	24	16.60%	0.027	0.298	**
	Apis mellifera L.	33	22.80%	0.052	0.337	***
Scollidae	*Vespula vulgaris*	18	12.40%	0.015	0.259	**
Syrphidae	*Syrphus corollae*	12	8.30%	0.007	0.206	*
Pieridae	*Pieris rapae*	9	6.20%	0.004	0.173	*
Pieridae	*P. brassicae*	21	14.50%	0.021	0.28	**
Formicidae	*Formica sp.*	28	19.30%	0.037	0.318	***
Total	145					
Dominance	0.164					
Shannon Entropy	1.870					
Community dominance	16.4%					

*=Less frequent visitors **=Frequent visitor ***=Most frequent visitors

A total number of six families and seven species was identified. Diversity of insect pollinators was observed and among others presence of honey bee was observed as most frequently visited pollinators (25,18). butterfly was the second most visible insect pollinator (13) and the lowest presence (6) was observed for the pollinator of *Pieris rapae* from the family Pieridae. The Shannon-Weaver diversity index in mustard field for species was 1.85, with the dominance was 0.169. There were 16.9 % community dominance for 7 observed species in mustard field (Table 5).

Table 5: Total insect diversity in mustard field (T_5).

Family	Species	Abundance (No. of insects/m^2	x	x^2	-x ln(x)	Frequency of visits
Apidae	*Apis cerana F.*	18	17.60%	0.031	0.306	*
	Apis mellifera L.	25	24.50%	0.06	0.345	***
Scollidae	*Vespula vulgaris*	12	11.80%	0.014	0.252	*
Syrphidae	*Syrphus corollae*	8	7.80%	0.006	0.2	*
Pieridae	*Pieris rapae*	6	5.90%	0.003	0.167	*
Pieridae	*P. brassicae*	13	12.70%	0.016	0.263	*
Formicidae	*Formica sp.*	20	19.60%	0.038	0.319	**
Total	102					
Dominance	0.169					
Shannon Entropy	1.850					
Community dominance	16.9%					

*=Less frequent visitors **=Frequent visitor ***=Most frequent visitors

4.1.2 Abundance of pollinators influenced with pollinator attractant crops:

Abundance of pollinators in mustard field bordered with dill (T_1)

4 species was found as insect pollinators influenced by mustard with linseed as pollinator attractant crop. Honey bee (24) was highest and the lowest number as insect pollinator species was observed for wasp (5). Very few Syrphid fly (5) and butterfly (12) was also observed (Table 6). Dill was observed as least considerable border crop to increase pollination of mustard.

Table 6: Abundance of pollinators influenced by mustard with Dill as pollinator attractant crop.

Common name	**Scientific name**	**Family**	**Presence of pollinator before insecticide application (No. of insects/m^2**	**Presence of pollinator after insecticide application (No. of insects/m^2**
Honey bee	*Apis mellifera* L	Apidae	24	7
Wasp	*Vespula vulgaris*	Scollidae	5	1
Syrphid fly	*Syrphus corollae*	Syrphidae	5	2
Butterfly	*P. brassicae*	Pieridae	12	2

Abundance of pollinators in mustard field (bordered with linseed T_2)

4 species was found as insect pollinators influenced by mustard with linseed as pollinator attractant crop. Honey bee (24) was highest and the lowest number as insect pollinator species was observed for wasp (5). Very few Syrphid fly (6) and butterfly (10) was also observed (Table 7). Among other pollinator attractant crops linseed performed poorly to attract insect pollinator to increase mustard pollination.

Table 7: Abundance of pollinators influenced by mustard with Linseed as pollinator attractant crop.

Common name	**Scientific name**	**Family**	**Presence of pollinator before insecticide application (No. of insects/m^2**	**Presence of pollinator after insecticide application (No. of insects/m^2**
Honey bee	*Apis mellifera* L	Apidae	24	8
Wasp	*Vespula vulgaris*	Scollidae	5	1
Syrphid fly	*Syrphus corollae*	Syrphidae	6	1
Butterfly	*P. brassicae*	Pieridae	10	3

Abundance of pollinators in mustard field bordered with coriander (T_3)

4 species was found as insect pollinators influenced by mustard with coriander as pollinator attractant crop. Honey bee (27) was highest, followed by butterfly (12) and the lowest number as insect pollinator species was observed for wasp (5). Considerable number of Syrphid fly (7) was also observed (Table 8).

