COMPARATIVE STUDY OF POLLINATION METHOD ON BOTTLE GOURD (*Lagenaria siceraria* L.) YIELD

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COMPARATIVE STUDY OF POLLINATION METHOD ON BOTTLE GOURD (*Lagenaria siceraria* L.) YIELD

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Dedicated to
My Beloved Parents

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CERTIFICATE

This is to certify that the thesis entitled, ***"COMPARATIVE STUDY OF POLLINATION METHOD ON BOTTLE GOURD (Lagenaria siceraria L.) YIELD"*** *submitted to the Department of Entomology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE (M.S.) IN ENTOMOLOGY,*** *embodies the result of a piece of bona fide research work carried out by* ***KAMRONNAHAR KHANAM RIMA*** *bearing* ***Registration No.11-04522*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma in any other institution elsewhere.*

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

Dated: June, 2017
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ABSTRACT

In Bangladesh, bottle gourd *(Lagenaria siceraria* L.*)* is the most important vegetable among different kinds of winter vegetables because of its nutritive value and economic return. The present study was designed in the experimental field of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka during December 2016 to May 2017, to determine the effect of different modes of pollination on fruit and seed characters, foraging behavior of major bee pollinators, various insect visitors and their relative abundance. The experiment was laid out in Randomized Complete Block Design (RCBD) with eight (8) replications and three (3) treatments (without bee pollination, hand pollination, and open pollination). A total of 12 insect species were recorded as visitors in bottle gourd ecosystem. Among them, *Formica sp*. was most abundant followed by *Therioaphis trifolii*, *Apis mellifera*, *Halictus sp*. and *Bactrocera*. *Cucurbitae*, but Syrphids were least abundant. The activity of insects was peaked between 8.00am-9.00am followed by 11.00am-12.00pm and 2.00pm-5.00pm. Least activity was recorded at night 8.00pm-9.00pm . The pollen or nectar foragers proportion of *Apis mellifera* revealed that there was significantly more number of nectar foragers (6.16/m^2/10 min) as compared to pollen foragers (5.27/m^2/10 min). The pollen foragers were highest during morning (6.75/m^2/10 min) whereas the nectar foragers were maximum during noon (6.74/m^2/10 min). The highest percentage of fruit set was observed in hand pollination (71.52%) while the lowest in open pollination (60.85%) and without honey bee pollination (45.65%). Misshapen fruit percentage was highest in without honey bee pollination (23.35%) while the lowest in open pollination (21.87%) and hand pollination (15.01%). Significantly lowest percentage of healthy fruits (78.25%) resulted in without honey bee pollination. Similarly the fruit weight (2200.54 g), number of seeds per fruit (185), fruit diameter (60.8 cm), fruit length (89.7 cm) and weight of 1000-seeds (88.3 g) were found maximum in hand pollination among the three form of pollination as compared to open and without bee pollination.

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LIST OF COMMONLY USED ABBREVIATIONS

FULL WORD	ABBREVIATION
And others	*et al.*
Bangladesh Bureau of Statistics	BBS
Bangladesh Agriculture and Research Institution	BARI
Cultivar	cv.
Degree Celsius	^{o}C
edest (means That is)	i.e.
Figure	Fig.
Gram	G
Micro gram	Mg
Micro mol	µM
Milligram/litre	mgL^{-1}
Namely	Viz.
Parts/million	Ppm
Percentage	%
Seconds	Sec
Species (plural number)	spp.
Variety	var.

CHAPTER I

INTRODUCTION

Bottle gourd (*Lagenaria siceraria* L.) belongs to the family Cucurbitaceae having chromosome number 2n = 22. It is native in South Africa. In Bangladesh, the fruit of bottle gourd is called lau or in the Chittagong and Sylhet region kodu or xodu and is served with rice as a common dish. It is one of the most important cultivated cucurbits in Bangladesh. It is grown in summer season as well as in rainy season (Yadav *et al.,* 2010).

The bottle like shape of fruit and used as container of wines and spirits in the post gave it, the common name bottle gourd. It is monoecious, highly cross pollinated, annual vine with soft tendril. The amount of cross pollination ranges from 60 to 80% (Choudhary, 1979). The shape of bottle gourd fruits are cylindrical, round oval and oblong.

Bottle gourd generally is a winter crop, possibly tropical or sub-tropical in origin, rich in Carotene, Calcium and Vitamin C. In Bangladesh it is cultivated in about 10,000 ha, producing about 62,000 m tons; grown by direct sowing or transplanting 15 to 20 days old seedlings. Crops are ready for harvest within 55-60 days; yield ranges from 35-40 m tons/ha. Some varieties of bottle gourd are BARI Lau-1, Khet lau and Hazari. Bottle gourd is primarily used in making curry with different fishes. Its leaves and vines are also used as vegetables. The tender bottle gourd is used to prepare sweet dishes with sugar and milk.

