

**EFFECT OF PESTICIDE APPLICATION TIMING ON HONEY  
BEE FORAGING IN MUSTARD FIELD AND ROLE OF HONEY  
BEE ON MUSTARD YIELD**

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**EFFECT OF PESTICIDE APPLICATION TIMING ON HONEY BEE  
FORAGING IN MUSTARD FIELD AND ROLE OF HONEY BEE ON  
MUSTARD YIELD**

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**CERTIFICATE**

This is to certify that the thesis entitled **EFFECT OF PESTICIDE APPLICATION TIMING ON**

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**MUSTARD YIELD** ,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 bona fide research work carried out by JOYDEB KUMAR PAUL, Registration No.: 11-04356, 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 has been availed of during the course of this investigation has duly been acknowledged.

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The Author

# **EFFECT OF PESTICIDE APPLICATION TIMING ON HONEY BEE FORAGING IN MUSTARD FIELD AND ROLE OF HONEY BEE ON MUSTARD YIELD**

## **Abstract**

The study was conducted in the field at Nagarpur, Tangail, Bangladesh, during November 2016 to February 2017 to evaluate the effect of pesticide application timing on honey bee foraging in mustard field and role of honey bees on mustard yield. Honey bee (*Apis mellifera*) was found as the main insect pollinator during mustard flowering season. The results on the effect of pesticide application timing P<sub>1</sub> = 8.30-9.30 am, P<sub>2</sub> = 9.30-10.30 am, P<sub>3</sub> = 10.30-11.30 am, P<sub>4</sub> = 11.30-12.30 pm, P<sub>5</sub> = 12.30-1.30 pm, P<sub>6</sub> = 1.30-2.30 pm, P<sub>7</sub> = 2.30-3.30 pm and P<sub>8</sub> = 3.30-4.30 pm on honey bee foraging and netting without bees (T<sub>1</sub>) and netting with bees (T<sub>2</sub>) compared with control (T<sub>3</sub>) in mustard field to observe yield differences. At the hour of 10.30-2.30 highest number of honey bees was recorded in mustard field. As a result, optimum time for pesticide application was recorded at the hour of 8.30-10.30 and also the application of pesticide during this time decreased the percent of mortality of honey bees. It was observed that honey bees helped mustard pollination, decreased the flowering period and increased the number of pod per plant. Pod length of mustard was higher as well. The most impressive result was recorded in mustard yield. Mustard yield was considerably higher in honey bee foraging plots.

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## LIST OF ABBREVIATIONS AND ACRONYMS

<b>Abbreviation</b>	<b>Full meaning</b>
BADC	Bangladesh Agriculture Development Corporation
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
BCPC	British Crop Production Council
CV	Coefficient of Variation
°C	Degree Celsius
d.f.	Degrees of Freedom <i>et al.</i>
EC	Emulsifiable Concentrate
FAO	Food and Agriculture Organization
gm	Gram
ha	Hectare
IPM	Integrated Pest Management
CRSP	Collaborative Research Support
J.	Journal
Kg	Kilogram
LSD	Least Significant Difference
Mg	Milli gram
MI	Milli liter
MP	Muriate of Potash
%	Percent
RCBD	Randomized Complete Block Design
SAU	Sher-e-Bangla Agricultural University
TSP	Triple Super Phosphate
WP	Wettable Powder

# CHAPTER-I

## INTRODUCTION

Mustard (*Brassica* spp.), belongs to the family Cruciferae, is an important oil seed crop, cultivated for edible oil throughout Bangladesh. It is one of the leading oil seed crops in the world as well as in Bangladesh. It plays a vital role in human nutrition. It is used as a condiment, salad, green manure and fodder crop, and leaf and stem as a vegetable in the various mustard growing countries of the World (FAO, 2004). In Bangladesh, more than 361.909 thousand metric tons of rape and mustard produced from a total of 787.025 thousand acres of land during 2015-2016 (BBS, 2017). Domestic production of edible oil almost entirely comes from rapeseed and mustard occupying only about 2.73% area of total cropped area in Bangladesh (BBS, 2017). The annual oil seed production of 0.933 million tons of which the share of rapeseed-mustard was 0.362 million tons, which comes about 69.94% of the total edible oil seed production (BBS, 2017). Mustard occupied the top of the list in respect of area and production compare to other oilseed crops grown in Bangladesh (Abraham, 1994). 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 (Singh *et al.*, 2005 and Mohapatra *et al.*, 2010). Honeybees are very important a social insect known as the most economically valuable insect because of its honey production and pollinating activities (Lawal and Banjo, 2010). The principal role of honeybee in Agriculture is pollination. These insects are of great economic importance because they not only produce honey and bee wax but also act as primary pollinating agents of many agricultural and horticultural crops. They are among the most important pollinating insects found within orchards and modern agricultural systems (Williams, 1994; Morse and Calderone, 2000). There are many species of honeybee, but four species are common these are *Apis florea*, *Apis dorsata*, *Apis cerana* and *Apis mellifera*.are commons. Due to domestic nature, *Apis mellifera* is the most popular worldwide and can be easily reared, and safely migrated from one place to other for pollination and honey production (FAO, 1986). Like other honeybee species *A. mellifera* has a high flight range for foraging (maximum 2-3 km away from its colony) (Abrol, 1997). Foragers take care of bringing from the environment everything that the colony needs to the hive: pollen, nectar, water and propolis

(Ameco, 2012). Of the 100 crops that provide 90% of the world's food, 71 are bee pollinated, and honey bees (*A. mellifera*) are the managed pollinator conscripted to provide the necessary pollination services for most of these crops (United Nations Food and Agriculture Organization, 2005). Honey bees, like other insects, are reasonably sensitive to a range of chemical insecticides (Devillers *et al.*, 2002; Stefanidou *et al.*, 2003; Hardstone and Scott, 2010), and bees close to agricultural areas are particularly vulnerable to pesticide exposure through multiple routes (Krupke *et al.*, 2012). Over the past decade, there has been a sharp increase in the number of honey bee colony losses in the United States, often exceeding 30% per year (Lee *et al.*, 2015).

Beekeepers renting their colonies for pollination, or making honey on or in close proximity to agricultural crops, are concerned about pesticide exposure and its potential negative impacts on their colonies. This includes sublethal impacts that may affect forager performance and are more difficult to diagnose. Pesticide use, apart from loss of natural vegetation cover (Winfrey *et al.*, 2009), has been cited as one of the major drivers of the recent decline in pollinator populations (Brittain *et al.*, 2010; Mullin *et al.*, 2010; Henry *et al.*, 2012; Whitehorn *et al.*, 2012). A comparison of native bee species responses across a pesticide use gradient using combinations of biomarkers (Badiou-Bénéteau *et al.*, 2012) would be a crucial and valuable contribution towards developing a more accurate pesticide regulatory framework.

A number of physiological biomarkers of xenobiotics have been investigated in various animal species (BadiouBénéteau *et al.*, 2012). Antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) are of vital importance in an organism's defense against oxidative stress (McCord and Fridovich, 1969; Fridovich, 1982; Khessiba *et al.*, 2005; Schriever *et al.*, 2008; Mamidala *et al.*, 2011), and both have been associated with pesticide toxicity in insects (Landa *et al.*, 1991), frogs (Czarniewska *et al.*, 2003) and also in freshwater clams (Connors, 2004). A number of antioxidant enzymes, such as SOD, CAT, glutathione S-transferase, glutathione peroxidase and glutathione reductase, have been reported to occur in insects (Ahmad *et al.*, 1991; Felton and Summers, 1995; Joannis and Storey, 1996).

Increased levels of anti-oxidant enzymes would therefore be indicative of the organisms' attempt at coping in an oxidative stress environment. Free radicals act on important macromolecular structures of the organism and can cause severe damage to the physiology of the organism by interfering with cell components, including proteins, lipids, and DNA (Akhgari *et al.*, 2003; Ranjbar *et al.*, 2005). Imbalance of free radicals within the body can affect processes like lipid peroxidation that are vital to the survival of an animal (Akhgari *et al.*, 2003). Hence, elevated levels of CAT and SOD could be an adaptive response to this imbalanced scenario as a protective mechanism (Akhgari *et al.*, 2003). Conceiving all thoughts and ideas, the present study has been undertaken with the following objectives:-

**Objectives:**

1. to study the effect of honey bee foraging on mustard yield,
2. to find out the yield of mustard in absence of honey bees or other pollinating agents
3. For finding out the optimum time of pesticide application in mustard yield to reduce mortality rate of the natural pollinating agents.

## CHAPTER-II

### REVIEW OF LITERATURE

A number of studies regarding effect of pesticide application timing on honey bee foraging in mustard field and role of honey bee on mustard yield have been done and reported in Bangladesh and elsewhere in the world. However, studies in this area appeared very limited in Bangladesh. For a better understanding, clear conception and to know the results of previous research works on impact of pesticide application at the time of honey bee foraging in mustard field and the yield of mustard, the relevant available literature have been reviewed and presented below:-

#### Honey bee

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).



**Plate 1:** *Apis mellifera*, one of seven recognized species of honey bee

He also observed that, in the Netherlands, the European, Western, or Common honey bee (*A. mellifera*) is native species. The subspecies *A. mellifera mellifera*, which exists in The Netherlands, is also known as the European dark bee. About 8,000 years ago, after the last ice age, this species spread over the whole of Europe from the Mediterranean (Kleinjans, *et al.*, 2012). It can be assumed that, in The Netherlands and large parts of Europe, the native dark bee does currently not exist as a pure subspecies in the wild anymore (Blacquièrè *et al.*, 2009). In most ecosystems bees (Hymenoptera: Apiformes) are the primary pollinators of flowering plants (Kearns *et al.*, 1998; Aizen and Feinsinger, 2003; Ashman *et al.*, 2004). Of particular social

interest is the reliance of fruit, seed, and nut crops on apiformes, particularly managed honey bee (*A. mellifera* L.) populations (Klein *et al.*, 2007).

## **Nomenclature**

Phylum: Arthropod

Class: Insecta

Order: Hymenoptera

Family: Apidae

Genus: *Apis*

Species: *Apis mellifera*

## **Castes of honey bee**

Two sexes (male (i.e. drones) and female) and two castes of female bees (queens and workers) make up the population of a beehive, each having its own characteristics, roles, and responsibilities within the hive. Upon closer examination, the three types of honey bees have a different appearance (Kleinjans, *et al.*, 2012).

## **Queen**

Within a hive, there is only one queen. It is a female bee with a fully developed reproductive system. The queen mates only once with several drones, and then remains fertile for life. A queen can live for 3 to 5 years and can lay up to 2,000 eggs per day. Fertilized eggs become female (workers) and unfertilized eggs become male (drones). When the queen dies or becomes unproductive, the other bees will initiate the development of a new queen. For queen bees, it takes 16 days from egg to emergence (BYBA, 2011).



