STUDY ON POLLEN COLLECTION EFFICIENCY OF EUROPEAN HONEYBEE (*Apis mellifera)* FROM MUSTARD FLOWER

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CERTIFICATE

This is to certify that the thesis entitled **Study on Pollen Collection Efficiency of European Honeybee (*Apis mellifera)* from(*Apis mellifera)* from Mustard Flower**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 Abir Ahmed, Registration No.: 10-04067, 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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DEDICATED TO

MY

BELOVED PARENTS

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STUDY ON POLLEN COLLECTION EFFICIENCY OF EUROPEAN HONEYBEE (*Apis mellifera)* FROM MUSTARD FLOWER

ABSTRACT

A study was conducted in Sirajgonj district during the mustard growing time of the year 2015-2016. It was found that honey bee foraging efficiency in mustard flower was highest (13 and 25 respectively) in terms of number of bees/m²/min and number of flowers visited/min. Average weight of single pollen loads were observed at the second harvest period (8-13 days) which obtained the highest (7.74mg/peiece) weight and the highest (9.54 g/day/hour) amount of pollen was weighed in the second harvesting period (8-13 days). The highest ranges (7.7 to 11.25 and 7.25 to 8.85 respectively) of pollen loaded bee foragers were available at 11.00 hours to 12.00 hours and 12.00 hours to 13.00 hours. It was also observed that the entry frequency ranged from 19.18 to 22.92 number/minute and the exit frequency ranged from 19.26 to 22.78 number/minute. In the poly hive super the highest (136 g) pollen yield was obtained from site 2 i.e. Ullapara, Sirajgonj and there is no yield in traditional bee hives. Pollen harvest best time is also in 11.00 hour of day time.

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CHAPTER I

INTRODUCTION

Pollen is the male germ of a flower. Field bees collect pollen from anthers of flowers and attach the grains to their hind legs, which act as pollen baskets (corbiculate). In the process of collecting pollen, bees inadvertently carry out the function of pollination of the various plants they visit.Bee gathered pollens are rich in proteins (approximately 40% protein), free amino acids, vitamins, including B-complex, and folic acid http:// Food matteers. TV/Articles-1/10-Amazing-Health-Benefits-Of-Bee-Pollen).

Pollen is the sole protein food of a honeybee colony harvested by bee foragers in their natural environment. In order to satisfy its needs a honeybee colony uses from 12 kg to more than 35 kg of pollen (Hodowla Pszczó, 1983). The presence of pollen in the nest is a prerequisite for normal colony development. It is important for regular growth and development of the brood. The rich amino acid composition of the pollen protein and other valuable pollen constituents made pollen one of products recovered from the bees harvested by man. So it is often referred to as bee pollen (Wilde and Wilde, 2002).

Valuable dietary, prophylactic and even curative properties of pollen caused the demand for the product to be on the increase so more and more attention has been paid to pollen recovery from bees. Capture of pollen and pollen cells also contribute to add income in honey farming. Consequently, it is important to get an understanding of all the factors which have an impact on the pollen collection efficiency of bee colonies and how the factors relate to one another. Pollen can be collected from bees in two forms: as pollen loads – granules formed by the bees from fresh pollen and as bee bread – a product of milk fermentation processed by the bees in the combs.

Foragers working outside the bee hive are usually specialized to harvest particular materials in order to increasing their work efficiency. It was demonstrated that 60% of them were nectar foragers, 25% were busy collecting pollen and 15% bring the two materials at the same time and also act as deliverers of water and propolis raw materials(Free, 1960). However, worker bees have been observed to be highly adaptable to current conditions and colony requirements which manifest it self as a change in the proportion of nectar vs. pollen foragers (Free, 1960).

Honey bees (*Apis mellifera*) are dependent on the supply of floral pollen and nectar. The activity of the honey bee is controlled largely by ambient conditions. In some habitats where the weather fluctuates annually, as in the "sub-tropical region", the warm season coincides with the lack of flowers and is considered as the "Dearth period". In the cold season, when the flowers appear, the bees are at full activity, collecting and storing food, along with fulfilling their reproductive duties. Although bees are physiologically capable of being active in hot deserts, they suffer from the lack of food sources and water, the latter being used for cooling hives in addition to its physiological function. Honey bees are thus restricted to areas where blooming occurs at least for part of the year (Echazarreta and Paxton, 1997). Proper colony management should ensure adequate honey reserves but sometimes carbohydrate supplement feeding also become necessary. Whenever colonies have little honey reserves, they should be fed with artificial foods. Carbohydrate foods have some value for stimulating queens to begin laying eggs, but no carbohydrate will support sustained egg laying or brood rearing in the absence of pollen or a protein supplemental food (Shimanuki, 1971).

A. mellifera carries heavier pollen, less aggressive and produce more honey than the native bee *A. cerana*. It is less prone to swarming for beekeepers who naturally hope to lose their colonies as rarely as possible. Like other honey bee species *A. mellifera* has a high flight range for foraging. A worker of this species may fly maximum 2-3 km away from its colony (Abrol, 2006). There is a general agreement that introduction of the exotic *A. mellifera*, in Northern India, Bangladesh, Pakistan and Thailand is now the basis of flourishing apiculture industries. This exotic bee species produces three times more honey than the native, *A. cerana* and is more suited to modern bee management technology (Verma, 1990).All types of flowers are not suitable in yielding similar quality or quantity of nectar and pollen. Some are rich in nectar rather than pollen and vice versa. Furthermore, all types of nectar or pollen are not similarly accepted to the worker honey bees. When flowers enriched by nectar and abundant in the nature and the pollen quantity or quality is poor, alternative pollen i.e. pollen substitute should be supplied artificially to maintain the population of the colony. Similarly, nectar substitute should be provided to the colonies if good quality natural nectar is rarely available. For successful management and rearing of honey bees in dearth period, it is imperative to adapt beekeeping measures for colony development.

The annual cycle of colony development of European honey bee (*A. mellifera*) is described in detail in many independent studies in temperate climates from North America (Farrar, 1937; Avitabile, 1978). Limited brood rearing may be initiated during winter months and brood rearing leading to colony expansion is often initiated before nectar and pollen become available. Furthermore, queen rearing is essential for improving existing stock, but has not been practiced successfully with *A. mellifera* in spite of many attempts. This species is very new in Bangladesh and the information in this country regarding this species is also scanty.

In Bangladesh, at present 517 apiaries are available and total 1551 professional beekeepers are directly involved in this sector. Total 42,911 single managed bee boxes are present in the country (Sakhawat, 2013), and there is no data on pollen harvesting from bee colonies. By utilizing pollen trap, pollen loads from the legs of worker bee can be collected. Mustard is one of the important pollen and nectar producing plant.