It is an economically important crop cultivated worldwide for vegetable purpose or medicinal purpose. It is also used for decorative purposes as a bottle, utensil or pipe when it matures. Numerous health benefits are reported in bottle gourd including its anti-cancerous, cardio protective, diuretic, aphrodisiac, general tonic, antidote to certain poisons and scorpion stings, alternative purgative and cooling effects (Badmanaban and Patel, 2010).

It can also be used to cure pain, ulcers and fever and is used for pectoral cough, asthma and other bronchial disorders using prepared syrup from the tender fruits. Bottle gourds are known to lower cholesterol, triglyceride, low density lipoproteins, pain and inflammation (Ghule *et al.,* 2006), free radicals and oxidation (Deshpande *et al.,* 2008; Kubde *et al.,* 2010).

Hundred grams of tender fruit contains 96 g water, 0.2 g protein, 0.1 g fat, 2.5 g carbohydrate, 0.6 g fiber, 0.5 g minerals, 12k.cal, 20 mg calcium, 10 mg phosphorus, 0.7 mg iron, 0.3 mg thiamine, 0.01 mg riboflavin and 0.2 mg niacin (Gopalan *et al.,* 1982). The seed kernels contain 45% oil, and about 35% protein. Their seeds are good sources of lipids and proteins (Achu *et al.,* 2005; Loukou *et al.,* 2007). The fruit make delicious supplement to the human diet but the contents are considered of little nutritive value. It is grown for its tender fruits, basically used as vegetable. The tender edible fruits are also prepared into sweets, pickles, and other delicious preparations. The dried fruits are used as containers, utensils, fishing floats and some musical instruments.

Bottle gourd major producing countries are India, Sri Lanka, Indonesia, Malaysia, Philippines, China, Hong Kong, Tropical Africa, Colombia, and Brazil. In India it shares area of 107.89 thousand ha and production 2052.23 thousand metric tons. The sex expression, sex ratio and fruit set are one of the most important problems in cucurbits. Most of the cucurbitaceous crops are monoecious in nature; bear more male flowers and less female flowers separately on the same plant. Female flower appears later. Growth, flowering and sex expression is generally influenced by climate, soil moisture, nutrient application and management practices.

Bottle gourd mainly depends on insects for pollination as the male and female structures do not occur in the same flower and pollen grains are large and sticky to be carried by wind. The mechanical transfer of pollen is essential to bear fruits (Free, 1993; McGregor, 1976). The male flowers usually appear first and produce nectar and pollen, whereas female flowers produce more nectar and there by attract more bees compared to male flowers (Nepi and Pacini, 1993). Bottle gourd flowers open at night and usually last only one night. Pollen viability in a newly opened male flower is about 92 percent but by the time it closes that same morning the viability will be 75 percent and by the next day it will be only 10 percent (Nepi and Pacini, 1993). Thus, it is important for a female flower to be pollinated as early as possible on the day it opens while pollen is still viable. Hence, pollination especially insects are the important agents for successful fertilization. Utilization of pollinators is considered as the one of the cheapest and eco-friendly approach in maximizing the yield of cross pollinated crops (Free, 1993).

Amongst Cucurbitaceae, bottle gourd, *Lagenaria siceraria* have important contribution for the overall popularity. Being a monocious crop, bottle gourd is strictly cross pollinated and mainly depends on various pollinating agents, essentially insects for its pollination.

In view of the above aspects the present investigation entitled, “Comparative study of pollination method on bottle gourd yield” has been undertaken with the following objectives.

1. To identify various types of insect visitors and their abundance in bottle gourd flowers.
2. To study the effect of different modes of insect pollination on fruit yield of bottle gourd.

CHAPTER II

REVIEW OF LITERATURE

Bottle gourd is one of the most important vegetable crops grown in all parts of Bangladesh. It is a leading commercial crop grown widely throughout the tropical and sub-tropical parts of the world. But in Bangladesh research effort on bottle gourd seem to be poor. In order to increase production many factors influence the yield and quality of bottle gourd crop. One of the most important considerations is successful pollination. It is a well-documented fact that bottle gourd requires insect pollination to set fruit. Insect visitors of bottle gourd are numerous but not all of them are important pollinators. Of all the insect visitors mainly honey bees are major pollinators of bottle gourd (Connor and Martin, 1969; Woyke and Brownikowska, 1984). Honey bees adopt their foraging behavior according to the floral structure of the crop and factors like corolla shape, color and its attractiveness, rewards as pollen or nectar or both, concentration and amount of nectar etc. Development of distinct male (staminate) and female (pistillate) lines in recent years has accentuated the need for bee pollination (Lord, 1985).

Therefore, relevant information available in the literature pertaining to the pollination and comparative studies of bottle gourd were reviewed in this section. Moreover literatures related to the efficient multivariate techniques for diversity analysis were also reviewed in the following headings.