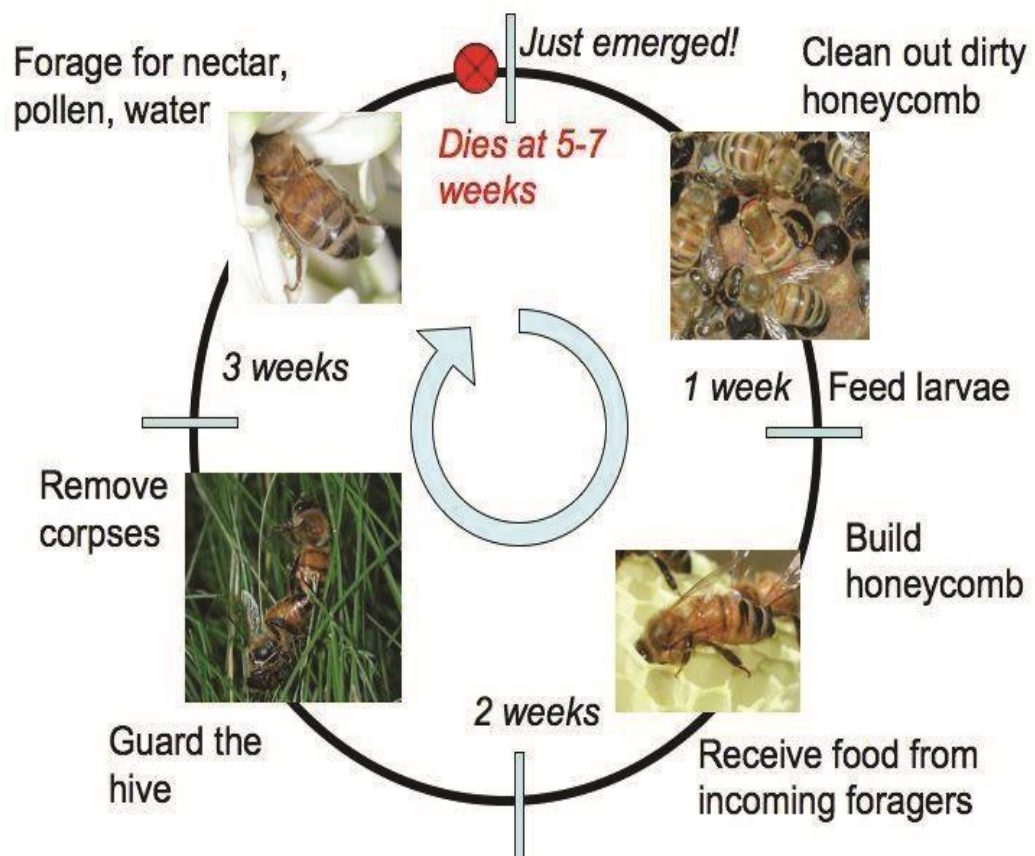
**Plate 2:** Three types of honey bees: worker (l), drone (m) and mature queen (r)

## **Worker**

A worker is a female bee of which the reproductive organs are undeveloped, due to a specific diet during its development stage and through the activity of queen

pheromone in the colony. The vast majority of honey bees are worker bees. Worker bees may live for 4- 9 months during the winter season, but only 6 weeks during the demanding summer months. For worker bees, it takes 21 days from egg to emergence (BYBA, 2011).

The worker bees sequentially take on a series of specific tasks during their lifetime, as depicted in plate 3. The activities of young bees start in the centre of the brood nest with the cleaning of cells and tending the brood. Subsequently, the workers go to the outer edges of the nest in order to pack pollen and store nectar. Until after about three weeks, workers become foragers for another 10- 20 days. Foragers take care of bringing from the environment everything that the colony needs in the hive: pollen, nectar, water and propolis. Some activities can be executed lifelong (e.g. patrolling, resting, and ventilating the nest).



**Plate 3:** The changing tasks during the life of a worker honey bee

### **Drone**

Drones are fertile male bees that are kept on standby during the summer for mating with a virgin queen. Because a drone has a barbed sex organ, which cannot be pulled

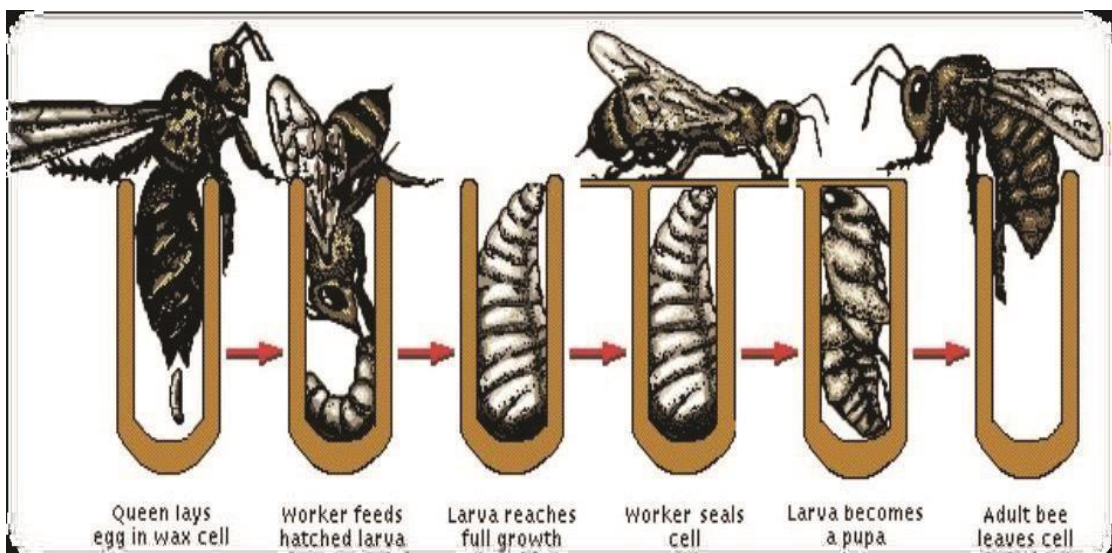
out of the female genital opening, mating is followed by death of the drone. For drones, it takes 24 days from egg to emergence (BYBA, 2011). Because drones are of no use in the winter, they are expelled from the hive in the autumn.

## Honey bee life stages

As with most advanced insects, honey bees exhibit a complete development or metamorphosis during their life: the young and the adults look very different. The life stages of a honey bee are egg, larva, pupa and adult (Plate 4). It is noted that the cells are depicted vertically, but in reality, they are oriented horizontally. The first three stages are also referred to as brood. Development from egg to adult in general takes two to three weeks (Stone, 2005).

### Egg

The eggs are described as having an appearance similar to sausage-shaped poppy seeds. Each egg has a small opening at the broad end of the egg, the micropyle, which allows for passage of sperm. Hatching takes place three days after egg laying. The queen can lay about 2000 eggs per day, and the colony can increase from a few thousand to tens of thousands of bees in several weeks (Tautz, 2008).



**Plate 4:** Honey bee life stages: from egg to larva, then to pupa and finally to an adult bee



## Larva

From hatching of the egg, the larval stage lasts for six days. Upon hatching, the larva is almost microscopic, resembling a small, white, curved, segmented worm lacking legs and eyes. It lies coiled on the bottom of the cell. Larvae are fed royal jelly and later bee bread, i.e. nutritional granules of pollen with added honey or nectar prepared by the workers (Plate 5). Each larva receives an estimated 10,000 feedings during this stage. Larval weight increases 5.5 times during the first daand approx.

1,500 times in 6 days.



**Plate 5:** Workers caring for larvae

**Plate 6:** Worker (s) and queen (l) pupae

The process of feeding and growing takes place while the cells are uncapped; the larvae spin their cocoons and change into pupae after workers have capped their cells (Winston, 1987). Larval stage durations vary: 5.5 days for queens, 6 days for workers, and 6.5 days for drones. Regardless of whether the larva is male or female, it moults five times during its larval stage (Stone, 2005).

## Pupa

The pupal stage is a stage of massive reorganization of tissues: the adult tissues develop from the imaginal discs carried by the larva. Organs also undergo a complete transformation; while the body changes from the wormlike larval body shape to the adult body shape with three distinct body regions (plate 6). The pupal stage lasts about 8- 9 days for workers and drones, and 4- 5 days for queens. It is followed by the final moult to the adult stage (Winston, 1987).

## **Adult**

As stated above, adult honey bees are either queens, workers or drones. The majority of honey bees that one sees outside of a hive are workers. A typical colony in mid-summer consists of up to 20,000-30,000 workers, 500 to 1,000 drones, and one queen.

### **Foraging of honey bees**

Honeybee, *A. mellifera* L. was reared in around Bangladesh Agricultural University campus in Mymensingh to study its life cycle, behaviour, 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).

Pollen, the primary dietary source of proteins, lipids, vitamins, and minerals, is essential to the physiological development of adult honey bees (*A. mellifera*). A varied pollen diet is vital to immune system maintenance, organ development, and colony succession via brood production. The reasons for the recent decline in honey bee populations are wide-ranging but include a lack of diverse nectar and pollen resources. Resource deficiency and colony fitness is well understood within natural and agricultural landscapes; few studies have determined the importance of a polyfloral diet for bees existing in areas of intense development. Focusing on honey bees in the city of Philadelphia, we investigated the range of plants utilized as pollen sources and if there are significant colony-level benefits to foraging diversity. We examined the pollen content of honey samples collected from 15 Philadelphia hives from August to November 2011. Late season fitness of colonies was assessed by measuring hive-area covered by brood found in sampled hives. The findings presented here shed light on taxa visited by honey bees in an urban ecosystem. Identification

and selection of plants shown to be principal pollen sources can be used to promote effective pollinator restoration programs in developing cities (Nicholson, C., 2012).

It is known that honey bees and other social insects strongly benefit from the communication between individuals to locate favourable food sources. By the so-called 'waggle dance', which is performed inside the beehive, direction and distance of nectar and pollen sources is effectively communicated. The recruitment of part of the honey bees to explore new sources further away from the nests allows them to collect food at considerable distances. The waggle dance behaviour is in particular effective to optimize the colony's ability to exploit the most favourable foraging patches in the environment (Beekman and Lew, 2007).

Beekman and Ratnieks (2000) studied long range foraging by honey bees by decoding waggle dance information from honey bees foraging at large blooming heather fields – these can be very attractive for bees – in England and concluded that the median distance foraged was 6.1 km, and the mean 5.5 km. Only 10% of the bees foraged within 0.5 km of the hive whereas 50% went more than 6 km, 25% more than 7.5 km and 10% more than 9.5 km from the hive. This study shows that bees are able to cover large distances in the particular case. Earlier studies showed smaller distances (average about 1 km). They assume that such distances are only found in situations where food quality per patch varies much and patches are large. Only in such cases large distance travelling can be rewarding.

Also by decoding waggle dances, Visscher and Seeley (1982) showed that honey bees regularly fly several kilometres from the hive. In their study, the most common distance was 600- 800 m. The mean was 2.3 km and the range enclosing 95% of the colony's foraging activity. Also much shorter distances were found in a study by Waddington *et al.* (1994), where the foraging range was 745- 1,413 m. In patchy landscapes, where food richness varies, temporally and spatially recruitment of foragers that explore larger distances can be profitable (depending on scarcity and patchiness of pollen nectar density).