From these points of views the proposed study was underlined-

- To study the foraging behavior in the mustard field and
- To collect bee pollen from mustard flower at blooming period.

CHAPTER II

REVIEW OF LETERATURE

There are many species of honeybee, but four species are common these are *Apis florae, Apis dorsata, Apis cerana* and *Apis mellifera*. Due to domestic nature, Apis *mellifera* is the most popular world wide and can be easily reared, and safely migrated from one place to other for pollination and honey production. *A. mellifera* carried heavier pollen, less aggressive, and produce more honey than the native bee *A. cerana*. It is less prone to swarming for bee keepers who naturally hope to lose their colonies as rarely as possible(FAO,1986). Like other honey bee species *A. mellifera* has a high flight range for foraging. A worker of this species may fly maximum 2-3 km away from its colony (Abrol,1997). *Apis mellifera* in Northern India, Bangladesh, Pakistan, and Thailand is now the basis of flourishing apicultural industries. This exotic bee species produces three times more honey than the native, *honeybee* and is more suited to modern bee management technology (Verma, 1990).

A review of relevant literatures on foraging tasks, foraging times, foraging distances, foraging preferences and pollen collection efficiency of *Apis mellifera* in the bee colony are presented as follows :

Background on polle foraging in honey Bees

Herbert (1992) found that honey bees collect pollen because it provides the colony with amino acids, vitamins, minerals, and lipids needed for brood production . Pasquale *et al.* (2013) found that nutritional quality of pollen has been linked with honey bee physiology, worker longevity, and tolerance of parasitism . Pollen stores within the hive are utilized extensively by nurse bees for rearing brood during the spring, summer, and fall. Pollen collection and storage levels may be important fitness metrics because they are related to bee physiology, colony brood production, and future colony size.

Jeffree (1957) studied a typical honey bee colony will collect 13–20 kg of pollen each year and store ≤ 1 kg of pollen within the hive. Pollen collection rates are regulated within the hive based on the quantity of stored pollen and pollen consumption rates. Emission of brood pheromone by larval bees may also stimulate pollen foraging found in a done by Pankiw (2004):. About 130 mg of pollen is required for rearing a single bee, and a colony may raise over 100,000 bees during the summer. Although individual honey bees maintain high species fidelity during specific foraging trips, a bee colony as a whole can collect pollen from a variety of plant species throughout a day. While foraging, bees mix pollen with regurgitated nectar or 'glue' honey and other secretions, which is the groomed from the body and packed into a specialized storage structure on each of the rear legs (the corbicula or "pollen baskets"). It is reasonable to suspect that some amount of passively acquired pollen (i.e., not from plants visited by the forager but incidentally encountered through carryover from other pollinators, wind transport, physical contact between flowers of different species, etc.) is incorporated into these pollen loads, but this study was not designed to distinguish such.

Foraging tasks

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). This organization is important in saving time and the efforts of the honey bee foragers.

Liang *et al.* (2012) found extensive differences between honey bee food scouts and the other foragers with regard to brain gene expression including catecholamine, glutamate, and γ-amino butyric acid signaling.

Klein and Seeley (2011) described that the under some ecological conditions, a temporal shift from foraging activity to sleeping (napping) may occur.

Beyaert *et al*. (2012) described that the night sleep of forager bees is very important and night sleep deprivation may impact the navigation memory of honey bees.

Dimou and Thrasyvoulou (2007) described that to the resource forager bees collect, foraging activity can be classified into water, nectar, pollen or resin foraging. On rare occasions, forager bees can also collect wax from scale insects, *Ceroplastes* sp.

The type of foraging, whether for pollen or nectar, is a colony-level trait with a genetic component (Hunt *et al*. 1995), and is affected by the genotype of bee strain (Pankiw *et al*. 2002). Also, these tasks depend on collective and individual decisions of forager bees. The prior experience at a feeding place plays a role during collective foraging (Fernandez and Farina 2005). For more details about the collective foraging of honey bees see Vries and Biesmeijer (1998). The authors also developed a model based on individual behaviour roles (e.g. previous information about food sources).

Another important factor for the type of foraging task is sucrose response thresholds. Pankiw and Page (2000) found differences between honey bee workers in their thresholds to sucrose; the lowest threshold was found in water foragers, then pollen foragers, then nectar foragers followed by foragers of both pollen and nectar. They correlated these differences with the division of labour of forager bees.

Simone - Finstrom *et al*. (2010) found that the sucrose response thresholds of the resin foragers were lower than pollen foragers. Thus, honey bee workers with low sucrose response thresholds start foraging behaviour for water and pollen earlier and at younger ages than workers with high sucrose response thresholds which forage for nectar (Pankiw 2005). The correlation between foraging behaviour and sucrose response thresholds (foraging behaviour syndrome) was reviewed by Pankiw (2005). The sucrose concentration response threshold is affected by rearing environment (Pankiw *et al*. 2002) and responds positively to bovine insulin treatments (Mott and Breed 2012).

Also, the change to pollen foragers is related to the colony conditions and foragers use their experience in trophallactic contacts to assess the pollen need of their colonies (Weidenmuller and Tautz 2002). Under shortages of pollen or in conditions of poor pollen quality, honey bee colonies increase the proportion of pollen foragers without increasing foraging rate (Pernal and Currie 2010). The foraging choice between pollen (protein) and nectar (carbohydrate sources) is influenced by insulin receptor substrate (IRS) as demonstrated by Wang *et al.* (2010). It seems that the foraging tasks are under the control of many factors and detailed studies on these factors are required.

Foraging time

It is known that the foraging activity of honeybees is initiated in early morning and finishes in the evening. In some studies, honeybee 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. Carrillo *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 foragersmin^{-1}) than during the morning period (17.66 foragers min^{-1}).

Yucel and Duman (2005) found that honeybee workers visited onion flowers from 8.15 to 16.30 h and the peak foraging was between 11.00 to 12.00 h.

Rahman and Rahman (1993) studies pollen gathering activity of *A. cerana* and *A. mellifera* and compared them in *B. napus*. In *A. cabana* colonies, the maximum area of stored pollen was recorded in February and the minimum in October. In *A. mellifera* colonies the maximum and minimum quantities were recorded in March and October, respectively. Throughout the year, the average of pollen collecting bees/day was significantly higher in *A. cerana* colonies than in *A. mellifera*.

Hossain (1992) stated that foraging activity of honeybees (*A. mellifera*) was greatest in January, October and November, with maximum pollen collection in January, February and November. Nils recorded during the monsoon (June-August). Correlations were given the number of pollen collectors and the number of foragers. There was a significant negative correlation between relative humidity (RH) and foraging and pollen collection, especially in June-August.