Table 8: Abundance of pollinators influenced by mustard with Coriander as pollinator attractant crop.

Common name	**Scientific name**	**Family**	**Presence of pollinator before insecticide application (No. of insects/m^2**	**Presence of pollinator after insecticide application (No. of insects/m^2**
Honey bee	*Apis mellifera* L	Apidae	27	9
Wasp	*Vespula vulgaris*	Scollidae	5	2
Syrphid fly	*Syrphus corollae*	Syrphidae	7	2
Butterfly	*P. brassicae*	Pieridae	12	3

Abundance of pollinators in mustard field bordered with Niger (T_4)

Total 4 species was identified as insect pollinators influenced by mustard with Niger as pollinator attractant crop. Honey bee (28) was highest, followed by butterfly (18) and the lowest number as insect pollinator species was observed for wasp (9). Noticeable number of Syrphid fly (15) was also observed (Table 9).

Table 9: Abundance of pollinators influenced by mustard with Niger as pollinator attractant crop.

Common name	Scientific name	Family	Presence of pollinator before insecticide application (No. of insects/m^2	Presence of pollinator after insecticide application (No. of insects/m^2
Honey bee	*Apis mellifera* L	Apidae	28	12
Wasp	*Vespula vulgaris*	Scollidae	9	3
Syrphid fly	*Syrphus corollae*	Syrphidae	15	6
Butterfly	*P. brassicae*	Pieridae	18	7

Abundance of pollinators in mustard field (T_5)

Total 4 species was identified as insect pollinator in mustard field. Among those number of honey bee (20) was highest, followed by butterfly (9) and the lowest number as insect pollinator species was observed for *Syrphus corollae* (4).

Table 10: Abundance of pollinators influenced with mustard.

Common name	Scientific name	Family	Presence of pollinator before insecticide application (No. of insects/m^2	Presence of pollinator after insecticide application (No. of insects/m^2
Honey bee	*Apis mellifera* L	Apidae	20	7
Wasp	*Vespula vulgaris*	Scollidae	5	1
Syrphid fly	*Syrphus corollae*	Syrphidae	4	1
Butterfly	*P. brassicae*	Pieridae	9	3

4.1.3 Time of foraging of insect pollinators

It was observed that the highest number of insect pollinators found at 11.30 am - 02.30 pm and lowest number of insect pollinators found at 5.00 pm. But it was clearly showed that maximum honey bee as well as others insect pollinators was observed in mustard field from 8.30 am to 05.00 pm for all border crops as pollinator attractant. (Table 11). As the optimum time of insect pollinator foraging was observed at 11.30 am and the least number of insect pollinator observed at 5.00 pm, insecticide application should be at 5.00 pm to avoid minimum pollinator drifting. Application of any insecticides at 5.00 pm affect lowest to the insect pollinators population.

Table 11. Time of foraging of insect pollinators influenced by bordered crops and insecticide application time.

Crop	Pollinator presence (No. of insects/m^2)	8:30 am (S_1)	11:30 am (S_2)	2:30 pm (S_3)	5:00 pm (S_4)
Mustard bordered with Dill (T_1)	Before insecticide application	18	46	39	17
	After insecticide application	8	23	20	8
Mustard bordered with Linseed (T_2)	Before insecticide application	19	45	38	12
	After insecticide application	9	25	21	7
Mustard bordered with Coriander (T_3)	Before insecticide application	23	51	39	13
	After insecticide application	11	27	18	7
Mustard bordered with Niger (T_4)	Before insecticide application	28	70	56	19
	After insecticide application	9	29	21	11
Mustard (T_5)	Before insecticide application	18	44	35	11
	After insecticide application	7	23	19	3