Ramsay *et al.* (1999) placed beehives at a distance of 800 m from a GM oilseeds rape field. Over 50% of the bees had GM pollen in their pollen loads, showing that this distance is easily covered by the majority of the bees. From this and other studies,

they conclude that pollen is easily collected from this favoured crop at ranges up to 2 km.

Steffan- Dewenter and Kuhn (2003) observed and decoded over a thousand honey bee waggle dances from colonies in simple and complex landscapes in different seasons. Overall, the mean distance was about 1.5 km and ranged from 60 m to 10 km.

Williams (2001) reviewed the role of bees in pollen and gene flow from GM plants. Referring to maximum flight ranges up to 10 km for honey bees, there is ample evidence that by far most pollen are deposited on nearby plants during foraging or brought to the colony within the range of a few hundred meters. Typically mean distances are around 300 m. Because of the skewed distribution (Plate 7) of flight distances and pollen transport, occasional transport of pollen over large distances is possible. For example, the majority of bees may forage within a range of 500- 1000 meter but a small fraction (that is hard to quantify) may forage at a distance of 5 km or more.

Of course, this affects the fraction of pollen transported over such distances but for some settings that can be relevant. In particular, when attractive patches such as flowering oilseed rape is within reach of the colony and other good food sources nearby are scarce.

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.

Because of the significant role of honey bees and bumble bees in the pollination and potential unwanted cross- pollination between fields, or related Brassica species outside the field, much research have been done on pollination transport and gene

flow patterns (Smith- Kleefman, *et al.*, (2005); Cresswell, *et al.*, (2002); Damgaard and Kjellsson, (2005); Luyten and De Jong, (2011)).

The results from empirical data or modelling data all point to the conclusion that pollen transport by flight and probability of cross- pollination exponentially decreases with distance from the pollen source. Therefore, even though honey bees or bumble bees may cover large distances under particular circumstances, the major activity and resulting pollination occurs within a distance of a few hundred meters (Beckie and Hall, 2008). On the other hand, potential cross- pollination can occur over large distances up to 3 or 4 kilometres, even though the probability of such a pollination is in the order of 0.01 to 0.001 %. Depending on the spatial arrangement of fields and the size of the bee population and the number of flowers to be fertilized, this still may result in a significant absolute number of crosspollinated flowers even though this may be a very small fraction of the total flower population. There is much evidence that honey bees can cover and hence transport pollen over large distances up to 10 km or more. 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 favourite 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). As Beekman and Ratnieks (2000) have shown for heather fields, which can be very attractive for bees, more than 50% of the bees of a colony could focus on such distant fields. However, no experimental evidence, sufficient data or field validated models are available that can give clues about the final quantitative impact for different crops. Handling low probabilities in a variable landscape context is extremely difficult and more experimental and modelling research is needed to get a better understand on what is really going on (Beckie and Hall, 2008). Direct measurement of labelled pollen transport and subsequent

pollination has not been evaluated in an experimental setting covering more than a few kilometres.

Pollen is generally harvested up to a range of 6 kilometres. The annual need for pollen of an average ('ten- frame') colony has been measured at 13- 18 kg (Brodschneider and Crailsheim, 2010), while a colony may collect a total of 10- 26 kg per year.

Hagler *et al.*, (2011) found that the foraging range of honey bees ranged from 45 m to 5983 m. Under desert conditions, water foragers can fly up to 2 km from their colonies to collect water (Visscher *et al.*, 1996).

The spatial arrangement of fields, bee colonies during the season, the variable flight activity of bees makes it very hard to determine a relation between distance and pollination probabilities that is valid for many different conditions (Steffan- Dewenter and Kuhn, 2003). Hence, setting distance criteria for preventing undesirable out- crossing always includes a political decision in addition to ecological arguments (Lezaun, 2011), especially as long as no more hard and convincing data is available. Foraging behaviour is one of the distinctive behaviours of honey bees, *Apis mellifera*. This behaviour is the link between the honey bee colony and the ambient environment. Therefore, various in-colony and out-colony factors have an impact on this behaviour, and many studies have been employed to investigate these factors. Foraging behaviour is not advantageous only for the colony and for plant pollination but also has other benefits. In contrast, some disadvantages have also been discovered to be linked with foraging activity. Practically speaking, the control over this behaviour is very important to maximize colony products as well as to increase other agricultural benefits. This paper presents a review on foraging activity including; the regulation of foraging tasks, factors impacting this behaviour, foraging preference, variations between subspecies, monitoring methods as well as the possible methods for controlling this behaviour. As concluded from this review, more work needs to be performed in order to elucidate certain aspects of foraging behavior (Abou-Shaara, 2014). The forager bees can be classified into two categories; scout bees which search for the best food resource and the reticent bees which wait in the beehive until the scout bees return and give them information about the food source by dancing. The reticent bees, in general, range from 40–90% of the total forager population (Nest and Moore, 2012).

It is known that the foraging activity of honey bees is initiated in early morning and finishes in the evening. In some studies, honey bee workers started foraging activity at 6.17 am (Joshi and Joshi, 2010) but this commencement time can be greatly impacted by the region. Under desert conditions, Alqarni (2006) found that a higher number of foragers left the colonies at 8 am than at 10 am. In general, the foraging activity fluctuates during the day from the morning until the evening. ReyesCarrillo *et al.* (2007) found high pollen collection in the early morning while low amounts of pollen were collected in the afternoon. Pernal and Currie (2010) reported a higher foraging rate mean during the afternoon period (36.02 foragers/min) than during the morning period (17.66 foragers/min). 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. Foragers have the ability to remember the time of the day at which the higher food resources are available as found with *Sysirinchium palmifolium* plants (Silva *et al.*, 2013) and such ability may correlate with foraging activity peaks. In general, the normal foraging interval at the same feeding site is less than 5 min (Yang *et al.*, 2008) and bees spend different times per flower depending on the plant species. The time spent per flower was 6.92, 6.50 and 5.54 s for Chinese cabbage, broccoli and kohlrabi, respectively (Sushil *et al.*, 2013).

From 1850 until now, the number of bee colonies in The Netherlands has steadily decreased from 200,000 to approximately 80,000 in summer and 40,000 in winter. These colonies, kept by beekeepers, essentially represent the continuation of the original population of wild honey bees (Blacquièrre *et al.*, 2009).

## **Factors affecting foraging of honey bees**

There are numerous factors that may impact foraging activity (e.g. onset and end time, foraging interval and peaks) as explained in the next paragraphs.

### **Behavioral factors**

There are many factors that can impact foraging activity. These factors can be divided into two major groups: in-colony factors and out-colony factors. The first group (in-colony factors) include: queen presence and case (virgin or mated). Higher foraging activity with less pollen collection was found in colonies headed by virgin queens

than colonies headed by mated queens while lower foraging activity and pollen collection were found in queenless colonies than in colonies with a mated or virgin queen (Free *et al.*, 1985b). Also, foraging activity is impacted by colony strength and brood rearing activity (Amdam *et al.*, 2009; Abou-Shaara *et al.*, 2013), and the degree of pollen need (Weidenmuller and Tautz, 2002). Beehive type also has an impact on the foraging activity of honey bees (Abou-Shaara *et al.*, 2013). The infection of honey bee foragers with diseases and parasites such as *Nosema* sp. or *Varroa destructor* may result in the inability of foragers to return to their colonies or increased time to return (Kralj and Fuchs, 2006; Kralj and Fuchs, 2010). The genotype of honey bee strains (e.g. high and low pollen-hoarding bees) strongly affected foraging behaviour for nectar or for pollen (Pankiw *et al.*, 2002). The inheritance of high pollen-hoarding behaviour is a recessive trait unlike honey storing behaviour, which shows a more dominant pattern (Page *et al.*, 1995). Beside these factors, ovariole number can influence nectar collection by honey bee workers (Siegel *et al.*, 2012).

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.

Other factors may also have an impact on foraging behaviour. For example, foraging distance was found to be affected by the time of year (Steffan- Dewenter and Kuhn, 2003; Beekman *et al.*, 2004). Pearce *et al.* (2013) found no considerable effects of moving beehives from their location to another location as far as 26 km from their original site on honey bee foraging activity. Sushil *et al.* (2013), meanwhile, found that foragers spent less time in a flower under open conditions than in net house conditions. Brittain *et al.* (2013) observed alterations in honey bee foraging behaviour in California almond orchards due to the presence of other bee species communities. Picard-Nizou *et al.* (1995) found no effects of oilseed rape (*Brassica napus* L.) genetically modified by the introduction of a chitinase gene to enhance disease resistance on the foraging behaviour of honey bees (*Apis mellifera* L.). In general, the time of the year, the presence of other bee species and the study conditions should be



taken into consideration in study of foraging behaviour. Clearly, moreover, more studies on genetically modified plants are required.

### **Environmental factors**

With respect to environmental factors which influence foraging activity, *A. mellifera* bees were observed to commence their foraging activity at ambient temperatures with a mean of 6.57°C (Tan *et al.*, 2012) while in another study this value was found to be 16°C (Joshi and Joshi, 2010). At ambient temperatures of about 20°C, the highest activity was recorded (Tan *et al.*, 2012) while at 43°C the lowest foraging activity was found (Blazyte-Cereskiene *et al.*, 2010) as well as at or below 10°C (Joshi and Joshi, 2010). Further, a significant negative correlation ( $r = -0.09$ ) was found between foraging activity and temperature (Abou-Shaara *et al.*, 2013). Thus, it is expected that foraging activity is influenced passively by elevated temperature as found by Cooper and Schaffer (1985) with pollen foragers. In contrast, relative humidity had less of an effect on flight activity (Joshi and Joshi, 2010). Further investigations are required in order to elucidate these phenomena.

It was also found that other environmental factors can have an impact on foraging activity. Collins *et al.* (1997) found no impact of solar ultraviolet-B (UV-B) on the foraging activity of honey bees on two species of mustard, *Brassica nigra* and *B. rapa* grown under controlled conditions. However, Mattu *et al.* (2012) reported that altitude influenced foraging commencement and cessation time, duration of foraging activity and trips as well as the number of flowers visited per minute. Further, Sharma and Kumar (2010) found a negative effect of an electromotive field on foraging behaviour. Surprisingly, diesel exhaust can diminish the foraging efficiency of honey bee workers by reducing the ability of worker bees to recognize floral odours (Girling *et al.*, 2013).

### **Factors of natural enemies**

Foraging behaviour can also be influenced by natural enemies of honey bees. In the United Kingdom Kirk *et al.* (1995) found that the pollen beetle *Meligethes aeneus* (Nitidulidae) influenced the foraging behaviour of honey bees on oilseed rape flowers: forager bee preferred fully open flowers without beetles on them. Foraging activity can also be affected by the presence of predators (e.g. hornets) and a

reduction in the foraging visits by 55–79% and residence times by 17–33% was previously reported (Tan *et al.*, 2013). Also, the presence of bee-eaters impacted passively on foraging activity (Ali and Taha, 2012).

The accidental introduction of the Varroa destructor mite in the early 1980s gave the final blow to wild colonies of the honey bee in Europe. Varroa mites are external parasites and are the most important pest of honey bees around the world. The mites, which are about the size of a pinhead (approx. 1,5 x 1,1 mm), use specialised mouthparts to attack developing bee larvae or adult bees, resulting in deformed bees, reduced lifespan and ultimately the destruction of the colony (DAFF, 2011).