Ghoniemy and Abu-Zeid (1992) observed that more bees (*Apis mellifera*) foraged for nectar than for pollen in 2 cultivars of swederape (*Brassica napus*). The average number of flowers visited per minute for nectar collection reached it speak (10.14) at mid day, whereas for pollen collection the peak (2.55) was at 9:00-10:00 h.

Hossain (1992) stated that foraging activity of honeybees (*A. mellifera*) was greatest in January, October and November with maximum pollen collection in January , February and November. Maximum values for pollen collection was recorded during the monsoon (June-August) in litchi flower. Jay (1974) observed that more pollen was collected in the morning than in the afternoon.

Pollen selection criteria used by honeybees may include such factors as pollen age, moisture content, color, pH, pollen grain size, physical configuration of the grains and odour (Stanley and Linskens 1974).These parameters are independent of the nutritive value of the pollen and could potentially influence its selection by foragers.

Baker (1971) found that the pollen collections were higher when honey collections were more. He also remarked that correlation between pollen collection and brood area was significant in starved colonies in which brood rearing was depressed. It was observed from his experiment that adequate stores of honey were important for pollen collection.

Bisht and Pant (1968) observed on litchi flowers that the number of pollen gathering bees was the highest during the months of January, February and March. They observed lesser activity of the bee during April, August, September and October. The number of pollen gathering bee reduced further during November and December. Pollen gathering activity is dependent upon the availability of pollen yielding flowers and the environmental conditions like sunrise, sunset and day temperature etc. Pollen gathering activity is, therefore, the interaction of available flora and these interactions presumably determine the pattern of pollen gathering activity. They also remarked that there was a negative correlation between the time when the bees started pollen gathering trips and the maximum temperature of the day. There was a positive correlation between relative humidity of the day and the periods when the bee started pollen gathering.

Jhajj and Goyal (1979) worked on the comparative behaviour as pollen forager of *A. mellifera* and *Apis cerana* (F.) in rape plant (*B. napus*). They observed that number of bees of either species collected mixed pollen of on all foraging trips on the same day, or one each of the 5 days observed. Pollen availability decreased from morning to afternoon and some pollen foragers of both species then changed to nectar collection in the next morning. The two species showed similar behaviour in pollen foraging, with minor differences.

Adlakha and Sharma (1975) reported that at Nagrota, *A. mellifera* was active with in 21.28°C range, inactive below 12.88°C; *A. cerana* was active even at lower temperatures. Foraging range of *A. mellifera* L. was 3-4 times higher than *A. cerana*.

Foraging distance

The energy hypothesis which suggests that foragers estimate the feeder distance (food resource) based on the spent energy during foraging flight is now considered to be incorrect and another hypothesis based on optical flow was suggested (Esch and Burns 1996). Both hypotheses can be considered as integrated explanations in as much as the energy spent during flight as well as the speed motion of the ground image received by the retina are both essential for estimating distance as well for distance calculation. The mean foraging distance for *A. m. carnica* was 1526.1 m while foraging distances of pollen-collecting bees had a mean of 1743 m in simple landscapes and 1543.4 m in complex landscapes (Steffan-Dewenter and Kuhn 2003). The mean of foraging distances for small colonies of *A. m. mellifera* was 670 m and for large colonies it was 620 m in July, while the values were 1430 m for small colonies and 2850 m for large colonies in August (Beekman *et al*. 2004).

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). It seems that the foraging distance for colonies in thesame region is impacted by race, colony strength, food resource, month and the time of the day.

Foraging preference

Forager bees prefer the collection of water, nectar, pollen or resin from some resources over others. There are many examples of foraging preference; only a few examples are presented here. Water foragers were noticed to prefer continuous water sources than stable ones as well as large water containers than small ones (Abou-Shaara 2012). Also, forager bees have a preference to collect water from some unusual sources (e.g. cow dung) over clean water (Butler 1940). Nectar foragers sometimes prefer one food source over another as well as the specific position of one flower over another. Sushil *et al.* (2013) found that more honey bee foragers visited broccoli followed by kohlrabi and finally Chinese cabbage with 6.05, 5.35 and 5.05 bees/plant, respectively.

Mayer and Lunden (1988) found more nectar foragers on the top of the flowers of Manchurian crabapple than red delicious apple.

Fohouo *et al.* (2008) found the highest number of forager workers was on *Syzygium guineense* var. *guineense* and the lowest number on *Psorospermum febrifugum*. Also, Weaver (1965) detected differences in honey bee foraging behaviour on hairy vetch (*Vicia vitlosa* Roth) flowers; some bees used the flower base while others use the flower mouth. Honey bees have a preference for apple tree branches located in the middle of trees rather than for those branches located higher up or lower (Mattu *et al*. 2012). Similarly, pollen and resin foragers prefer some resources over others. More studies are required to fully uncover the preference behaviour of forager bees.

Foraging behaviour of honey bee subspecies

Differences between foraging activity as the number of bees leaving the hives were found between three honey bee subspecies; Yemeni, Italian and Carniolan honey bees, with a higher foraging activity of Yemeni then Italian and finally Carniolan honey bees under desert conditions (Alqarni 2006).

Ali (2011) also found a higher foraging rate for Yemeni honey bees than Carniolan honey bees during June and August and at different monitoring times; 6–7 am, 11–12 am and 4–5 pm. The same trend was found by Abou-Shaara *et al.* (2013), where Yemeni honey bees had higher foraging activity than Carniolan honey bees under desert conditions. In contrast, no clear impact of bee race was found for ARS Russian or Italian honey bees with respect to the percentages of pollen foragers or flight activity (Danka *et al.* 2006). The differences between the foraging activity of honey bee sub species can be explained partly by the variations in their morphological characteristics. Bees with large wings were reported to have higher flying ability than small ones (Mostajeran *et al.* 2006).

Higginson *et al.* (2011) found that bees with damaged wings had less foraging trips and flew closer to the hive than healthy ones. Positive correlations were found between foraging activity and sealed brood area as well as bee number (Abou-Shaara *et al.* 2013). Also, the adaptation of honey bee subspecies to certain environmental conditions may influence the foraging activity (Alqarni, 2006). Forager workers of Yemeni and Carniolan honey bee sub species, under laboratory conditions, showed different abilities to tolerate different temperatures and relative humidity gradients (Abou-Shaara *et al.* 2012). However, until now only relatively few studies have been performed on honey bee subspecies.

Factors impacting foraging activity

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 queen less 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 *Varrao 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 lowpollen-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 (see, preference of honey bees paragraph). 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.

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).

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 bees 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).

Insecticides may also influence foraging behaviour. Yang *et al.* (2008) reported effects of sub lethal 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 ng/bee or more for clothianidin and 1.5 ng/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.

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 melli-fera* 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.