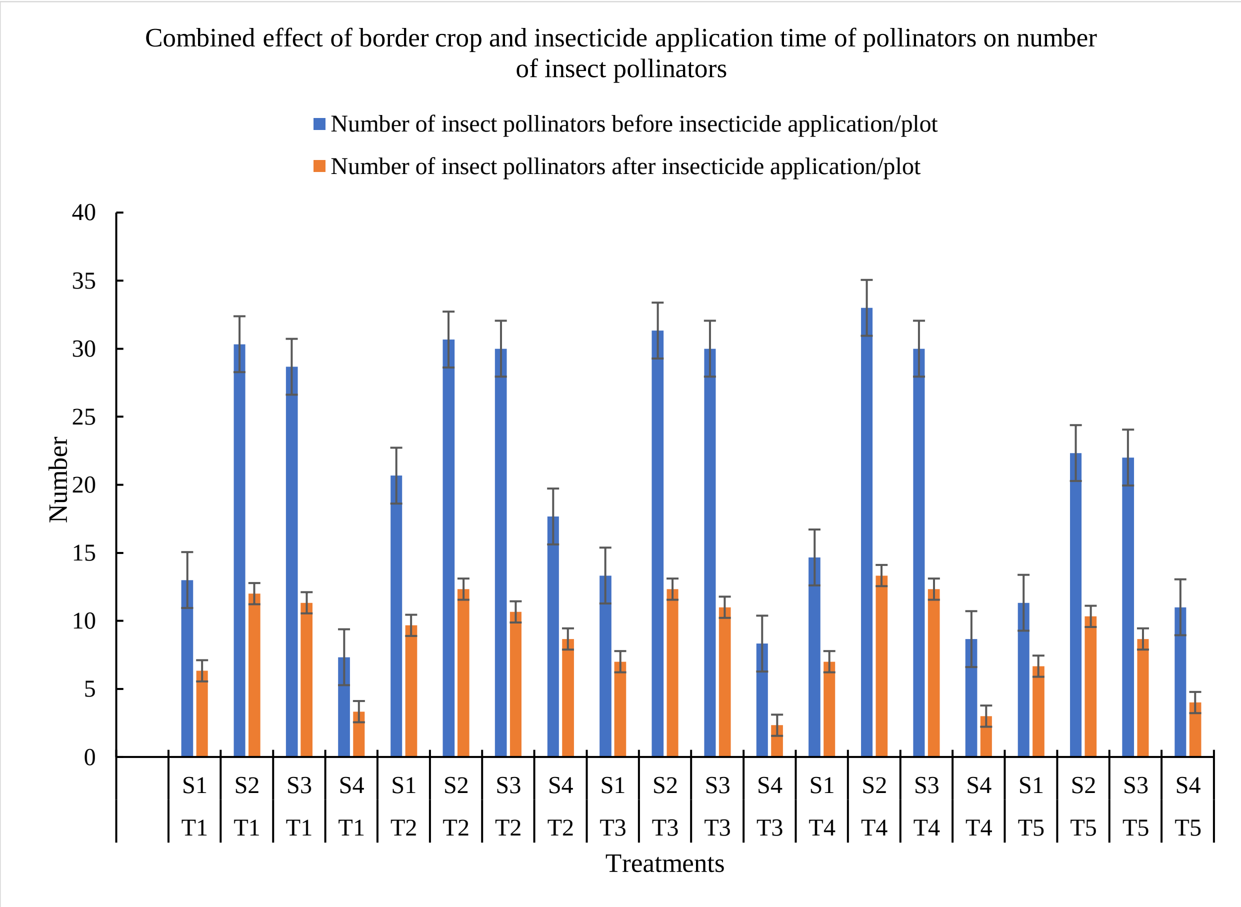
4.1.4 Number of insect pollinators before and after insecticide application/plot

For the effect on insect pollinator before insecticide application S_2- Spray insecticide at 11.30 am resulted the highest (29.53) number of pollinators and S_4- Spray insecticide at 5.00 pm showed lowest insect pollinator (10.60) (Table 12). For the effect of time on insect pollinator as after insecticide application S_2- Spray insecticide at 11.30 am resulted the highest (12.07) number of pollinators and S_4- Spray insecticide at 5.00 pm showed lowest insect pollinator (4.26) (Table 12). Results indicates that, insecticide application at 5.00 pm affect lowest to the insect pollinators population.