2.1 Diversity of insect visitors

Morimoto *et al.* (2004) reported that 22 species of four orders were the major pollinators of bottle gourd in Kenya. Kauffeld *et al.* (1978) collected insects from cucurbits, which belonged to 37 species (29 were identified). Honey bees collecting nectar were the most numerous visitors, and few of them carried pollen. Cervancia and Bergonia (1991) observed that most common visitors of cucumber were *Xylocopa chlorina*, *Xylocopa philippinensis*, *Megachile atrata and Apis dorsata*. They were most abundant from 10.00-11.00 h. Sajjanar *et al.* (2004) found that a total of 24 species of insects visited the cucumber flowers in which hymenoptera were predominating. Among honey bees, *A. dorsata* was the most frequent visitor. Rana *et al*, (2006) found that main visitors of cucumber were small ants (15.7), followed by *Apis mellifera* (4.32), bumble bees (3.34), *Apis cerana* (1.8), Nomia (0.96) and Syrphids (0.78).

Avila *et al.* (1989) studied the time of effective pollination in fields producing hybrid seed of squash (*Cucurbita pepo*) and observed that *Apis mellifera* was the most abundant insect pollinator in the morning, followed by *Trigona spinipes* and *Chrysomelid Diabrotica speciosa*.

Brett and Sulivan (1972) observed several species of solitary bees, visiting the flowers of *Citrullus lanatus*, watermelon but it was observed that the honey bees were the principal pollinators.

Njoroge *et al.* (2004) studied pollination ecology in *Citrullus lanatus*, which is a species vulnerable to pollination loss and observed that this species depends heavily on the honey bees (*A. mellifera*) for pollination. Other pollinators identified include Xylocopa bees, Halictid bees, Hypotrigona bees, flies and beetles.

Free (1993) reported that the insects such as ants, thrips, beetles and solitary bees have been identified as possible pollinators of cucurbits but, it is generally recognized that honey bees (*Apis mellifera*) were the most important pollinators in commercial crop production. Rust *et al.* (2003) collected 6 families, 15 genera and 43 species of bees on *Ecballium elaterium* (cucurbitaceae). Numerically dominant species were *Lasioglossum malachurum*, *Apis mellifera* and *Ceratina cyanea*.

Grewal and Sidhu (1978) observed the insect visitors of cucurbits in Punjab and found that *Apis florea*, *Apis dorsata*, *Apis mellifera* and solitary bees, were main visitors of the crop.

Grewal and Sidhu (1978) reported that honey bees *Apis florea* and solitary bees of Halictidae were the most abundant bee visitors (65 and 23%, respectively) of muskmelon in Punjab. Rao and Suryanarayana (1988) found that *Apis cerana* comprised 87% of the pollinating insects of watermelon; the others included *Apis florea* and *Trigona iridipennis*.

Jaycox *et al.* (1975) observed, honey bees, black solitary bees, or bumble bees visiting pumpkin (*Cucurbita moschata*) flowers. *Apis florea* and *Apis dorsata* were the most abundant bees visiting *Cucurbita pepo*, solitary bees belonging to the Anthophoridae, Xylocopidae, Megachilidae and Halictidae were also present (Grewal and Sidhu, 1978). *Apis spp* were also found to be the most important pollinators of *Cucurbita pepo* in Bangalore, India (Girish, 1981); the relative proportion of *Apis cerana*, *Apis dorsata* and *Apis florea* present were 87:10:3, respectively, the number of solitary bees was negligible.

Joseph (2005) and Behera *et al.* (2010) suggested that for a commercial fruit and seed production, pollination management for this crop is essential and the use of hand pollination or the introduction of honey bee colonies in enclosures in India is recommended.

Kumar (2002) reported that 12 insect species belonging to eleven families under four orders were found visiting the blossoms of wanga, tinda and cucumber at Hissar. Nepi and Pacini (1993) observed that most gourd flowers open at night and usually last only one night. Pollen viability in a newly opened male flower is about 92 percent but by the time it closes that same morning the viability will be 75 percent and by the next day it will be only 10 percent.

Malerbo *et al.* (1999) reported that most frequent insects on watermelon flowers were ants (37.2%), followed by stingless bees *Melloons sp*. (32%), *Trigona sp.* (9%), flies (9%), Beetles (77%) and Butterflies (5.1%).

Tsyganov *et al.* (1953) considered one bee equal in value to 11,000 thrips as pollinators of cucurbits. McGregor and Todd (1952) observed other insects (e.g. native bees, thrips and ladybird beetles) on the flowers, but found their activity was not conducive to pollination and obtained no evidence that they contributed to fruit set. Skrebtsova (1964) stated that honey bees are the only pollinators present in many U.S. fields and represent 84-96 percent of insect pollinators on cucumber.