### **Factors of chemical insecticides**

Insecticides may also influence foraging behaviour. Yang *et al.* (2008) reported effects of sublethal doses of imidacloprid on the foraging behaviour 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 neonicotinoids are so widely used.

Pesticides have been cited as one of the major drivers of pollinator loss. However, little is known about pesticide impacts on natural populations of native honey bee species. This study looked into the effect of pesticides 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 pesticide use gradient. This study reports, for the first time, an increase in expression of xanthine oxidase in an invertebrate system (honey bees) exposed to pesticides (Chakrabarti, *et al.*, 2015).

In *A. dorsata*, it was observed that CAT activity had significantly increased from D<sub>2</sub> onwards, whereas SOD activity had only significantly increased at treatment D<sub>5</sub> compared to control (D<sub>0</sub>). This could be because ROS comprises a number of elements and not just superoxide anion (Bouayed and Bohn, 2010). However, all ROS elements are eventually converted to H<sub>2</sub>O<sub>2</sub>, where CAT is predominant for removing H<sub>2</sub>O<sub>2</sub> at the terminal end (Mueller *et al.*, 1997; Barbeta *et al.*, 2004).

The xanthine oxidase catalyses conversion of hypoxanthine to xanthine and also mediates its subsequent conversion to uric acid (Aranda *et al.*, 2007). Superoxide anion (O<sub>2</sub><sup>-</sup>) is an important byproduct of this reaction (Aranda *et al.*, 2007).

Their synergistic action is important in helping individuals to combat oxidative stress. CAT activity is a biomarker of exposure to an oxidative stress (Khessiba *et al.*, 2005). CAT has been identified as a key player in dealing with ROS (Mamidala *et al.*, 2011), and elevated levels of CAT have previously been shown in *A.mellifera* following laboratory exposure to xenobiotic compounds (Badiou- Bénéteau *et al.*, 2012).

From behavioural alteration (Whitehorn *et al.*, 2012) to neuro-physiological changes (Palmer *et al.*, 2013) in adults as well as in broods (Henry *et al.*, 2012), a number of recent studies have established deleterious responses of honey bees to pesticide toxicity. As has previously been reported, *A. mellifera* is deficient in its genome for expression of detoxifying enzymes (Claudianos *et al.*, 2006), and given this apparent deficient detoxification system, other detoxification systems in honey bees become important.

In 2004 Iwasa *et al.* working at North Carolina State University, determined the LD<sub>50</sub> concentrations for several insecticides applied topically to adult honey bees in the laboratory. Of seven neonicotinoids tested, they found that imidacloprid was most toxic at 17.9 mg/bee. Clothianidan and thiamethoxam were close behind at 21.8 and 29.9 mg/bee, respectively. These were followed by dinotefuran (75.0 mg/bee) and nitenpyram (138 mg/bee). Acetamiprid and thiacloprid, which have slightly different chemical structures, were much less toxic to bees.

The concentrations found in this study were comparable to those found in other crops. The fieldcollected pollen of sunflowers treated with Gaucho has been found to contain 3 µg/kg imidacloprid (Bonmatin *et al.*, 2003), and the pollen of seed-treated rape was found to contain 4.4-7.6 µg/kg (ScottDupree and Spivak, 2001).

Sublethal effects can be temporary or permanent. Furthermore, they may affect multiple stages of the life cycle, not just the adults. Haynes (1988) pointed out that, “The assumption that a colony of honey bees is healthy simply because no increase in mortality is noted immediately after exposure to an insecticide may not be valid”.

As a case in point (Bonmatin *et al.*, 2005) demonstrated that although acute levels of imidacloprid are seldom carried back to the hive in pollen, the chance for chronic and sublethal exposure to brood is significant even when contaminated pollen is mixed with clean pollen.

According to Rortais *et al.* (2005), “Such impacts might affect honeybees by disrupting their cognitive capacities (i.e. the learning and orientation abilities) and behaviors (i.e. the collection of food). In such condition, a forager might not be able to return to the hive and, as it relies on the colony for its survival, might die within a few hours. Therefore, the initial sublethal effect might eventually become lethal to honey bees.” Furthermore, pesticides at sublethal levels have been shown to suppress the honey bee immune system (Frazier *et al.*, 2008). And, according to Peters *et al.* (2010), minute amounts of pesticide in the parts per billion range can cause morphological changes, immune deficiencies, heart deformities, and reproductive abnormalities.

Beginning in 1995, beekeepers in France noticed increased mortality of bees working in fields of maize, rape, and sunflowers (Comité Scientifique et Technique, 2003), a phenomenon that spurred indepth research into the effect of imidacloprid on honey bees. Since then, a number of nations have placed restrictions on its use or have banned it altogether in certain crops (Suchail *et al.*, 2003). Several types of imidacloprid toxicity have been described. Acute toxicity (LD<sub>50</sub>) has been measured at concentrations from 3.7 to 40 µg/kg. Mortality of 50% can also be achieved by chronic exposure to imidacloprid at 0.1 to 10 µg/kg for 10 days. Sublethal toxicity has been observed beginning at 1 µg/kg in an adult bee. The ranges are due to variations in treatment and measurement protocols and natural variability in honey bee populations (Bonmatin *et al.*, 2005).

Davis *et al.* (1988) found that the systemic insecticides carbofuran and dimethoate affected larval development at concentrations that were sublethal to adults. In their experiments, pre-measured concentrations of the insecticides were mixed with royal jelly and fed to larvae at various life stages. They found that although adults appeared to be unaffected by carbofuran at 1.25 µg/g royal jelly, dosages as low as 0.625 µg/g caused mature larval weights to be significantly lower than the controls. At 1.25 µg/g the number of potentially viable pupae was also lower than the controls. Dai, *et al.*, (2010) examined the sublethal effects of two pyrethroids bifenthrin and deltamethrin on the growth and development of *Apis mellifera ligustica*, the most common subspecies of honey bee in the United States. The pyrethroids are synthetic forms of pyrethrin, the insecticide derived from certain chrysanthemums. They are potent neurotoxins which typically cause paralysis in the target organisms. Pyrethroids are problematic because they are widely available in both commercial and consumer formulations, and because they are often considered safe and natural alternatives to the organophosphates.

Several studies indicated that the neonicotinoid insecticides are found in pollen at levels that affect learning and cognition in bees (Chauzat *et al.*, 2006, Halm *et al.*, 2006, Desneux *et al.*, 2007). Since these sublethal levels are substantially below the regulatory adult LD<sub>50</sub> for these chemicals, spraying at these levels is not prohibited by the EPA.

### **Effect on yield of mustard for honey bees foraging**

Mustard (*Brassica* spp.), family cruciferae are the major oilseed crop grown throughout Bangladesh for edible oil. It is an open pollinated crop and honeybees are effective pollinators for open pollinated crops because of a lot of nectar and pollens are available on the flowers of mustard. The studies were conducted during *rabi* 2013-14 and 2014-15 at ARS in India, Kota to enhance the yield of mustard through honey bee pollinator. Mustard variety “Bio-902” was grown following all recommended agronomic practices without spraying through the crop season. The colonies of honeybee (*Apis mellifera*) were placed in cage measuring 10X10 sq. meters before the initiation of flowering. The present study contained three pollination treatments viz., Plants caged Pollinator Exclusion (PE), Plants caged with bee hive (BP) and Plants

kept open to all pollinators (OP). The comparative data pertaining to modes of pollination in mustard crop revealed that highest values of mean no. of siliqua/plant (186.44), no. of seeds/ siliqua (13.82) and seed yield (20.54 q/ha) were obtained from OP followed by BP and it was recorded lowest in PE. The introduction of honeybees in agricultural crops plays a vital role in pollination which in turn resulted in higher production of seed yield as well as honey production (Patidar, *et al.*, 2017).

Ahmed and Rehman (2002) observed in mustard that the number of siliquae per plant was significantly higher in OP (189.60-190.24) over PWI (120.93-120.69). Thapa (2006) found in Indian mustard about 11 percent increased in pod setting. Verma and Joshi (1983) reported that on mustard bloom, honeybee pollination increased the number of seeds by 4.07 per pod. Delbrassinne and Rasmont (1988) reported that intensive pollination of *Brassica juncea* linn. By *A. mellifera* increased the number of seeds per pod (12.22%), Panda *et.al.* (1989) obtained average seed number / pod was 10.80 whereas without insects were 5.90. Ahmed and Rehman (2002) found that the number of seeds per siliqua (12.04-12.60) percent higher than PWI in the two varieties of rape seed.

Sanas *et al.*, (2014) also found that *A. mellifera* increased the number of seed per pod (23.27%) in mustard under Konkan condition of Maharashtra. There are many studies showing 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).

# CHAPTER-III

## MATERIALS AND METHODS

The experiment was conducted during November 2016 to February 2017 to find out the effect of pesticide application timing on honey bee foraging in mustard field and role of honey bee on mustard yield. The details of the materials and methods that used to conduct the study are presented below:

### **Location**

The study was conducted in the field at Nagarpur, Tangail.

### **Climate**

The climate of study site is under the subtropical climate, characterized by three distinct seasons, the winter season from November to February and the pre-monsoon period or hot season from March to April and the monsoon period from May to October (Edris *et al.*, 1979).

### **Soil**

The soil of the experimental area belongs to the Modhupur Tract (UNDP, 1988) under AEZ No. 28 and was dark grey terrace soil. The selected plot was medium high land and the soil 20 series was Tejgaon (Anon, 2010). The soil characterized by poor fertility and impeded by internal drainage. The pH of the experimental soil ranged from 5.5 to 6.2 (Anon, 2010).

### **Mustard variety and its characteristics**

Mustard seeds of variety Tori-7 were selected for this experiment. The variety was local one and improved by the Bangladesh Agricultural Research Institute (BARI) in the year of 2004. The plant height of this variety ranges 60-75cm and the life cycle is 75 -75 days when cultivated in robi season.

### **Land preparation**

The experimental plot was opened in the first week of November 2016 with a power tiller, and was exposed to the sun for a week, after which the land was harrowed, ploughed and cross-ploughed several times followed by laddering to obtain a good tilth. Weeds and stubble were removed, and finally obtained a desirable tilth of soil for sowing of mustard Seeds.

## Experimental design and layout

The experiment was conducted considering seven treatments and laid out in a Randomized Complete Block Design (RCBD). Each treatment was replicated three times. Field trials were conducted during the winter season in the field at Nagarpur, Tangail. Mustard (*Brassica napus* var. Bari Sarisha- 7) was cultivated for this experiment. The unit plot size was 25 m x 12m. The distance between plots and blocks were 0.75 m and 1.0m, respectively. Row to row distance for mustard was 50 cm. similar distance was maintained when every seeds were sown, respectively.

## Fertilizers and manure application

The fertilizers N, P, K, S, Zn and B in the form of Urea, TSP, MP, Gypsum, Zinc sulphate and borax, respectively were applied. The entire amount of TSP, MP, Gypsum, Zinc sulphate and borax were applied during the final preparation of land. Urea was applied in two equal installments at final land preparation and at 30 days of seed sowing. The dose and method of application of fertilizers are shown in Table 1 (Anon., 2005).