Table 12. Number of insect pollinators before and after pesticide application/plot influenced by the time of pesticide application

Treatments	Number of insect pollinators before insecticide application/plot	Number of insect pollinators after insecticide application/plot
S_1	14.60 c	7.33 c
S_2	29.53 a	12.07 a
S_3	28.13 b	10.80 b
S_4	10.60 d	4.26 d
CV%	13.86	17.84
LSD (0.5)	0.86	0.58

[In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly by LSD at 0.05 level of probability; S_1- Spray insecticide at 8.30 am, S_2- Spray insecticide at 11.30 am, S_3- Spray insecticide at 2.30 pm and S_4- Spray insecticide at 5.00 pm]



[Vertical bars showing error bars; T_1 - Mustard bordered by Dill (Shova), T_2 - Mustard bordered by Linseed (Neela), T_3 - Mustard bordered by Coriander (BARI Dhonia 1) and T_4 - Mustard bordered by Niger; S_1- Spray insecticide at 8.30 am, S_2- Spray insecticide at 11.30 am, S_3- Spray insecticide at 2.30 pm and S_4- Spray insecticide at 5.00 pm]

Figure 1. Combined effect of border crop and insecticide application time of pollinators on number of insect pollinators before and insecticide application/plot.

4.2 Effect of Mustard crop bordered by other crops

4.2.1 Total number of pod/plant

In terms of pod per plant of mustard plant with border crops were greatly influenced by the presence of pollinators. At the different treatments there were no significant variation was observed (Table 13). The highest number of pod/plant (25.50) was recorded in T_4 - Mustard bordered by Niger and lowest number of pod/plant (21.66) was recorded in T_5 - Mustard without border crop (Control).

4.2.2 Length of Pod

The highest length of pod (5.31 cm) was recorded in T_4 - Mustard bordered by Niger and lowest length of pod (4.60 cm) was recorded in T_5 - Mustard without border crop (Control).

Table 13. Effect of Mustard crop bordered by other crops.

Treatments	Total number of pod/plant	Length of pod (cm)	Dry weight of seed/plant (%)	Total seed weight/plant (g)
T_1	23.67 a	4.91 a	5.22 b	1020.80 a
T_2	24.30 a	5.14 a	4.69 c	1110.70 a
T_3	23.33 a	5.21 a	4.68 c	965.60 a
T_4	25.50 a	5.31 a	5.87 a	1123.00 a
T_5	21.66 a	4.60 a	4.46 c	922.70 a
CV%	13.86	17.84	10.30	14.19
LSD (0.5)	5.72	0.81	0.46	482.39

[T_1 - Mustard bordered by Dill (Shova), T_2 - Mustard bordered by Linseed (Neela), T_3 - Mustard bordered by Coriander (BARI Dhonia 1), T_4 - Mustard bordered by Niger and T_5 - Mustard without border crop (Control)]

4.2.3 Dry weight of seed/plant (%)

The highest dry weight of seed/plant (5.87%) was recorded in the treatment T_4 - Mustard bordered by Niger and lowest dry weight of seed/plant (4.46%) was recorded in T_5 - Mustard without border crop (Control).

4.2.4 Total seed weight/plant

Total seed weight/plant of mustard significantly influenced by different pollination conditions with the influence of bordered crops (Table 13). The highest seed weight/plant (1123 g) was recorded from treatment T_4 - Mustard bordered by

Niger. The lowest seed weight/plant (922.70 g) was recorded from treatment T_5 – Mustard without border crop (Control).

4.3 Effect of time of insecticide application in mustard yield

4.3.1 Total number of pod/plant

In terms of pod per plant of mustard plant with time of insecticide application were greatly influenced by the presence of pollinators. At the different treatments there were significant variation was observed (Table 14). The highest number of pod/plant (25.97) was recorded in S_4- Spray insecticide at 5.00 pm considering the least presence of pollinator at that time. Lowest number of pod/plant (22.75) was recorded in S_1- Spray insecticide at 8.30 am.

4.3.2 Length of Pod

The highest length of pod (5.26 cm) was recorded in the treatment S_4- Spray insecticide at 5.00 am and lowest length of pod (4.84 cm) was recorded in S_1- Spray insecticide at 8.30 am. (Table 14)

Table 14. Effect of time of insecticide application in mustard yield.