Monitoring of foraging activity

Foraging activity is measured by employing different parameters including, the foraging commencement or/and cessation time (Joshi and Joshi 2010; Mattu *et al*. 2012; Tan *et al.* 2012); the number of bees returning to the beehive (Beekman *et al.* 2004; Pernal and Currie 2010; Ali 2011) or leaving beehives (Alqarni 2006) or both (Abou-Shaara *et al.*

2013); the peak and fluctuations of foraging over time (Malerbo-Souza 2011); foraging speed and foraging distance (Steffan-Dewenter and Kuhn 2003); or estimation of foraging distance by decoding of the waggle dance (Pearce *et al.* 2013).

Other parameters related to foraging activity and the visiting of plants include, the number of foragers per flower (Sushil *et al.* 2013); the number of visited flowers per forager (Mattu *et al.* 2012); and time spent per flower (Sushil *et al.* 2013); nectar and pollen collection method from the blooms (Mackenzie 1994); the position of the forager bees on or at the side of the flower (Mayer and Lunden 1988; Mattu *et al*. 2012); the position of visited branches and flowers (Mattu *et al.* 2012); the proportion of pollen or nectar foragers relative to total foragers; foraging type; the load of pollen and pollen type; concentration of crop nectar sucrose (Pearce *et al*. 2013); and competition with other pollinators (Mackenzie 1994; Brittain *et al*. 2013).

Sushil *et al.* 2013 alsodid studies to monitor foraging activity under net conditions (Sushil *et al*. 2013). Marking and recapturing forager workers has been used in certain studies (Akinwande and Badejo 2009).

Hagler *et al*. (2011) used self-marking devices for studying the foraging range of honey bees on an alfalfa seed production field.

Colin *et al*. (2004) developed a method to quantify the foraging activity of small colonies of honey bees confined in insect-proof tunnels using video recording. Pollen foraging activity can be monitored with pollen traps (Reyes-Carrillo *et al*. 2007).

In some studies, syrup foraging rate was investigated by Paleolog 2009. Harmonic radar can also be used in recording the flight paths of foraging honey bee workers (Riley and Smith 2002; Riley *et al*. 2007). A standard protocol for monitoring foraging behaviour was presented by Scheiner *et al*. (2013) and other protocols for studying plant pollination by honey bees were reported by Delaplane *et al*. (2013). However, according to the objectives of a given study, any of the previously mentioned parameters can be used.

During the monitoring of foraging activity there are some important factors that should be taken into consideration including, the equal strength of the studied bee colonies especially the number of brood and pollen frames; the presence of any diseases in the studied colonies; the time of day and year; temperature and relative humidity as well as the presence of bee competitors or predators. Forager bees can be collected from the hive entrance by using forceps in front of the colonies as well as using an aspirator (Yucel and Duman 2005). Also, specific devices (e.g. Bee scan) can be used for counting forager bees (Scheiner *et al*. 2013).

Importance of foraging activity

Beside of the basic importance of foraging activity for honey bee colonies in collecting pollen, nectar, water and resin there are numerous reports of its importance for plant pollination (e.g., Young *et al*. 2007) especially for plants where honey bees are the primer pollinator. A vast number of species were found to be honey bee-pollinated plants including, highbush blueberry; apple and pears; almonds; Cantaloupe; rape varieties; and others (e.g. Boylan-Pett *et al*. 1991; Mayer and Lunden 1988; Reyes-Carrillo *et al.* 2007; Blazyte-Cereskiene *et al*. 2010). In a study by Sushil *et al*. (2013) found honey bees have a key role in increasing the seed production of three crops: broccoli, kohlrabi and Chinese cabbage. Also, an increase in the seed quality and quantity of onion, *Allium cepa*, cultivar Valencia was found (Yucel and Duman 2005).

Mishra *et al*. (2013) found other benefits besides pollination to be mediated by foragers; namely the deposition of nitrogen (in faeces) on plants during visits. They found about 2.27 to 2.69 g nitrogen per month as the mean production rate of bee frass by a 5000-bee colony. Forager bees also have the ability to distribute certain biocontrol agents including *Erwinia herbicola* Eh252 of fire blight onto apple flowers as well as onto nashi flowers (Cornish *et al*. 1998). To maximize the benefit of forager bees in spreading biocontrol agents, a new high-performance ‘Triwaks’ dispenser was developed (Bilu *et al.* 2004). The foraging activity of honey bees is very important as a bioindicator for indirect studies of environmental contamination with pesticides (e.g. Balayiannis and Balayiannis 2008). Foraging bees can even be trained using proboscis extension reflex conditioning for the detection of TNT. The foraging activity of honey bees has also been used to help monitor flowering plant species in an area. Foraging bees can also be used in the identification of pest infestation (e.g. fruit flies; Chamberlain *et al.* 2012). Beekeepers can benefit from the foraging behaviour of their colonies by fixing pollen traps or venom collection boards in front of hives to collect pollen or bee venom, respectively.

Foraging behaviour also has importance in computer science. It is known that forager bees can select their food sources in an optimal way although many food resources may be available (Thuijsman *et al*. 1995). Thus, honey bee foraging behaviour and related skills in food scouting and collection (Swarm intelligence) was used in computer science to solve many optimisation problems. Swarm intelligence is currently an important field in Artificial Intelligence (Kumar and Govindaraj 2013). Baig and Rashid (2007) presented an algorithm based on the swarming of honey bees called Honey Bee Foraging (HBF), which they proposed as useful for multimodal and dynamic nature optimisation problems.

Controlling foraging activity

It has been found that treatment with certain chemicals can enhance foraging activity. Pankiw (2004) found, using a suspended glass plate containing synthetic brood pheromone in isopropanol that colonies treated with this brood pheromone had higher ratios of pollen to non-pollen foragers entering colonies 1 h after the treatment. Mott and Breed (2012) found that bovine insulin treatments increased the threshold of the bees' sucrose response and significantly decreased the age at which foraging activity commenced for winter worker bees and summer nurse bees, respectively. Also, Schulz et al. (2002) found an earlier commencement of foraging in young bees in colonies treated with octopamine. Additionally, the pollination mediated by honey bees, *A. mellifera,* can be improved by the presence of other bee species in the orchards as found by Brittain *et al.* (2013) in California almond orchards. In addition, the use of modified beehives as demonstrated by Abou-Shaara *et al*. (2013), can improve foraging activity.

Similarly, Free *et al*. (1985a) found that treatment of oil-seed rape, field beans and sunflower heads with 2-heptanone and isopentyl acetate (honey bee alarm pheromones) were repellent to honey bee foragers. Kirk *et al*. (1995) found that the simulation of adult beetles using black spots on flower petals deterred nectar-foraging honey bees from landing on the flowers. Also, certain pesticides are repellent to honey bees.