**Table 1.** Dose and method of application of fertilizers in mustard field

Fertilizers	Dose (kg/ha)	Application (%)	
		Basal	Top dressing
Urea	300	50	50
TSP	180	100	--
MP	100	100	--
Gypsum	180	100	--
Zinc sulphate	07	100	--
Borax	15	100	--

## Date of sowing

The seeds of mustard were sown in sole and in intercrop plot on 26 November 2016.

## Cultural practices

After establishment of seedlings, all other intercultural operations such as, thinning, weeding, irrigation were accomplished as per as when necessary for better growth and development of the mustard crop. Single irrigation was applied just once before flower initiation. Plots were provided with well-arranged drainage facilities as prevention process of removing excess rain water if any. Weeding was done twice in



the field to keep the plots free from weeds to ensure better growth and development of the crops.

The newly emerged weeds were uprooted carefully at flowering stage by mechanical means.

### **Net Setup**

For the experiment net was set up over selected plot at 18 December 2016, so that only honey bees present in the selected plots and no insect especially honey bees interpreted the selected plots. Net size was 25 m x 12m x 1.5m.

### **Box Setup**

For the experiment boxes were set up over selected plots in 31<sup>st</sup> December 2016.

### **Data collection**

The data on the following parameters were recorded at different time intervals:

- Plant height/ plot
- Total number of Pod/plant
- Length of Pod/plant
- Total number of seed/pod
- Thousand seed weight/plot
- Number of flower/plant
- Number of honey bee before pesticide application/plot
- Number of honey bee after pesticide application/plot
- Time of bee foraging
- Weight of seeds/plot.

### **Procedure of recording data**

#### **1. Plant height/ plot**

Plant height was measured from each plot from randomly selected ten plants. Then average plant height/plot was counted.

#### **2. Total number of Pod/plant**

Total number of pod was counted from each plot from randomly selected ten plants also. Then average number of pod/plant was counted.

#### **3. Length of Pod/plant**

Length of pod per plant was measured from randomly selected ten plants. Then average pod length/plant was measured.

#### **4. Total number of seed/pod**

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

#### **5. Thousand seeds weight/plot**

Thousand seed weight per plot was counted from each plot from randomly selected ten plants.

#### **6. Number of flower/plant**

Total number of flower was counted from each plot from randomly selected ten plants. Then average number of flower/plant was counted.

#### **7. Number of honey bee before pesticide application/plot**

Total number of honey bee before pesticide application was counted from each plot using sweeping net at least five times at each plot.

#### **8. Number of honey bee after pesticide application/plot**

Total number of honey bee after pesticide application was counted from each plot using sweeping net at least five times at each plot.

#### **9. Time of bee foraging**

Time of honey bee foraging was recorded by observing bee box and field from each plot.

### **Harvesting, threshing and cleaning**

Mustard was harvested at the maturity (93 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 of each plot was threshed separately, cleaned, sun dried, weighed and packed.

### **Statistical analysis**

Data were analyzed using IBM SPSS 25 software for proper interpretation. The data recorded on different parameters were subjected to analysis of variance (STAT Graphics Centurion XV) and means were compared by Duncan's Multiple Range Test (STAT Graphics Centurion XV) at 5% level of significance.

## **CHAPTER IV**

### **RESULTS AND DISCUSSION**

The results on the effect of pesticide application timing  $P_1 = 8.30-9.30$  am,  $P_2 = 9.30-10.30$  am,  $P_3 = 10.30-11.30$  am,  $P_4 = 11.30-12.30$  pm,  $P_5 = 12.30-1.30$  pm,  $P_6 = 1.30-2.30$  pm,  $P_7 = 2.30-3.30$  pm and  $P_8 = 3.30-4.30$  pm on honey bee foraging and netting without bees ( $T_1$ ) and netting with bees ( $T_2$ ) compared with control ( $T_3$ ) in mustard field to observe its yield. The results of the present study have been discussed and possible interpretations are furnished and presented in this chapter under the following sub headings:

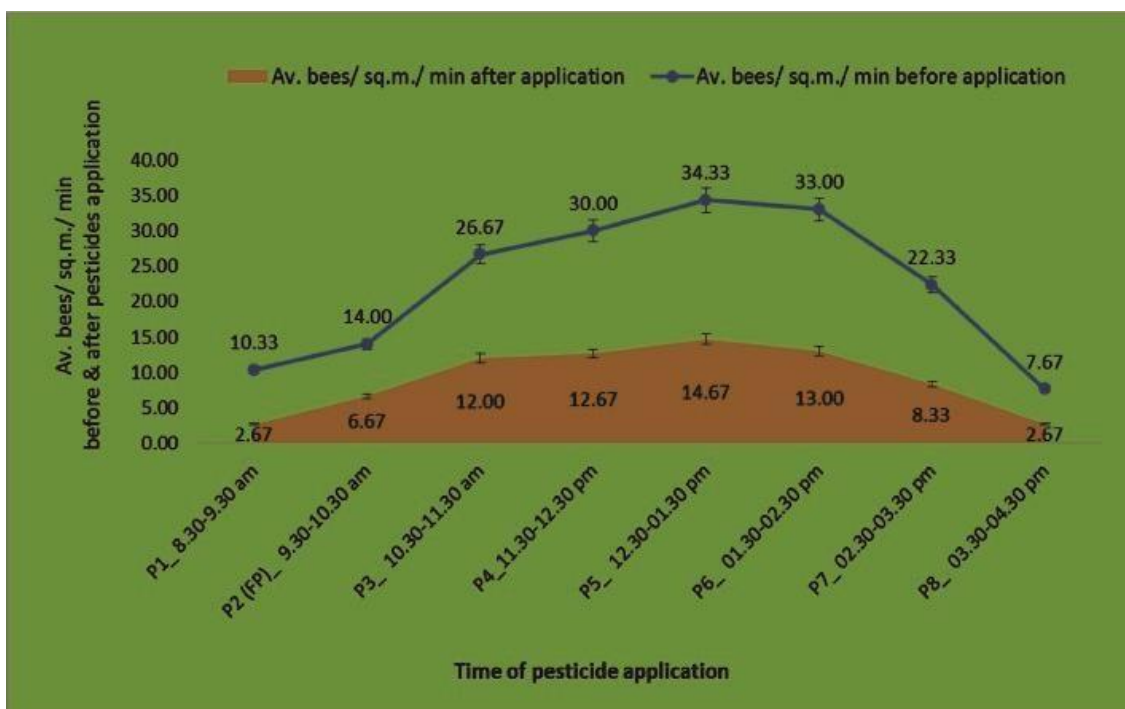
#### **Effect of pesticide application on honey bees foraging**

##### **Number of honey bee**

Highest number of honey bee (34.33 Av. bees/ sq. m./ min) was recorded at 12.30-1.30 pm ( $P_5$ ) before pesticide application which was statistically different from those of all other periods. The second highest number of honey bee (33.00 Av. bees/ sq. m./ min) was recorded at 1.30-2.30 pm ( $P_6$ ) before pesticide application. On the other hand, the lowest number of honey bee (7.67 Av. bees/ sq. m./ min) was recorded at 3.30-4.30 pm ( $P_8$ ) before pesticide application which was statistically different from those of all other periods (Figure 1).

After pesticide application in mustard field, the highest number of honey bee (14.67 Av. bees/ sq. m./ min) was observed at 12.30-1.30 pm ( $P_5$ ) which was statistically different from all other periods. The second highest number of honey bee (13.00 Av. bees/ sq. m./ min) was recorded at 1.30-2.30 pm ( $P_6$ ).

The lowest number of honey bee (2.67 Av. bees/ sq. m./ min) was recorded at 3.30-4.30 pm ( $P_8$ ) which was statistically similar with 8.30-9.30 am ( $P_1$ ) (2.67 Av. bees/ sq. m./ min) (Figure 1).

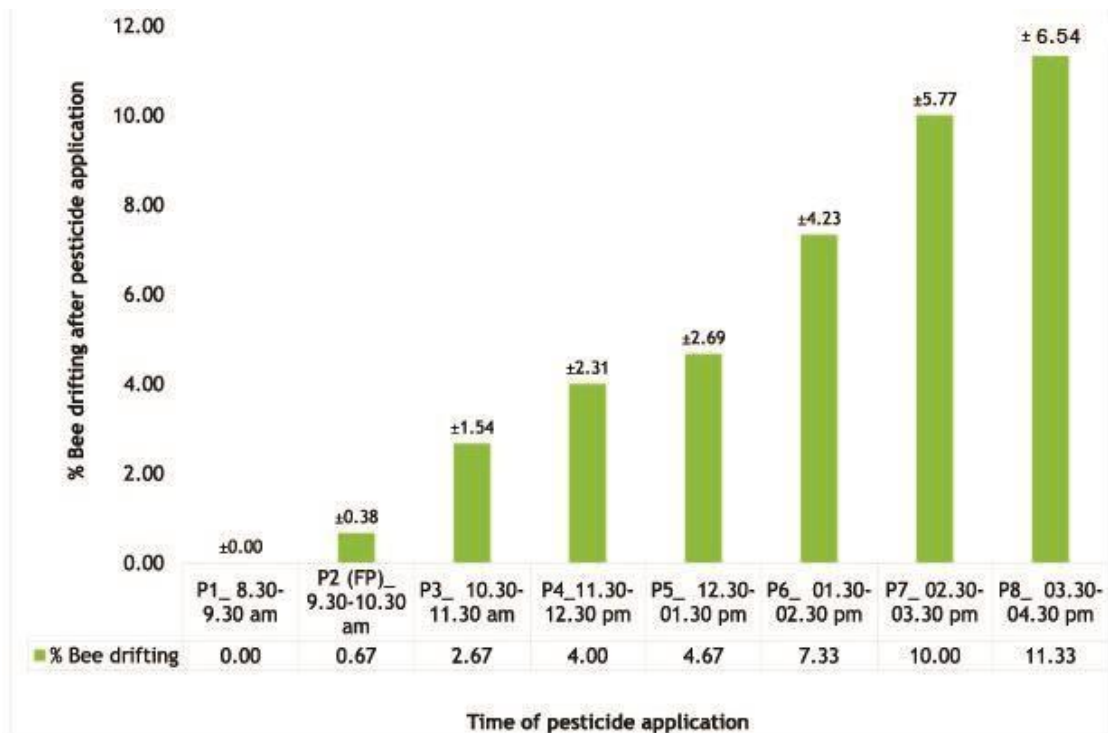


**Figure 1:** Number of honey bees/ square meter/ minute before and after pesticide application at different period of pesticide application

From the Figure 1 it was showed that the highest number of honey bee found at 12.30-1.30 pm and lowest number of honey bee found at 3.30-4.30 pm. But it was clearly showed that maximum honey bee observed in mustard field from 10.30 am to 2.30 pm.