2.2 Foraging behavior of insect visitors

2.2.1 Proportion of nectar and pollen foragers

Buchmann (1983) stated that tomato flowers do not produce nectar, so pollen is the only resource provided to bees as a floral reward. McGregor and Todd (1952) observed that pollen and nectar collection in cantaloupe by the honey bees, both in cages and outside usually began as soon as the flowers opened. Pollen collection reached a peak by about 11 a.m. and tapered off rapidly after midday with little collection after 2 p.m. Nectar collection also reached a peak about 11 a.m. and tapered off much less rapidly with some activity as late as 6 p.m.

In most of these species, staminate flowers offer nectar and pollen while the pistillate flowers offer only nectar as floral rewards to pollinators (Free, 1993). Shemetkov (1960) in Russia and Amaral *et al.* (1963) in Brazil reported that bees collected cucumber pollen heavily from 8 to 10 a.m. and nectar from 10 a.m. to noon. Bees collected pollen on cucumber in early morning and switched to nectar later in morning. Few honey bees collected pollen from cucumber flowers and that also in small loads. Nectar was the prime attractant (Collision and Martin, 1975).

Sajjanar *et al.* (2005) observed that under caged conditions, pollen foragers of *Apis cerana* initiated activity by 06.00 h. The activity was at a peak (6 bees/m^2/5 min) by 10.00 h and then declined gradually till 18.00 h whereas, nectar foragers initiated activity by 07.00 h remained in low number initially but picked up activity by noon to attain a peak by 13.00 h at 6.89 bees/m^2/5 min followed by gradual decrease in activity.

The presence of ruderal plant species in the vicinity of the crop or among the tomato plants which offer this resource (nectar) becomes an additional attraction to these agents (Gaglianone *et al.,* 2015). *Apis cerana, Apis florea and Melipona spp*. started collecting watermelon pollen from 8.30 h and reached a peak in numbers on the crop at 10.30 h (Bhambure, 1958). The watermelon flowers were fully opened by 7.00 h and most were fully closed by 14.00 h. Peak pollen collection occurred at 9.00 h and decreased thereafter (Rao and Suryanarayana, 1988).

Selcuk *et al.* (2010) observed that lepidopteron preferred to forage on yellow (29%) flowers, followed by pink (28%) and white colored flowers (19%). Sanduleac (1959) found that cultivars of *Cucurbita maxima, C. pepo and C. moschata* were worked intensively by bees from 06.00 to 12.00 h daily and the numbers of bees reached a peak between 08.00 and 09.00 h, the male flowers were preferred to female flowers indicating that they were collecting pollen deliberately. Hernandez and Lemus (1999) observed that honey bee activity on pumpkin was greatest from 9.00 to 10.00 h foraging both for nectar and pollen.

2.2.2 Floral preference of honey bees

73% of cultivated species relies on the action of the bees for pollination. Their action can influence the quality of fruits and seeds produced (FAO, 2004). Amaral *et al.* (1963) concluded that bees show no preference for staminate over pistillate flowers in cucumber. Connor (1969) stated that even when honey bees visit staminate flowers, the primary objectives is to collect nectar and that cucumbers are visited for pollen largely when other sources of pollen are absent. Stephan (1970) also reported that bees get very little pollen from cucumber.

Honey bees showed a significant preference for pistillate flowers in *Cucurbita pepo* which increased the chance of pollination. *Peponapis pruinosa* preferred staminate flowers. However, *Peponapis pruinus* worked the flowers more rapidly than honey bees (Tepedino, 1981). Rust *et al.* (2003) observed that most visits (97%) on *Ecballium elaterium* were to staminate flowers. Observation of foraging by honey bees (*Apis mellifera*) showed that they began to visit *Cucurbita pepo* flowers as soon as they opened with a foraging peak at 7.00-9.00 h, male flowers were visited first, but female flowers received more visits (Nepi *et al.*, 1996)

2.2.3 Foraging rate and foraging speed.

Shrivastava and Shrivastava (1991) studies on white flower gourd/bottle gourd (*L. siceraria*) crop revealed that flower visitors mainly recorded during night time included lepidopteron and coleopterans.

In U.S.A. honey bee foraging increased rapidly from 8 a.m. to peak activity at midday then sharply fell to low levels by 4 p.m. Most activity was confirmed between 10 a.m. and 2 p.m. (Connor and Martin, 1970). In USA most honey bees visits to cucumber crops occurred between 9.00 and 16.00 h with a peak between 11.00 and 12.00 and a secondary peak between 14.00 and 15.00 (Kauffeld and Williams, 1972; Collision and Martin; 1979). The first visit a flower received after opening was of longer duration than later visits; thereafter the time per flower visit tended to decrease during the day, reflecting the amount of nectar present, with a mean of 11.4 sec (Connor *et al.,* 1975).