CHAPTER III

MATERIALS AND METHODS

The present study on the pollen collection efficiency of *Apis mellifera* L. was done is Sirajgonj district. Three upazilla i.e. Ullahpara, Shahzadpur and Tarash were selected for the study. The experimental duration was 15 November 2015 to 15 January 2016. Peak mustard flower blooming period was selected for data recording. Data collection regarding the predetermined parameters and the analysis of data was performed to measures the foraging efficiency of honey bee. The materials required and the methodology of the application of treatments and determining various parameters are described under the following sub-headings:

Agro-forest situation:

The study was conducted during the mustard flower blooming season 15 November 2015 to 15 January 2016. Three apiary situated in Shahjadpur, Ullahpara and Tarash upazilla of Sirajgonj district and each of the site were consisting of 60, 101 and 75 bee hives respectively under the vegetation of jujube, mango, and palm trees. The apiary distantly surrounded by the lake and other tree plantation. There are some others herbs and shrubs are available surrounding the apiary. All these trees provide deep vegetation around. The main sources of nectar and pollen was mustard and the field of each area were abundant to support each of the bee colonies as well as apiary.

Honey bee (*Apis mellifera* L.) colonies:

To study the foraging efficiency of honey bee, *A. mellifera* L. colonies, 10 uniform hives of the same species were selected. Each hive or colony is consisting of 7 frames (2 brood frames + 3 occupied/2built frames) and a feeder frame. All the frames were considered for data collection.

Design and layout of the Experiment:

The amount of pollen hoarded by bee colonies was inferred from the amount of pollen loads which were collected by means of entrance-mounted pollen trapswith a 5.00 mm mesh perforated plate. Each time pollen was collected at the sametime from 9:00 to 16:00 on every 2nd or 3rdday, regardless of the weather on a trapping day. In total, during the experimental period 21 pollen load trappings were made to produce a total of 210 pollen samples.

Each pollen portion was weighed accurately, all samples were dried at 42°C (in an incubator) and pollen loads were counted.

Honey bee foraging

Field with abundantly growing mustard in Shahzadpur, Sirajgonj, having an apiary of *A. mellifera*, was selected for study. Observations were taken during full flowering season of the mustard. Abundance of *A. mellifera* bees (number of bees/ m^2 /min.) was noted from randomly marked plots of one square meter of this crop after every 10 minutes starting from 9.00 up to 16.00 hours on each alternating day for 30 days. On remaining alternating days, number of flowers visited/min.(forging frequency) was recorded with the help of stop clock at above mentioned fixed timings of the day. Data of windy days was recorded separately. Honeybees with maximum pollen load were captured with help of forceps during foraging activity. Pollen loads were collected from their pollen baskets with help of camel hair brush in watch glass and weighted with help of electronic balance. Collected data was consolidated, tabulated and analysed statistically. The values of various parameters given in results are the average values (along with standard Error) of all the days of observation.

Area of stored pollen

Pollen loads delivered by foragers from the field are deposited directly into comb cellsclose to brood nest, and then nurse bees use the stored pollen to feed the brood, while the excess pollen remains stored. A grid frame is commonly used to determine the quantity of brood, pollen and honey.

The determination of area of stored pollen was made by removing the combs from each hive in turn, and shaking all bees off each comb (each comb was carefully inspected for presence of the queen before shaking; when she was located the queen was transferred gently) into her hive box. Each comb was then placed on a modified artist's easel, andeach side was photographed with a digital camera. All photos were subsequently downloaded onto a computer, and image analyzing software (Image Proplus version 3.0, Media Cybernetics Inc., Silver Spring, Maryland, USA) was used toestimate the area (in cm^2) within a trace of the outline of the stored pollen in each image.

The sum of the pollen areas of all combs in a hive was regarded as the total area of storedpollen for that hive.

Trapping pollen

Traditional 6 hives and 6 Polyhive super were taken from each of the experimental hive to observe the total pollen yield (g) of the hive. Traditionally bee keepers do not harvest pollen and the traditional hives had not facilities to trap pollen. In the polyhive super there is an inside pollen trapping system and the trapped pollen were collected and weighed for the total yield of pollen/hive/season (g).

Parasite and predator management:

To avoid bee mites every hive was smoked with tobacco leaves 2 times in a week. To protect from ant attack, base of the hive stand were rubbed with thick Vaseline mixed with Finish detergent/insecticide. To protect from wasps and bee eating birds, a full time day labour was appointed to kill the wasps with badminton racket bat and drive out the birds by making sound with an empty tin container.

Statistical analysis:

The results were analyzed statistically using Statgraphics ver. 5.0 software. Arithmetic means and standard deviations were calculated. The differences were tested for significance by means of Duncan's multiple range test.

CHAPTER IV

RESULTS AND DISCUSSION

Foraging behaviour of *A. mellifera* on mustard flower

Honey bee forages on mustard flower to collect nectar and pollen. Data were observed from the morning 9.00 hour to 16.00 hour. It was found that number of bees/m²/min was highest (13) at 12.00 and 13.00 hours of day time whereas, the lowest (5) number of bees/m²/min was observed at 16.00 hours of day time (Table 1). Average number of bees/m²/min was low in the morning and it reaches in peak over the time and from 14.00 hours of day bee foraging declined.

Table 1: Foraging behaviour of *A. mellifera* on mustard flower

Day hours	9.00	10.00	11.00	12.00	13.00	14.00	15.00	16.00
No. of bees/m²/ min (average)	8±0.036	9±0.03	11±0.02	13±0.145	13±0.02	8±0.002	6±0.031	5±0.073
No. of flowers visited/ min (average)	10±0.001	17±0.02	21±0.06	25±0.039	25±0.06	21±0.04	18±0.03	12±0.036

Similar pattern of honey bee foraging was observed in terms of number of flowers visited/min (Table 1). The highest number of flowers visited/min of honey bee was observed from the morning 9.00 hour to 16.00 hour. It was found that number of flowers visited/min was highest (25) at 12.00 and 13.00 hours of day time whereas, the lowest (10) number of flowers visited/min was observed at 16.00 hours of day time

(Table 1). Average number of flowers visited/min was low in the morning and it reaches in peak over the time and from 14.00 hours of day bee foraging declined.

From this table it is concluded that foraging effieciency i.e. number of bees/m²/min and number of flowers visited/min was low in the morning and evening but at noon time it was high.

Average weights of single pollen loads harvested in 2015-2016 with the over successive periods (mg/piece) :

Average weight of single pollen loads were observed during the mustard blooming season 2015-2016. Among the three harvest period it was found that the second harvest period (8-13 days) obtained the highest (7.74mg/peiece) mean weight of individial pollen and the third harvest period (14-21 days) obtained the lowest (6.43 mg/peiece) mean weight of individial pollen (Table 2).