### Drifting of honey bee

Highest percent of honey bee drifting after pesticide application (11.33 %) was recorded at the time of pesticide application at 3.30-4.30 pm (P<sub>8</sub>) which was statistically different from all other periods. The second highest percent of honey bee drifting after pesticide application (10.00%) was recorded at the time of pesticide application at 2.30-3.30 pm (P<sub>7</sub>). On the other hand, the lowest percent of honey bee drifting after pesticide application (0.00%) was recorded at the time of pesticide application at 8.30-9.30 pm (P<sub>1</sub>) which was statistically similar with percent honey bee drifting after pesticide application (0.67%) at the time of pesticide application at 9.30-10.30 am (P<sub>2</sub>) (Figure 2).

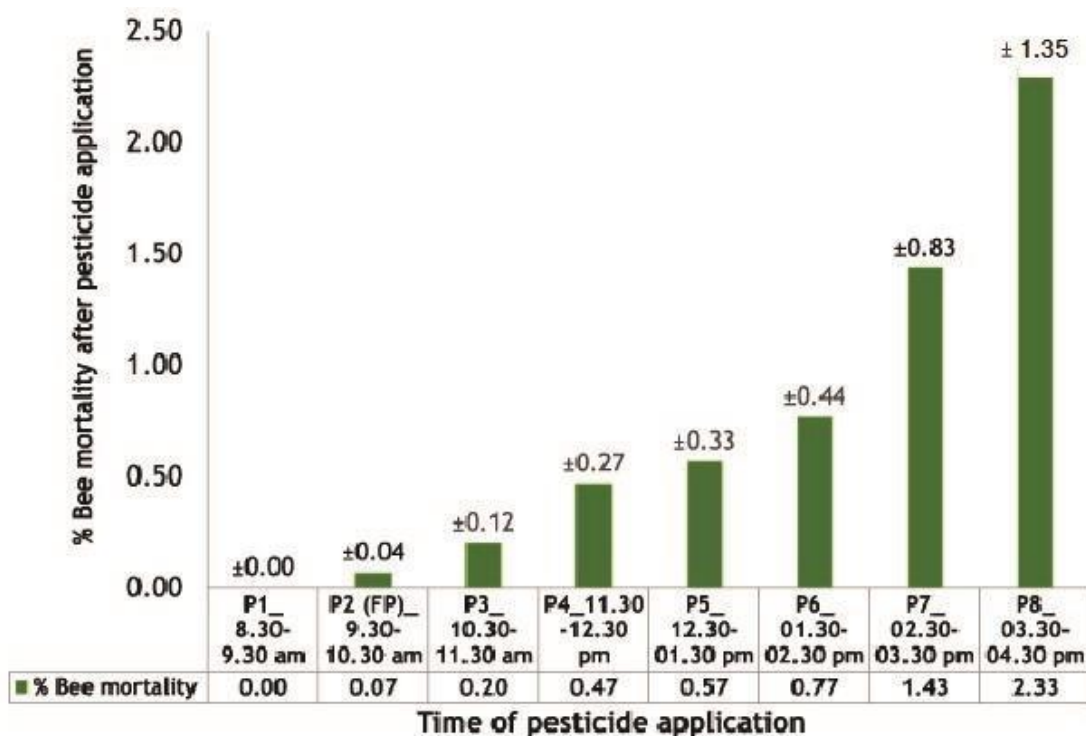


**Figure 2:** Percent of honey bee drifting after pesticide application at different period

From the above Figure 2 it was revealed that, time of pesticide application at 3.30-4.30 pm was most harmful time for honey bee foraging in mustard field and increased percent of honey bee drifting at this time. On the other hand, time of pesticide application at 8.30-10.30 am was less harmful for honey bee foraging in mustard field and also decreased percent of honey bee drifting during this time.

### **Mortality of honey bee**

Highest percent of mortality of honey bee after pesticide application (2.33 %) was recorded at the time of pesticide application at 3.30-4.30 pm (P<sub>8</sub>) which was statistically different from those of all other periods. The second highest percent of mortality of honey bee after pesticide application (1.43%) was recorded at the time of pesticide application at 2.30-3.30 pm (P<sub>7</sub>). On the other hand, the lowest percent of mortality of honey bee after pesticide application (0.00%) was recorded at the time of pesticide application at 8.30-9.30 pm (P<sub>1</sub>) which was statistically similar with the percent of mortality of honey bee after pesticide application (0.67%) at the time of pesticide application at 9.30-10.30 am (P<sub>2</sub>) (Figure 3).



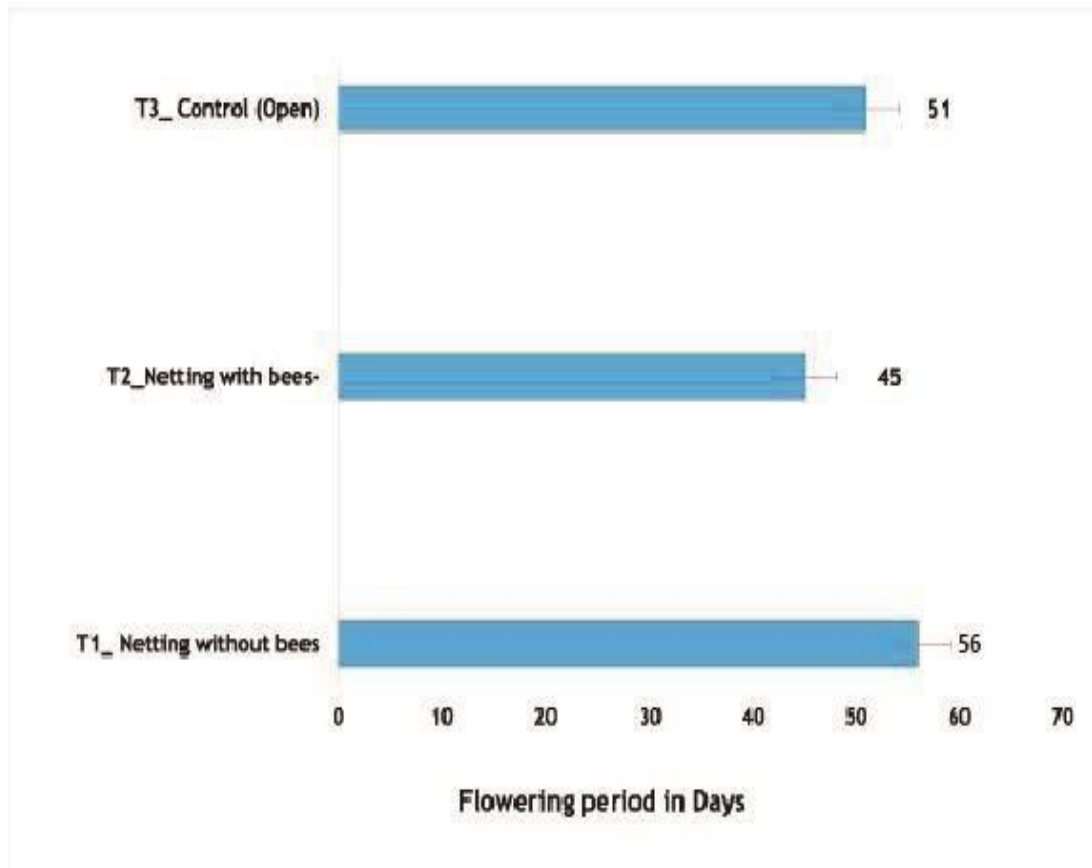
**Figure 3:** Percent of honey bee drifting after pesticide application at different period

From the above Figure 3 it was revealed that, time of pesticide application at 3.30-4.30 pm was most harmful time for honey bee foraging in mustard field and increased percent of mortality of honey bee at this time. On the other hand, time of pesticide application at 8.30-10.30 am was less harmful for honey bee foraging in mustard field and also decreased percent of mortality of honey bee during this time.

### **Effect of honey bee on yield contributing characters**

#### **Flowering period**

Higher flowering period of mustard was recorded at 56 days after sowing when mustard field was netted without bees (T<sub>1</sub>) which was statistically similar with T<sub>3</sub> (Control/open) treatment 51 days. On the other hand, lowest period of flowering was recorded 45 days at the treatment of netting of mustard field with bees (T<sub>2</sub>) (Figure 4).

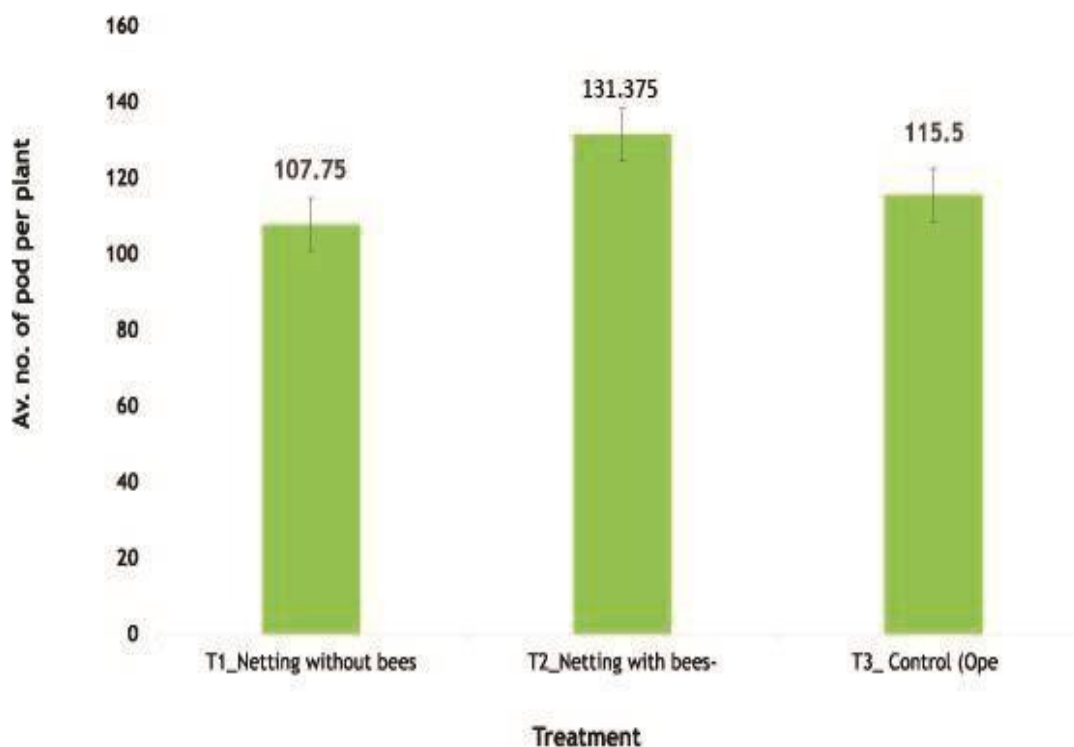


**Figure 4:** Flowering period in days of mustard at different treatments

From the above Figure 4 it was revealed that, honey bees helped on pollination of mustard and decreased the period of flowering stage of mustard than pollination without honey bees and open field.

### **Number of pod**

Highest number of pod per plant was recorded 131.375 pods per plant when mustard field was netted with honey bees and which was statistically different with other treatments. On the other hand, the lowest number of pod per plant was recorded 107.75 pods per plant when mustard field was netted without honey bees and which was statistically similar with control or open field (115.5 pods per plant) (Figure 5).