Treatments	Total number of pod/plant	Length of pod (cm)	Dry weight of seed/plant (%)	Total seed weight/plant (g)
S_1	22.75 b	4.84 a	5.73 b	1024.30 b
S_2	24.25 ab	5.16 a	4.60 c	977.70 b
S_3	23.00 b	5.11 a	3.85 d	990.70 b
S_4	25.97 a	5.25 a	5.83 a	1227.50 a
CV%	12.29	5.08	1.01	16.75
LSD (0.5)	2.48	0.21	0.04	148.93

[S_1- Spray insecticide at 8.30 am, S_2- Spray insecticide at 11.30 am, S_3- Spray insecticide at 2.30 pm and S_4- Spray insecticide at 5.00 pm]

4.3.3 Dry weight of seed/plant (%)

The highest dry weight of seed/plant (5.83 %) influenced by time of insecticide application was recorded in the treatment S_4- Spray insecticide at 5.00 pm and lowest dry weight of seed/plant (3.85 %) was recorded in S_2- Spray insecticide at 11.30 am. (Table 14)

4.3.4 Total seed weight/plant

Total seed weight/plant of mustard significantly influenced by different pollination conditions with the influence time of insecticide application (Table 14). The highest seed weight/plant (1227.5 g) was recorded from treatment S_4- Spray insecticide at 5.00 am. The lowest seed weight/plant (977.7 g) was recorded from treatment S_2- Spray insecticide at 11.30 pm.

CHAPTER 5

SUMMARY AND CONCLUSION

The experiment was conducted in the experimental field of Sher-e-Bangla Agricultural University, Dhaka-1207, during November 2019 to February 2020 to find out the optimum time of insecticide application in mustard field to reduce mortality rate of the natural pollinating agents of mustard. The experimental design was a split-plot design with three replicates and two factors Factor A: Main factor; Mustard crop bordered by other crops T_1 - Mustard bordered by Dill (Shova), T_2 - Mustard bordered by Linseed (Neela), T_3 - Mustard bordered by Coriander (BARI Dhonia-1), T_4 - Mustard bordered by Niger and T_5 - Mustard without border crop (Control); Factor B: Second factor; Insecticide: Actara 25WG@ (0.3gm/L; at the 15 days intervals) application timing where S_1- Spray insecticide at 8.30 am, S_2- Spray insecticide at 11.30 am, S_3- Spray insecticide at 2.30 pm and S_4- Spray insecticide at 5.00 pm.

A total number of six families and seven species was identified. Diversity of insect pollinators was observed and among others presence of honey bee was observed as most frequently visited pollinators (33,24). butterfly was the second most visible insect pollinator (21) and the lowest presence (9) was observed for the pollinator of *Pieris rapae* from the family Pieridae. The Shannon-Weaver diversity index in mustard field bordered with Niger for species was 1.87, with the dominance was 0.164. There were 16.4 % community dominance for 7 observed species in mustard field bordered with Niger. Diversity of insect pollinators was observed and among others presence of honey bee was observed as most frequently visited pollinators (25,18). butterfly was the second most visible insect pollinator (13) and the lowest presence (6) was observed for the pollinator of *Pieris rapae* from the family Pieridae. The Shannon-Weaver diversity index in mustard field for species was 1.85, with the dominance was 0.169. There were 16.9 % community dominance for 7 observed species in mustard field

It was observed that the highest number of insect pollinators found at 11.30 am - 02.30 pm and lowest number of insect pollinators found at 5.00 pm. But it was clearly showed that maximum honey bee as well as others insect pollinators was observed in mustard field from 8.30 am to 05.00 pm for all border crops as pollinator attractant. As the optimum time of insect pollinator foraging was observed at 11.30 am and the least number of insect pollinator observed at 5.00 pm, insecticide application should be at 5.00 pm to avoid minimum pollinator drifting. Application of any insecticides at 5.00 pm affect lowest to the insect pollinators population.