Rapp (1981) reported that honey bees started foraging on cucumber flowers at about 6.00 h and activity was highest from 9.00 to 12.00 h then it decreased early in the afternoon. The frequency of bee visits to pistillate was lower than to staminate, but the duration of visit was longer in pistillate (8 to 16 second), than on staminate (4 to 10 seconds). Cervancia and Bergonia (1991) observed high activity of pollinators from 10.0-11.00 h. They concluded that pollinators were most active when nectar secretion was highest. The lesser the time spent by a bee per flower, the greater its chance to pollinate more flowers.

Honey bee began to visit the flowers soon after they opened and were most numerous on fields between 8.00 h and 10.00 h; thereafter, they became steadily fewer until the flowers had closed (Goff, 1937; Adlerz, 1966). The duration of bee visits was different on male and female flowers. In 441 observations made in 1959, the longest single visit to a female flower was 20 sec and the mean visitation time was 5.7 sec. In 1003 observations on female and 989 observations on male flower made in 1960, the longest visits were 60 and 27 sec, and the mean visitation times were 8.0 and 5.7 sec, respectively, (Adlerz,1966). The time *A. cerana* pollen foragers spent per flower of watermelon, increased from 1-4 sec at 8.00 h to 8.2 sec at 12.00 h. The time spent in collecting nectar from a flower was less for staminate than pistillate flowers (Rao and Suryanarayana, 1988).

Nepi and Pacini (1993) observed that the male flowers usually appear first and produce nectar and pollen, whereas female flowers produce more nectar and there by attract more bees compared to male flowers.

Girish (1981) reported that there was very little difference in the time spent by bees in collecting nectar from pistillate and staminate flowers of *Cucurbita pepo*. However, the time spent tended to decrease from the time of the flower opening to closer. The time spent by a bee collecting nectar varied widely; the average time for female was 70 sec and for male 65 sec. The average time spent by pollen collectors on male flowers. In 441 observations made in 1959, the longest single visit to a female flower was 20 sec and the mean visitation time was 5.7 sec. In 1003 observations on female and 989 observations on male flower made in 1960, the longest visits were 60 and 27 sec, and the mean visitation times were 8.0 and 5.7 sec, respectively, (Adlerz, 1966).

The time *A. cerana* pollen foragers spent per flower of watermelon, increased from 1-4 sec at 8.00 h to 8.2 sec at 12.00 h. The time spent in collecting nectar from a flower was less for staminate than pistillate flowers (Rao and Suryanarayana, 1988). The average time spent by pollen collectors on male was 14 sec (Couto *et al.*, 1990).

Stanghellini *et al.* (2002) compared the activities of bumble bees and honey bees (*A. mellifera*) on field grown cucumber and watermelon and observed that bumble bees started foraging activity 15-40 minutes before *A. mellifera*; both species continued foraging until flowers closed in early afternoon. *B. impatiens* consistently visited more flowers per minute. Rana *et al.* (2006) observed that there was no varietal significant difference in number of cucumber flowers visited per minute or foraging time per flower by honey bee, *A. mellifera*. However, in the morning hours the bees spent significantly more time (10.95 sec) and visited less number (3.95 sec) of flowers as compared to noon and evening hours.

2.3 Amount of nectar sugar

Sihag (1990) stated that bottle gourd flowers were visited by *Xylocopa fenestrata* F., a dipteran, for pollen and nectar and acted as a good pollinator.

McGregor and Todd (1952) observed that the melon flowers opened between 7 and 8 a.m. and nectar secretion began at once. About 3 milligrams of nectar was produced by 11 a.m. after which secretion apparently ceased in the staminate flower. In the hermaphroditic flower secretion continued up to the afternoon, a total of 18 milligrams being produced. Shuel (1961) observed that nectar secretion in flowers is influenced by the maturation of stigma and stamens and also often by the age of flowers and is usually greater on the first day after the flower is open than later. The age and condition of flower also have an important effect on the secretion of nectar.

Collision (1973) found that in cucumber, *Cucumis sativus* L. maximum secretion occurred on the day of anthesis. Most flowers secreted no nectar on the second day. When male and female flowers occur on the same plant, one kind may secrete more nectar than other. Female flowers secreted more nectar than male flowers. Nemirovich-Denchenko (1964) reported that the average daily nectar yield of female and male flowers was 1.29 and 0.69 mg, respectively, and was greatest 3- 4 h after opening. Kaziev and Seidova (1965) found that a female cucumber flower secreted between 1.1 and 2.4 mg of nectar compared to between 0.9 and 1.6 mg by a male flower, the amount secreted depending to some extent upon the cultivar and environmental conditions.

Kropacova and Nedbalova (1974) observed that on an average a bee visited 4-5 blossoms/min and a flower was visited on average 28 times. The average sugar content in nectar was 2.3 mg/flower/day.