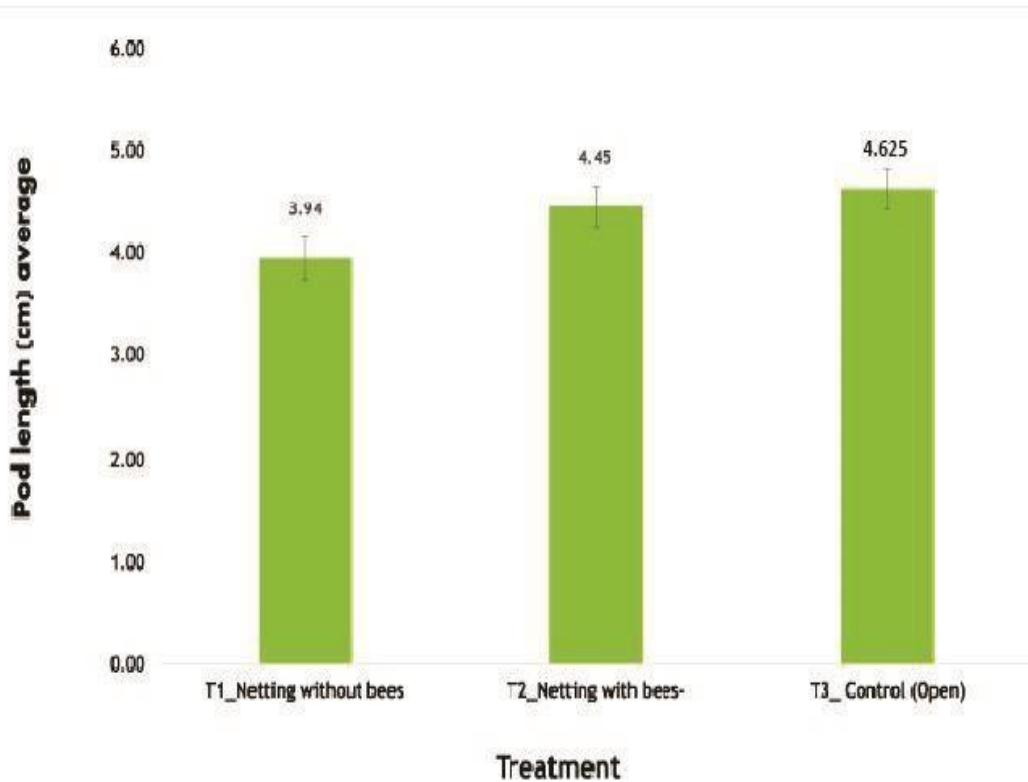
**Figure 5:** Figure Number of pod per plant at different treatments

Flowering period in days of mustard at different treatments From the above Figure 5 it was revealed that, honey bees helped on pollination of mustard and increased the number of pod per plant of mustard than pollination without honey bees and open field.

### **Pod length**

Highest number of pod length was recorded 4.625 cm. when mustard field was open (T<sub>3</sub>) which was statistically similar with 4.45 cm. pod length in case of netting with honey bees (T<sub>2</sub>). On the other hand, the lowest number of pod length was recorded 3.94 cm. when mustard field was netted with honey bees (Figure 6).



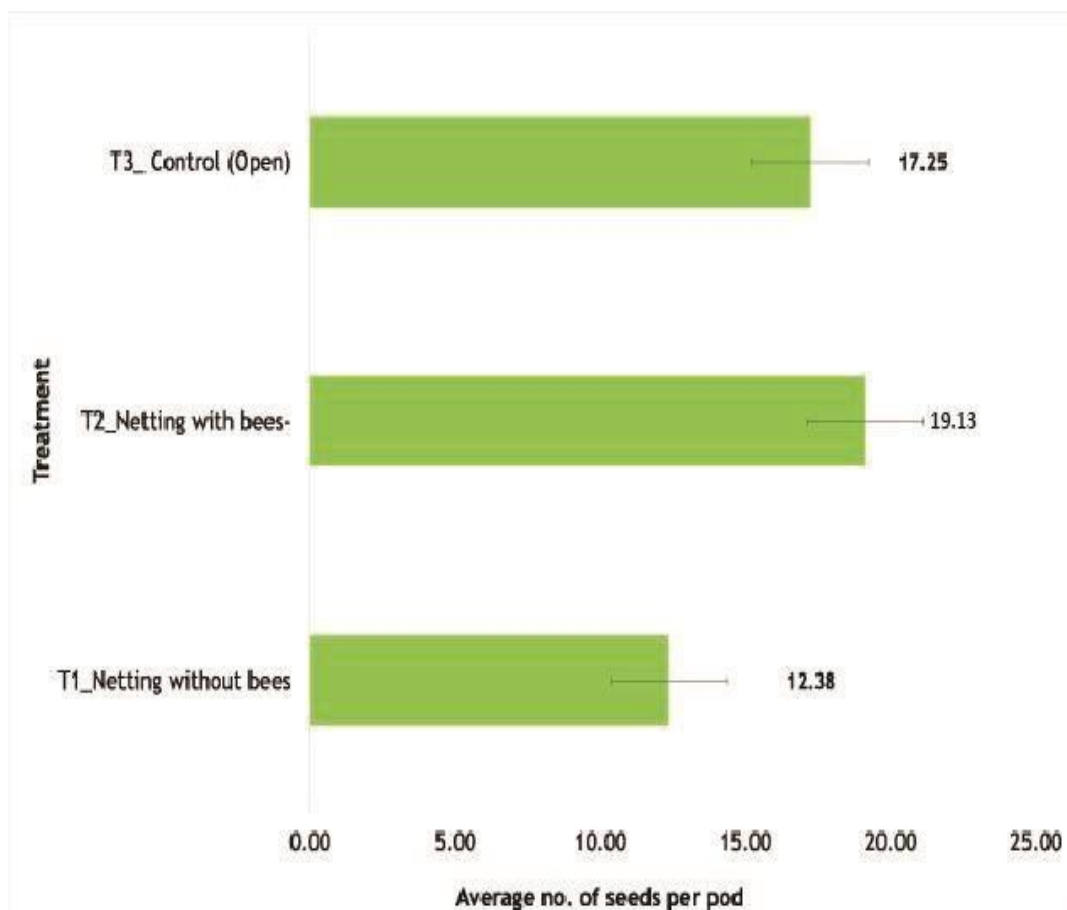


**Figure 6:** Pod length in different treatments

From the above Figure 6 it was revealed that, honey bees helped on pollination of mustard and open field increased pod length of mustard than pollination without honey bees in mustard field.

### **Number of seed per pod**

Highest number of seeds per pod was recorded 19.13 when mustard field was netted with honey bees ( $T_2$ ) which was statistically different from other treatments and followed by 17.25 seeds per pod in case of control or open mustard field ( $T_3$ ). On the other hand, the lowest number of seeds per pod was recorded 12.38 when mustard field was netted without honey bees (Figure 7).

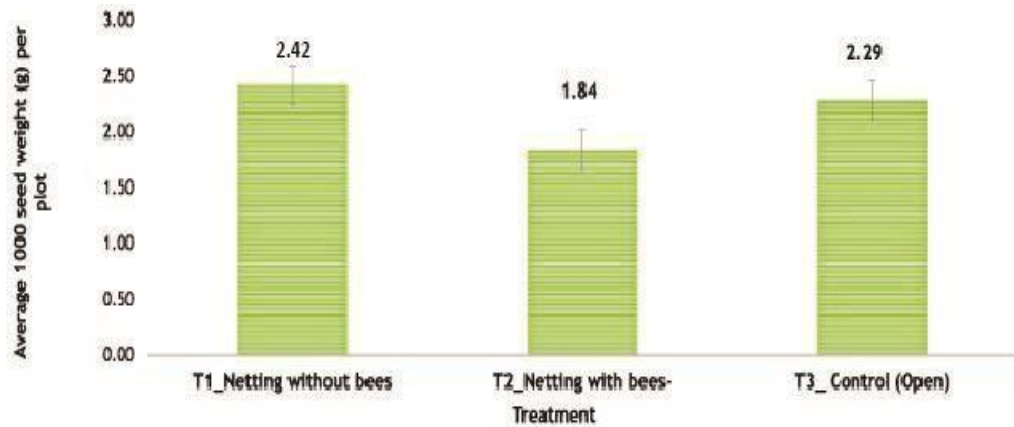


**Figure 7:** Average number of seeds per pod in different treatments

From the above Figure 7 it was revealed that, honey bees helped on pollination of mustard and increased the number of seeds per pod of mustard than pollination without honey bees and open field.

### **1000 seed weight**

Highest 1000 seed weight per plot of mustard was recorded 2.42 gm when mustard field was netted without honey bees ( $T_1$ ) which was statistically similar with 2.29 gm of 1000 seed weight per plot of mustard in case of control or open field ( $T_3$ ). On the other hand, the lowest 1000 seed weight per plot of mustard was recorded 1.84 gm when mustard field was netted with honey bees (Figure 8).

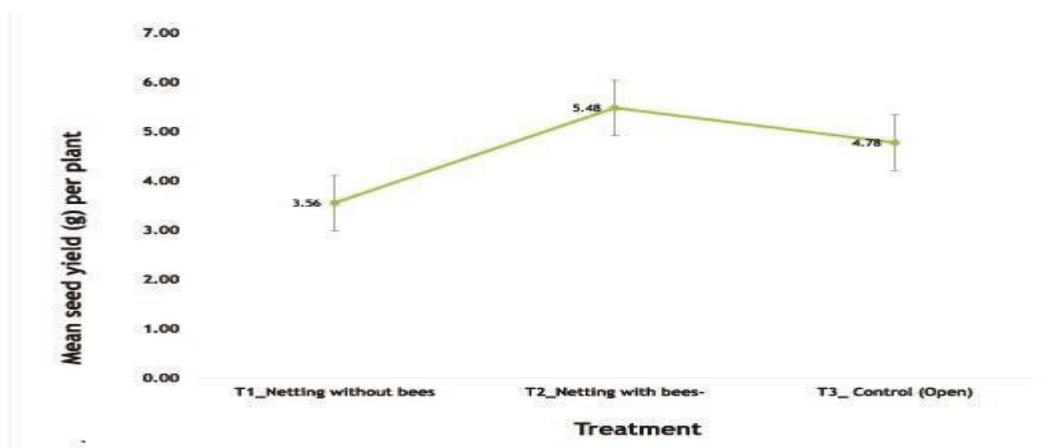


**Figure 8:** 1000 seed weight per plot in treatments

From the above Figure 8 it was revealed that, 1000 seed weight of mustard per plot was highest in netting without honey bees than netting with honey bees and open field.

### Seed yield per plant

Highest seed yield per plant of mustard was recorded 5.48 gm when mustard field was netted with honey bees (T<sub>2</sub>) which was statistically different from other treatments. On the other hand, the lowest seed yield per plant of mustard was recorded 3.56 gm when mustard field was netted with honey bees (T<sub>1</sub>) which was statistically similar with 4.78 gm of seed yield per plant of mustard in case of control or open field (T<sub>3</sub>) (Figure 9).