Table 2: Average weights of single pollen loads harvested in 2015-2016 with the over successive periods (mg/piece)

Harvest Period(days)	**Bee colony no.**										**$\bar{x}$**
	1	2	3	4	5	6	7	8	9	10	
1 – 7	6.37	7.46	7.19	6.98	7.28	8.90	8.43	7.20	6.58	7.49	7.39
8 – 13	6.78	8.33	7.73	7.89	7.16	8.32	8.15	8.27	7.34	7.43	7.74
14 – 21	5.01	7.36	5.51	6.91	5.96	6.10	7.75	7.45	6.48	5.74	6.43
$\bar{x}$	**5.68**	**7.75**	**6.39**	**7.11**	**6.67**	**7.25**	**8.06**	**7.64**	**6.77**	**6.61**	6.99
SD	1.05	1.39	1.33	1.08	1.23	1.67	1.07	1.12	1.07	1.23	1.24

Among the selected 10 bee colonies overall the highest (8.06mg) single pollen was observed in the 7th number of bee colony and the lowest (5.68mg) single pollen was observed in the 1st number of bee colony.

From this table it is concluded that foragers bee can load pollen from 5.68 mg to 8.06 mg pollen in her each pollen basket.

Average amounts of pollen harvested from individual bee colony

Pollen harvested from poly hive super with pollen trap in three different harvesting period was also observed in 10 bee colonies. Among the harvesting period, the highest (9.54 g/day/hour) amount of pollen was weighed in the second harvesting period (8-13 days). The lowest (3.2g/day/hour) amount of pollen was weighed in the third harvesting period (14-21 days) (Table 3).

Table 3: Average amounts of pollen harvested from individual bee colonies in 2015-2016 with the mustard flower blooming period being broken down into 3 stages (g/day) at 11.00 hour to 12.00 hour.

No. of colony	**Amount of trapped pollen(g/day)**									**Estimated pollen amount in the period**
	1 – 7(day)		8 – 13(day)		14-21(day)		Grand totals			
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	Total[1)]	$\bar{x}$	SD	
1	11.86	7.65	2.8	12.09	7.29	2.80	176.0	11.73	8.63	551.3
2	17.34	8.05	0.50	7.93	1.47	0.50	138.4	9.22	9.04	433.3
3	14.80	7.67	1.26	6.16	1.62	1.26	119.3	7.95	7.7	373.7
4	12.44	7.53	1.04	3.06	1.94	1.04	103.2	6.88	6.25	323.4
5	15.08	5.58	0.94	6.03	1.89	0.94	128.5	8.57	7.12	402.8
6	8.45	5.10	0.64	1.78	1.28	0.64	64.40	4.29	4.28	201.6
7	24.54	13.87	2.07	4.03	2.77	2.07	163.1	10.87	12.73	510.9
8	18.00	9.19	2.62	6.37	3.83	2.62	154.8	10.32	8.62	485.0
9	14.76	9.16	0.9	8.72	1.93	0.90	139.8	9.32	8.79	438.0
10	19.55	14.54	6.68	14.04	8.18	6.68	234.7	15.64	12.62	735.1
$\bar{x}$	5.68	8.86	9.54	7.04	3.20	1.97	142.2	9.50	8.61	446.5

1) - amounts of pollen from 21 trappings made during the mustard blooming period 2015-2016

Among the ten bee colonies the highest (510.9g) amount of estimated pollen was observed in the 7th number of colony and the lowest (323.4g) amount of estimated pollen was observed in the 4th number of colony (Table 3).

From the above table it may be concluded that second harvest interval or the mid time blooming of mustard flower produced highest amount of pollen. It might be for the maximum number of flowers were bloomed in the individual mustard plant in the blooming period.

Hourly variation of forager bee with pollen load :

Pollen loaded foragers were also observed in different hours of day. It was found that the highest ranges (7.7 to 11.25 and 7.25 to 8.85 respectively) of pollen loaded bee foragers were available at 11.00 hours to 12.00 hours and 12.00 hours to 13.00 hours. Rest of the hours had always lower number of bee count which ranges from 2.3 to 4.95.

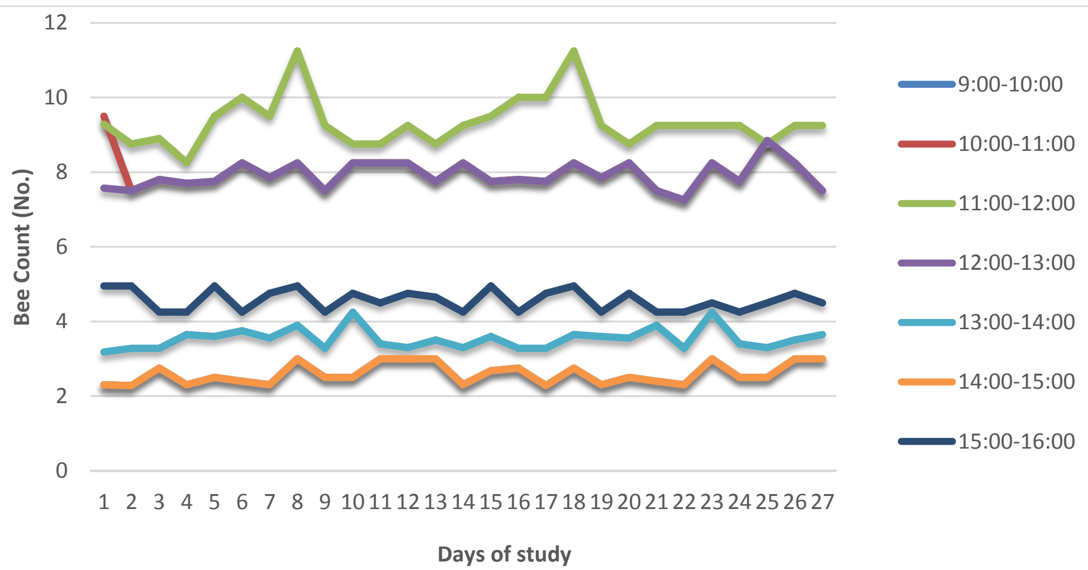

Figure 1. Hourly variation of forager bee with pollen load/minute

From the figure it is concluded that the best time of pollen harvesting during day is 11.00 hour to 13.00 hour.

Total foraging frequency/hour (with pollen load and without pollen load)

Foragers includes pollen collector, nectar collector, water collector and propolis collector. It was observed that all the collectors i.e. foragers activity was highest at 11.00 hours of day time (Fig. 3).