Free (1993) found that utilization of pollinators is considered as the one of the cheapest and eco-friendly approach in maximizing the yield of cross pollinated crops.

Kamler and Tronickova (1982) in Czechoslovakia found significant differences in nectar production between different monoecious cultivars of cucumber which on an average yielded 1.36 mg sugar per flower per day; in contrast flowers of gynoecious cultivars only 0.57 mg sugar per day. The average sugar content in cucumber nectar was 57.6±3.3 mg/flower.

Nepi *et al.* (1996) studied the nectar structure, nectar secretion, composition and insect visits in flowers of *Cucurbita pepo* cv. *Greyzini* in which anthesis lasts for only 6 hours. The nectar of female flowers were more abundant and richer in sugars and proteins than that of male flowers and was therefore more attractive to insect visitors. Flowers whose nectar was collected by bees fell the day after anthesis; unvisited flower fell after 3 days. Couto *et al*. (1990) reported that the ratio of male (M) to female (F), *Cucurbita pepo* flowers was over 7, but F produced 2.5 times more nectar and its sugar content was more than twice that of M nectar.

Nectar traits were compared between male and female flowers of *Cucurbita maxima* to determine any difference in the characteristics of the main reward offered to pollinators. Nectar chemical composition and sugar proportion were similar between flower types. Total nectar sugar production per female flower was threefold higher than per male flower and nectar removal did not have any effect on total nectar production in both flower morphs (Ashworth and Galetto, 2002).

2.4 Pollination requirements

Morimoto *et al.* (2004) from Kenya who reported that lepidopterans were the major pollinators of bottle gourd (*L. siceraria*).

Tarbaeva (1960) reported that melon stigma was more receptive for pollination 3-4 hours after anthesis. Nandpuri and Brar (1966) observed that in case of muskmelon maximum stigma receptivity prevailed 2 hours before anthesis and 2-3 hours after anthesis. Safarajan (1966) studied the effect of the age of stigma on fertilization in watermelon and obtained highest fruit set (37-44%) when newly opened flowers were pollinated between 7-8 a.m. both in intra varietal pollinations. He further observed that for getting maximum fruit set fresh pollen should be used.

Shakti *et al.* (1990) observed that anthesis in cucumber started at 5 a.m. and was complete by 8 a.m. with the maximum anthesis occurring between 6 and 7 a.m. The maximum anther dehiscence and stigma receptivity also occurred between 6 and 7 a.m. Stigmas become receptive 12 h after anthesis and remained so 24 h after opening of flowers at moderate temperature (Singh *et al.*, 2004).

The flowers of cucumber remained open only for one day. If they were not pollinated at that time the flowers aborted and dropped from the plants. When pollination occurred but was not complete fruit did not develop properly (Hodges and Baxendale, 1991).

Connor (1969) found that the best time of the day for effective cucumber pollination in Michigan was from 10 a.m. to 3 p.m. He also found that pollination was about equally effective when the pollen was placed on one or all the stigma lobes.

Pollination was adversely affected by high temperature and low humidity. Pollination after dehiscence up to 08.00 h was stated to be most effective (Seshadri, 1986). Pollination even after 24 hours were found effective in greenhouse conditions and no differences were found in seed set between flowers pollinated on the day of anthesis or those pollinated the following day (Munger, 1988).

The pollinators' action is extremely important in agricultural crops, being directly or indirectly responsible for about 1/3 of the food production consumed by human worldwide (Klein *et al.*, 2007, Ollerton *et al.*, 2011). Fruit and seed set in insect pollinated agricultural crops rely primarily on honey bees because of their ease in management and transportation. Gingras *et al.* (1997) observed that honey bees (*Apis mellifera*) were almost only pollinator to visit cucumber flowers in open pollinated plots in Quebec (Canada). And they also found that open pollinated plots produced significantly more fruits with superior weight and pollination rates than caged plots. Seyman *et al.* (1969) reported the importance of honey bees in cucumber production by obtaining increased fruit yield with increased exposure to bee activity. Introductions of honey bee colonies were recommended where populations of native insect pollinators were low (Cervancia and Bergonia, 1991).

In many fruit and vegetable crops the number of bee visitation can be the limiting step in obtaining optimal yield (Wolf *et al*., 1999). Shemetkov (1957) reported that the number of visits a flower received influenced the number of seeds and weight of fruit produced in cucumber; thus 2-8 visits per flower gave fruits of 221 g average weight and 60 seeds, and 50 visits per flower gave 500 g fruits with 140 seeds. Adlerz (1966) observed that *A. mellifera* visits were minimum for normal fruit development in cucumber.

Jaycox *et al.* (1975) found that as the number of bee visits increased from 1 to 12, the percent of fruit set in pumpkin increased from 6.5 to 64.5, or about a tenfold difference. The mean number of seeds and mean weight of the pumpkin also increased. Honey bee activity in commercial pickling cucumber fields should provide each flower on the day of anthesis with 15 to 20 flower visits, to achieve maximum fruit set for machine harvesting. A significant positive correlation was obtained between daily per cent fruit set and amount of pollen being distributed with each bee visit (Collision and Martin, 1975).