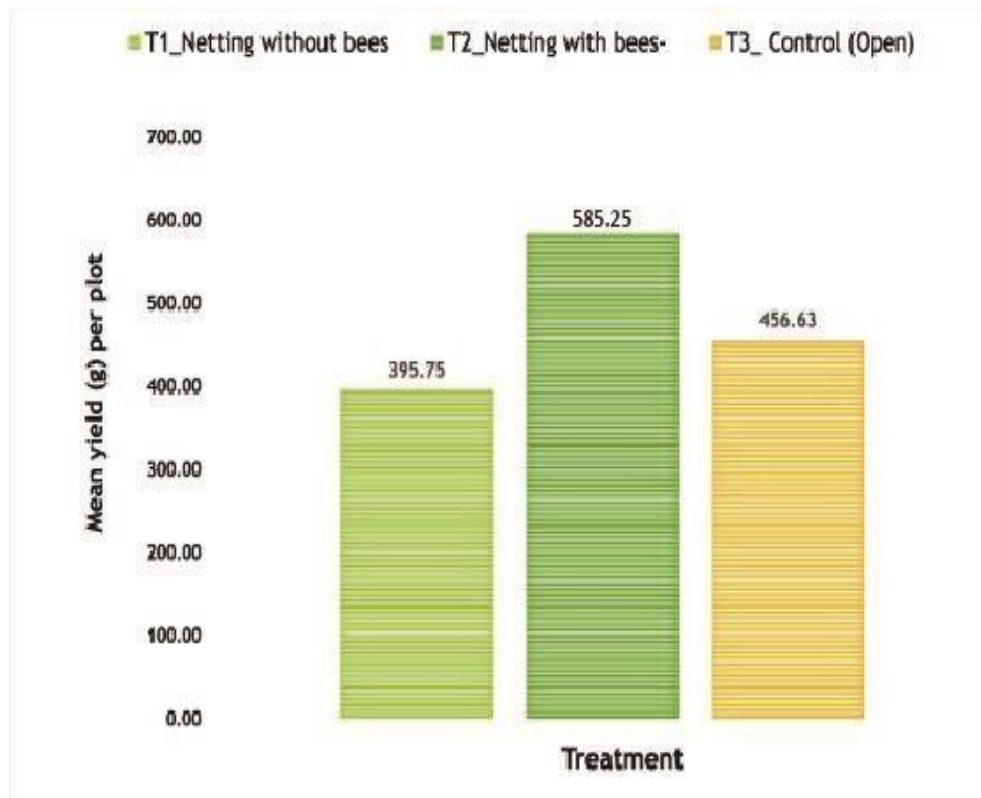


**Figure 9:** Mean seed yield per plant in different treatments

From the above Figure 9 it was revealed that, honey bees helped on pollination of mustard and netting with honey bees increased the mean seed yield per plant of mustard than field netting without honey bees and open field.

## Yield per plot

Highest yield per plot of mustard was recorded 585.25 gm when mustard field was netted with honey bees ( $T_2$ ) which was statistically different from other treatments. On the other hand, the lowest yield per plot of mustard was recorded 395.75 gm when mustard field was netted with honey bees ( $T_1$ ) which was statistically similar with 456.63 gm of yield per plot of mustard in case of control or open field ( $T_3$ ) (figure 10).



**Figure 10:** Yield of mustard per plot in different treatments

From the above Figure 10 it was revealed that, honey bees helped on pollination of mustard and increased the yield of mustard per plot than pollination without honey bees and open field.

## CHAPTER V

### SUMMARY AND CONCLUSION

The experiment was conducted in the field at Nagarpur, Tangail, Bangladesh during November, 2016 to February, 2017 to evaluate effect of pesticide application timing on honey bee foraging in mustard field and role of honey bee on mustard yield.

The highest number of honey bee found at 12.30-1.30 pm and lowest number of honey bee found at 3.30-4.30 pm. But it was clearly showed that maximum honey bee observed in mustard field from 10.30 am to 2.30 pm.

Time of pesticide application at 3.30-4.30 pm was most harmful time for honey bee foraging in mustard field and increased percent of honey bee drifting at this time. On the other hand, time of pesticide application at 8.30-10.30 am was less harmful for honey bee foraging in mustard field and also decreased percent of honey bee drifting during this time.

Time of pesticide application at 3.30-4.30 pm was most harmful time for honey bee foraging in mustard field and increased percent of mortality of honey bee at this time. On the other hand, time of pesticide application at 8.30-10.30 am was less harmful for honey bee foraging in mustard field and also decreased percent of mortality of honey bee during this time.

Honey bees helped on pollination of mustard and decreased the period of flowering stage of mustard than pollination without honey bees and open field. Honey bees helped on pollination of mustard and increased the number of pod per plant of mustard than pollination without honey bees and open field. Honey bees helped on pollination of mustard and open field increased pod length of mustard than pollination without honey bees in mustard field.

Honey bees helped on pollination of mustard and increased the number of seeds per pod of mustard than pollination without honey bees and open field. 1000 seed weight of mustard per plot was highest in netting without honey bees than netting with honey

bees and open field. Honey bees helped on pollination of mustard and netting with honey bees increased the mean seed yield per plant of mustard than field netting without honey bees and open field.

Honey bees helped on pollination of mustard and increased the yield of mustard per plot than pollination without honey bees and open field. Findings of the experiment reveal that 3.30-4.30 pm was the best time for honey bee foraging in mustard field and was not the best time for pesticide application on mustard field. On the other hand higher yield of mustard was found in case of honey bee pollinated mustard field.

## CHAPTER-VI

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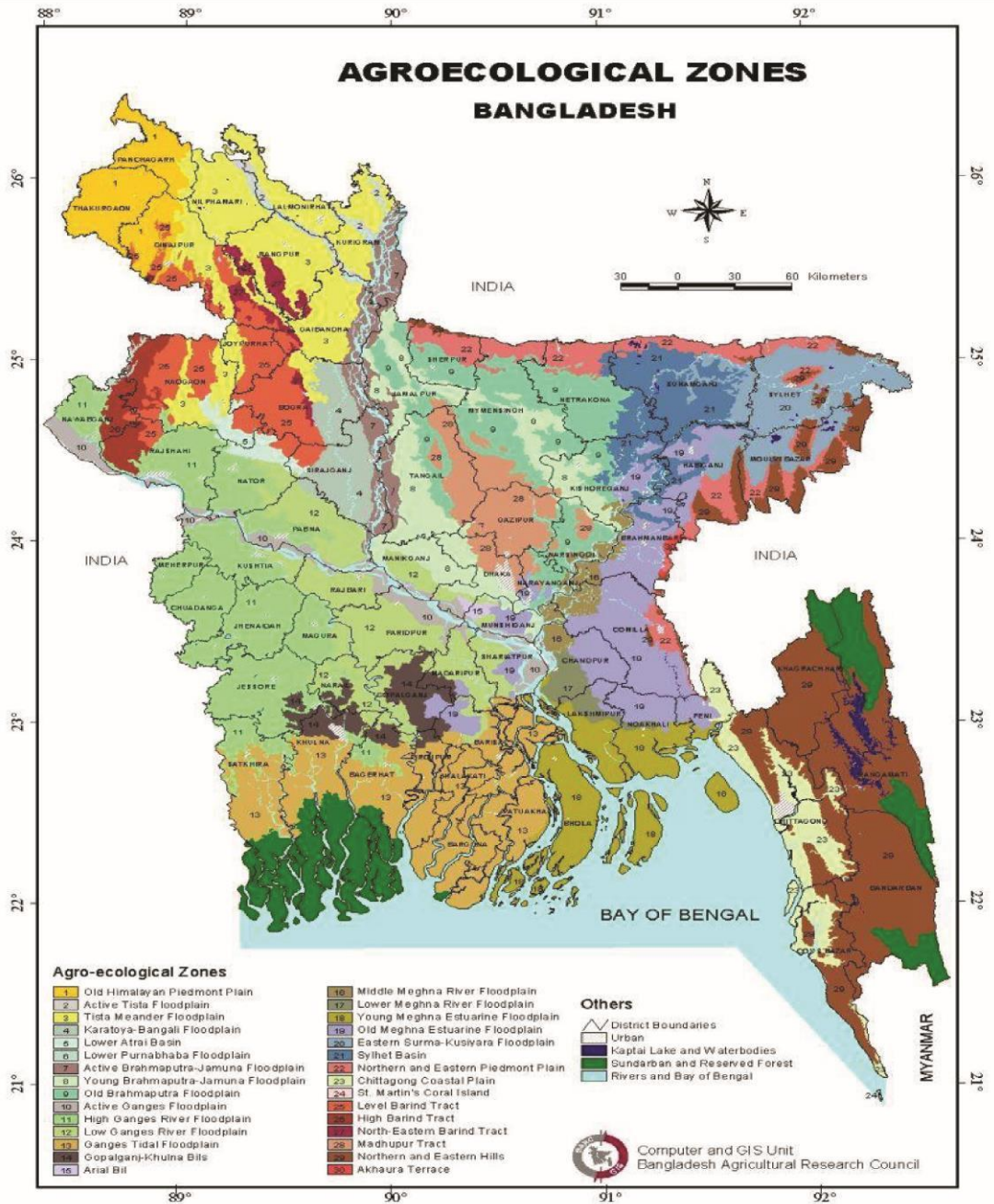
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# Chapter VII

## APPENDICES

### Appendix I. Agro-Ecological Zones of Bangladesh



**Appendix II. Monthly record of air temperature, rainfall and relative humidity of the experimental site during November 2016 to February 2017**

Months of observation	Air temperature (°C)		R. H. (%)	Rainfall (mm) (Total)
	Maximum	Minimum		
November, 2016	25.6	15.8	76	0
December, 2016	21.2	13.5	78	0
January, 2017	24.5	12.8	73	0
February, 2017	25.8	15.7	71	15

**Source: Bangladesh Meteorological Department (Climate and Weather Division), Agargoan, Dhaka-1207.**

### Appendix III: Geographical characteristics of the experimental field

Geographical Features	Characteristics
Location	Nagarapur, Tangail
AEZ	Madhupur Tract (28)
General Soil Type	Shallow red brown terrace soil
Land type	High land
Soil series	Tejgaon
Topography	Fairly leveled
Flood level	Above flood level
Drainage	Well drained
Cropping Pattern	Fallow- Tomato

Source: SRDI, 2013



**Appendix IV. The physical and chemical characteristics of soil of the experimental site as observed prior to experimentation (0-15 cm depth).**

CONSTITUENTS	PERCENT
Sand	26
Silt	45
Clay	29
Textural class	Silty clay

**Chemical properties:**

Soil characters	Value
Organic carbon (%)	0.54
Organic matter %	0.45
Total nitrogen (%)	0.027
Phosphorus	6.3 µg/g soil
Sulphur	8.42 µg/g soil
Magnesium	1.17 meq/100 g soil
Boron	0.88 µg/g soil
Copper	1.64 µg/g soil
Zinc	1.54 µg/g soil
Potassium	0.10 meq/100g soil

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