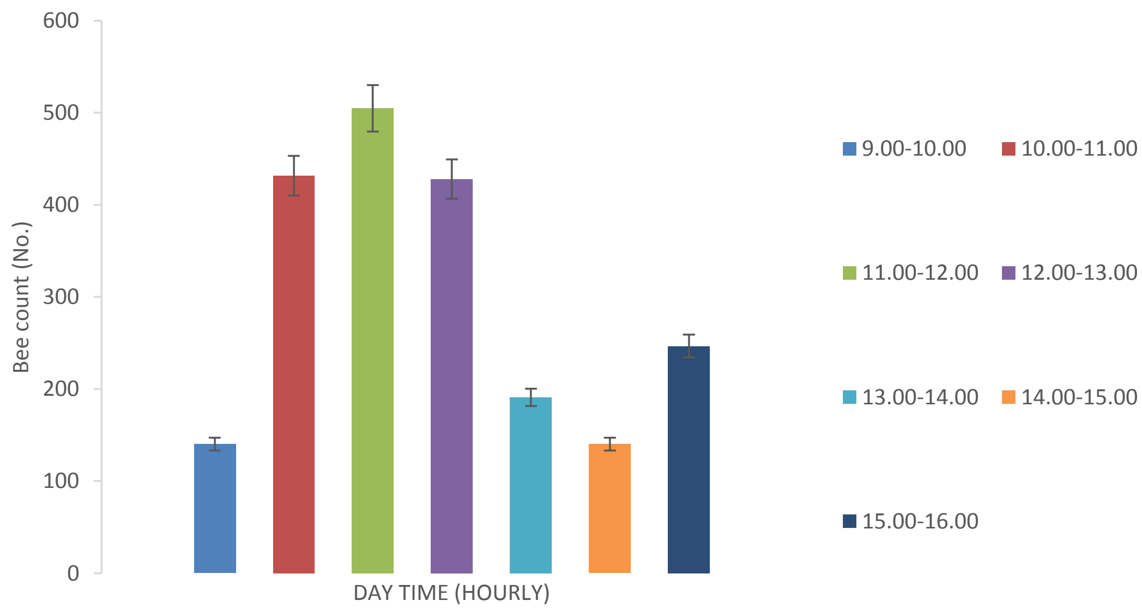

Figure 2. Total foraging frequency/hour (with pollen load and without pollen load)

Significantly the highest (504.86) number of forager at 11.00 hours and the lowest (140.18) at 9.00 hours of day time.

All the foragers of bees are actively move between 10.00 hours to 13.00 hours.

Bee entry and exit frequency from bee hive

Bee entry and exit from individual hive is important foraging behaviour of a bee colony. It was observed that bee exit and entry frequency of the hive is stable. The entry frequency ranged from 19.18 to 22.92 number/minute (Fig.3).

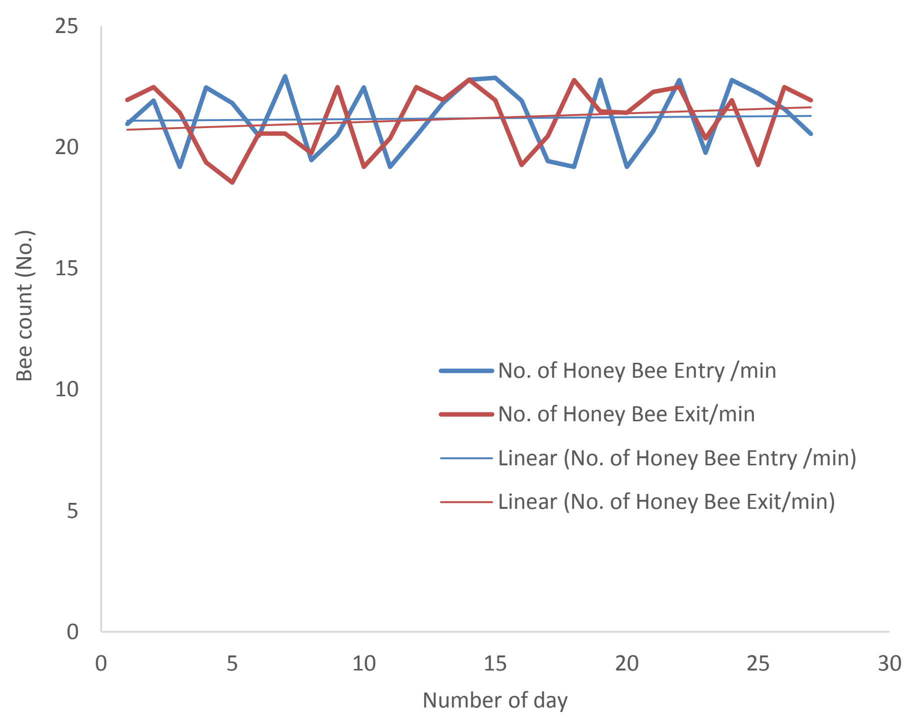

Figure 3. Bee entry/exit frequency

Again, It was also observed that bee exit frequency ranged from 19.26 to 22.78 number/minute (Fig.3).

From the above figure it is conluded that few number of bees were missed as the exit was lower than the entry. It might be the effect of bee drifting among the colonies.

Pollen yield/hive in three different locations of Sirajgonj

Three different sites of Sirajgonj district was abundant of bee pollen during the mustard flowering period. In traditional method no pollen was harvested and not a single colony was used by farmers for pollen collection. It was observed that in each site of Sirajgonj bee pollen can be harvested. It was observed that in the poly hive super the highest (136 g) pollen yield was obtained from site 2 i.e. Ullapara and the lowest (115.33g) pollen yield was obtained in site 3 i.e. Tarash (Fig. 4).