Collision (1976) and Stanghellini *et al.* (1997) have demonstrated that pistillate (female) cucumber flowers require 12 or more honey bee visits to set marketable fruit.

Gingras *et al.* (1999) observed that a single visit to a cucumber flower was sufficient, but flower that had the greatest number of visits and highest cumulative duration of visit also had the greatest cucumber yields. Prakash *et al.* (2004) also studied the effect of number of bee visits on fruit set and some fruit characters of cucumber and observed that a minimum of 10 bee visits are necessary to minimize the flower drop and increase fruit set rate and a minimum of 20 bee visits to get more fruit weight, fruit volume and number of seeds per fruit.

Musiiko (1941) indicated that hand pollination in cucumber using a small brush with a head of cotton at the end gave 30-35 percent higher fruit set. Tuljzenkova (1955) showed that glass house grown cucumbers when pollinated by bees, produced higher yield and better quality fruits than those produced by hand pollination. Stambera (1962) observed that in open set, bees were the excellent pollinators and increased yield and quality of cucumber seeds. Steinhauer (1970) reported that honey bees increased cucumber yield by 39 percent as compared to the fields where bee activity lacked. Sevgican (1976) pointed out that hand pollination in cucumber resulted in earliness and improved fruit quality. Robinson and Hefferman (1980) stated that maximum seed set was obtained when pollen was applied generously to whole stigmatic surface.

Kauffeld *et al.* (1975) obtained increased quantity and quality of cucumber (*Cucumis sativus* L.) fruits with honey bees in caged vs. field studies. Woyke and Brownikowska (1984) observed that the number of honey bees on fields where hives had been introduced was from 1.5 to 5.0 times as high as on fields without hives; on the later fields honey bees constituted between 23 and 81% of total insects. Significant correlation coefficients were found between cucumber yield and the number of honey bees foraging on the flowers.

Alex (1957 b) reported that the calculated average yield of three plots in a cucumber field caged without honey bees, three plots caged with honey bees and three plots not caged was 80, 409 and 472 kg, respectively. He attributed the set in the cages without honey bees to pollination by small ground nesting solitary bees. The mean yield, calculated in kilograms per hectare, during five years of a plot, caged without honey bees, a plot caged with honey bees and a plot not caged were 1754, 4683 and 5787 respectively. Kauffeld and Williams (1972) in Wisconsin, USA, found that plots of cucumbers caged with honey bees yielded a mean of 64 kg fruit compared to only 15 kg of misshapen fruit in a plot caged without bees.

Cervancia and Bergonia (1991) observed that in the cucumber plants caged with honey bees, caged to exclude bees and uncaged treatment the fruit set were 75, 33 and 58%, respectively; the mean fruit weights were 0.87, 0.36, 0.60 kg, and mean numbers of filled seeds per fruit were 203, 51 and 134. The introduction of honey bee colonies to cucumber is recommended when the populations of native insect pollinators are low.

Rafiq (1992) recorded the number of pistillate and staminate flowers, fruit set and fruit weight in 30 plants of cucumber, 15 of these plants were covered with muslin cloth to prevent pollination by honey bees while the remaining plants were left exposed. Fruit weights were greater (2.69 kg/plant.) for honey bee pollinated plants than for self-pollinated ones (2.03 kg/plant).

Prakash *et al.* (2004) recorded significantly higher fruit weight (1210 g), number of seeds per fruit (482) and fruit volume (1315 ml) in cucumber (*Cucumis sativus*) with 20 bee visits compared to 15 bee visits which resulted in 1110 g fruit weight, 1205 ml fruit volume and 448 seeds per fruit. A minimum of 10 bee visits are necessary to minimize fruit drop and increase fruit set rate and a minimum of 20 bees visit to get more fruit weight, fruit volume and number of seeds per fruit.

Mann (1953) found that in muskmelon (*Cucumis melo* L.) open pollinated flowers produced large fruits and had more seeds than hand pollinated flowers. Awasthi (1969) reported 98 percent and 68 percent fruit set in Kakri (*Cucumis melo*) following natural pollination and hand pollination, respectively. Sakamori *et al.* (1977) while working on muskmelon found no difference in fruit size and quality following open pollination and hand pollination, respectively.

McGregor and Todd (1952) observed that from the plots caged to exclude bees only 4 marketable melons were obtained from 160 plants. On similar plots caged with bees 180 marketable melons were produced. This highly significant difference established the necessity of bees in commercial melon production.

In Australia, Williams (1987) found that plots (2 x3 m) of melons caged to exclude bees, caged with honey bees and not caged yielded 20, 26 and 27 melons, respectively, with a mean weight of 0.68, 1.11 and 1.10 kg each and total weights of 13.4, 28.5 and 29.4 kg per plot.