In terms of pod per plant of mustard plant with border crops were greatly influenced by the presence of pollinators. At the different treatments there were no significant variation was observed. The highest number of pod/plant (25.50) was recorded in T_4 - Mustard bordered by Niger and lowest number of pod/plant (21.66) was recorded in T_5 - Mustard without border crop (Control). The highest length of pod (5.31 cm) was recorded in T_4 - Mustard bordered by Niger and lowest length of pod (4.60 cm) was recorded in T_5 - Mustard without border crop (Control). The highest dry weight of seed/plant (5.87%) was recorded in the treatment T_4 - Mustard bordered by Niger and lowest dry weight of seed/plant (4.46%) was recorded in T_5 - Mustard without border crop (Control). Total seed weight/plant of mustard significantly influenced by different pollination conditions with the influence of bordered crops. The highest seed weight/plant (1123 g) was recorded from treatment T_4 - Mustard bordered by Niger. The lowest seed weight/plant (922.70 g) was recorded from treatment T_5 – Mustard without border crop (Control).

In terms of pod per plant of mustard plant with time of insecticide application were greatly influenced by the presence of pollinators. At the different treatments there were significant variation was observed. The highest number of pod/plant (25.97) was recorded in S_4- Spray insecticide at 5.00 pm considering the least presence of pollinator at that time. Lowest number of pod/plant (22.75) was recorded in S_1- Spray insecticide at 8.30 am.

The highest length of pod (5.26 cm) was recorded in the treatment S_4- Spray insecticide at 5.00 pm and lowest length of pod (4.84 cm) was recorded in S_1- Spray insecticide at 8.30 am. The highest dry weight of seed/plant (5.83 %) influenced by time of insecticide application was recorded in the treatment S_4- Spray insecticide at 5.00 pm and lowest dry weight of seed/plant (3.85 %) was recorded in S_4- Spray insecticide at 5.00 pm. Total seed weight/plant of mustard significantly influenced by different pollination conditions with the influence time of insecticide application. The highest seed weight/plant (1227.5 g) was recorded from treatment S_4- Spray insecticide at 5.00 pm. The lowest seed weight/plant (977.7 g) was recorded from treatment S_3- Spray insecticide at 2.30 pm.

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Appendices

Appendix I. Morphological characteristics of the experimental field.

Morphological features	**Characteristics**
Location	Sher-e-Bangla Agricultural University Agronomy research field, Dhaka
AEZ	AEZ-28, Modhupur Tract
General Soil Type	Shallow Red Brown Terrace Soil
Land type	High land
Soil series	Tejgaon
Topography	Fairly leveled

Appendix II. The initial physical and chemical characteristics of soil of the experimental site (0 - 15 cm depth).

Physical characteristics	
Constituents	Percent
Sand	26
Silt	45
Clay	29
Textural class	Silty clay
Chemical characteristics	
Soil characters	Value
pH	5.6
Organic carbon (%)	0.45
Organic matter (%)	0.78
Total nitrogen (%)	0.03
Available P (ppm)	20.54
Exchangeable K (me/100 g soil)	0.10

Appendix III. Monthly meteorological information during the period from November, 2019 to February, 2020

Year	Month	Air temperature (^{0}C)		Relative humidity (%)	Total rainfall (mm)
		Maximum	Minimum		
2019	November	28.10	11.83	58.18	47
	December	25.00	9.46	69.53	00
2020	January	25.2	12.8	69	00
	February	27.3	16.9	66	39

Source : Meteorological Centre, Agargaon, Dhaka (Climate Division)

Appendix IV. Analysis of variance of the data on number of pod/plant, length of pod and weight of pod.

Source of variation	Degrees of freedom	Mean square number of		
		Number of pod/plant	Length of pod	Weight of pod
Replication	2	66.08	0.25	48.69
Factor A	4	4.02	0.22	52.15
Factor B	3	25.18	0.38	223.20
Factor A×B	12	5.70	0.50	15.77
Error	30	8.68	0.07	38.92

Appendix V. Analysis of variance of the data on Seed weight/plant, Number of pollinator before insecticide application and Number of pollinator after insecticide application.

Source of variation	Degrees of freedom	Mean square number of		
		Seed weight/plant	Number of pollinator before insecticide application	Number of pollinator after insecticide application
Replication	2	102940	4.19	1.27
Factor A	4	67542	6.19	1.74
Factor B	3	163284	1604.80	239.13
Factor A×B	12	26931	0.67	0.63
Error	30	31243	0.33	0.56