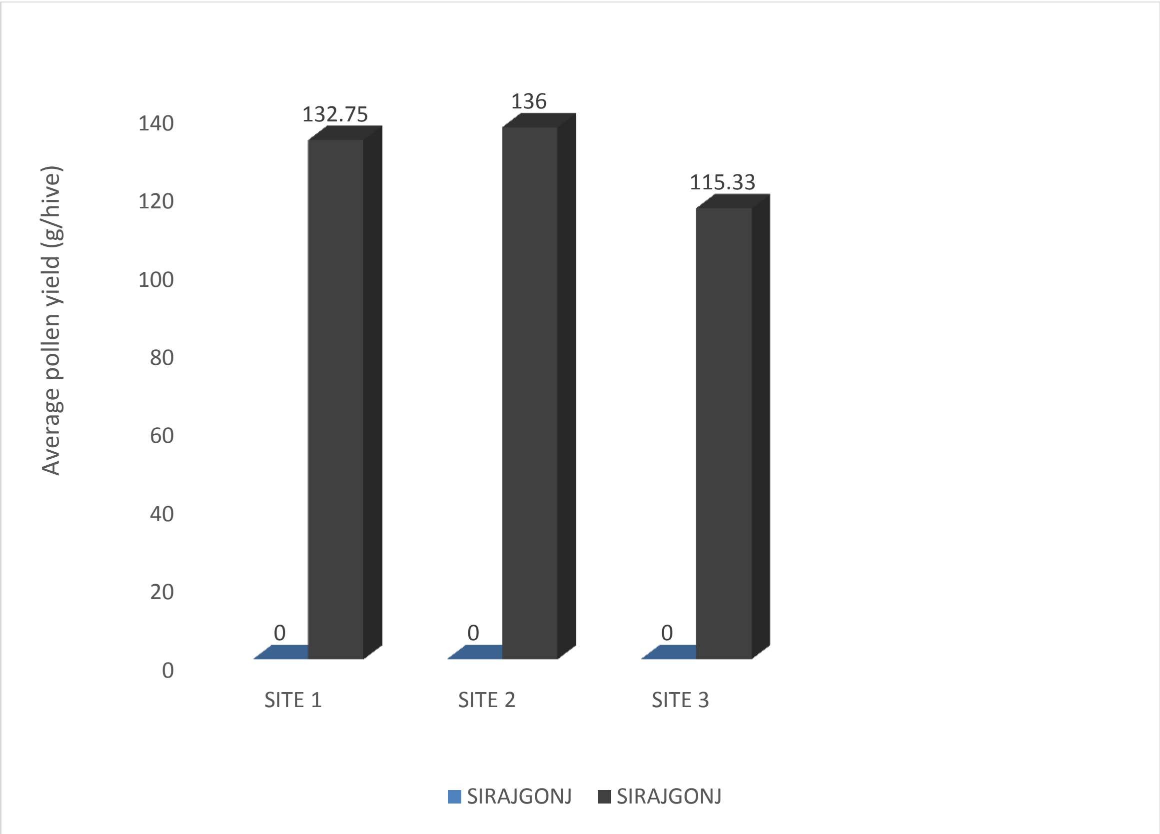

Figure 4. Pollen yield/hive in three different locations of Sirajgonj

There was no yield in traditional bee hives and it significantly differed from the modern poly bee hives with super.

Stored pollen inside the hive as bee bread

Stored pollen inside the bee hive was also observed in 10 number of colonies in two bee developmental life cycle. In each of the hives intital stored pollen was low in 1st week and it reaches at peak in the 2nd and 3rd week and then declined in the 4th, 5th and 6th week. The highest stored pollen area covered by bees was 380.6 cm²/colony in the seconds week.

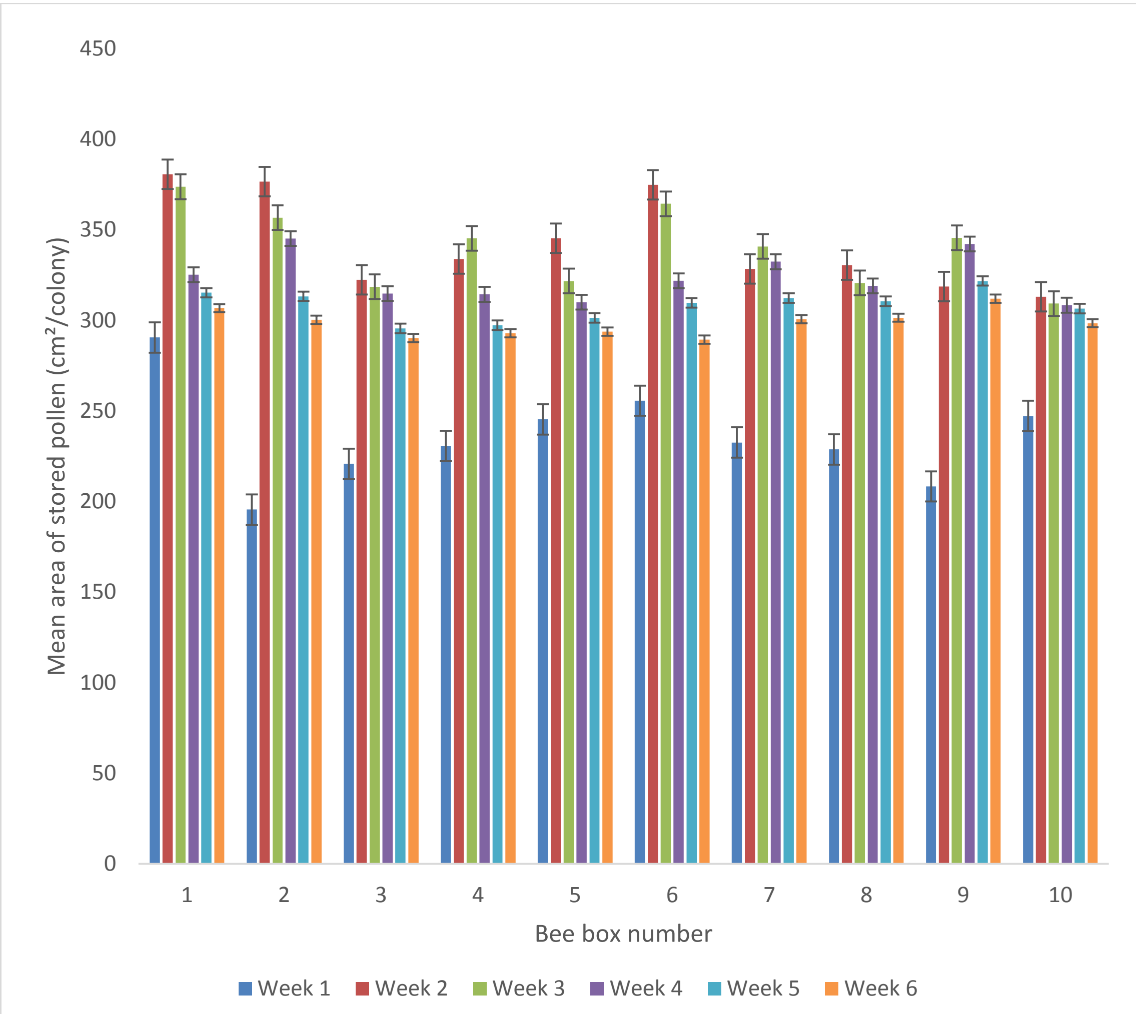

Figure 5. Stored pollen inside the hive as bee bread

The lowest stored pollen area covered by bees was 195.5 cm²/colony in the first week.

CHAPTER V

SUMMARY AND CONCLUSION

Honey bee foraging efficiency in mustard flower was studied to find out the foraging efficiency of honey bee in mustard flower at blooming period and to discover the yield of pollen in traditional and modern poly bee hive with super. It was found that during day time between the hours 11.00 and 13.00 the bee foraging was higher in comparison to other hour time of the day. Pollen harvest best time is also in 11.00 hour of day time. Again the modern poly hive super is signicantly efficient in terms of pollen production.

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