Gaye *et al.* (1991) observed that honey bees advanced the initial harvest date and early yield of muskmelon crop. Individual fruit weight also increased and was highly correlated with total seed weight. In greenhouses in Japan, honey bee pollination of muskmelons was as effective as hand pollination; attaining up to 98% fruit set, and was more economical (Sakamori *et al.*, 1977).

In Belgium, Lemasson (1987) compared the yield of melons grown in glasshouses with and without honey bees. The presence of honey bees increased the fruit set, 18.5 : 2.5 %; the weight of fruit per plant, 2664 :1469 g the weight per fruit, 621 :491 g, and the mean number of fruits per plant, 43 : 29. Garcia *et al.* (1998) evaluated the effects of no pollination, manual pollination and pollination by *A. mellifera* in green house trial with netted melons and observed that percentage of fruit drop was greater in non-pollinated plants than in pollinated plants. Bee pollination resulted in the highest fruit and seed weights (711.51 and 21.28 g, respectively); these were significantly higher than in manually pollinated plants (622.38 and 15.79). Fruit diameter, average number of seeds/fruit and thickness were similar in manually and bee-pollinated plants.

Dasgan *et al.* (1999) studied comparative behavior of honey bees and bumble bees in pollination of melon (*Cucumis melo* L.) and found that the yields obtained from honey bee pollinated and bumble bee pollinated melon plants were similar. Sarehane (1994) observed that melons open to pollination by bees had higher sugar concentrations and were firmer than those isolated from bees.

Kato and Couto (2002) studied the importance of insect pollination in melon. Some flowers were bagged to prevent insect visitation and others were left open. There was no fruit production in the covered treatments, and it was evident that honey bees are important and effective pollinators of melons for fruit production (quality and quantity).

Adlerz (1960) observed no difference in fruit set in water melon from hand pollination and open pollination methods. He further observed that fruit set was directly correlated with length of ovary (12mm -31mm). It was reported that fruit set and yield after hand pollination in watermelon was comparable with that of open pollination. He recorded higher fruit set (32.3%) from pollination between 8-9 am followed by pollination at 9-10a.m. (25.97%) and positive correlation between melon weight and number of seeds per fruit.

Shrivastava (1990) revealed that bottle gourd flowers were pollinated by the bug, *Cyrtopeltis tenuis*, sphingid moths, the beetle *Epilachna punctata* and pyralid moths.

Nath and Vashistha (1969) studied fruit set and fruit development in watermelon (*Citrullus lanatus*) and observed better fruit set (60.6-75%) from open pollination followed by hand pollination (50-66.6%).

Obhayashi (1976) recorded no difference in fruit set and yield of water melon plants pollinated by hand and those open pollinated. There was 10% more fruit set on the plots where honey bee colonies were placed than control plots caged to exclude bees (Mouzin *et al.*, 1980).

Rao and Suryanarayana (1988) found no fruit in 4x8m plots of *C. lanatus* caged without insect pollinators; a mean of 31.5 fruits of 4.3 kg mean weight were produced in cages with *A. cerana* colonies; and a mean of 22.8 fruits of 3.9 kg mean weight were produced in plots not caged.

Heemert *et al.* (1987) obtained a mean yield of 13 fruits/plant, which was the same as that produced by hand pollination in a 9 week trial. In a second trial lasting 18 weeks, bee pollination resulted in 53.4 fruits/plants and hand pollination 56.9 fruits/plants. The weight of fruits and the weight and number of mature seeds/melon were significantly higher from plots visited by bees than from those where bees were excluded (Brewer, 1974).

Stanghellini *et al.* (1998) compared bumble bees (*Bombus impatiens*) and honey bees (*A.mellifera*) at 4 visitation levels to pistillate flowers of watermelon. Bee visitation had a strong weight were produced in cages with *A. cerana* colonies; and a mean of 22.8 fruits of 3.9 kg mean weight were produced in plots not caged. Cucurbits mainly depend on insects for pollination as the male and female structures do not occur in the same flower and pollen grains are large and sticky to be carried by wind. The mechanical transfer of pollen is essential to bear fruits (Free, 1993; McGregor, 1976).

Heemert *et al.* (1987) obtained a mean yield of 13 fruits/plant, which was the same as that produced by hand pollination in a 9 week trial. In a second trial lasting 18 weeks, bee pollination resulted in 53.4 fruits/plants and hand pollination 56.9 fruits/plants. The weight of fruits and the weight and number of mature seeds / melon were significantly higher from plots visited by bees than from those where bees were excluded (Brewer, 1974).

Stanghellini *et al.* (1998) compared bumble bees (*Bombus impatiens*) and honey bees (*A. mellifera*) at 4 visitation levels to pistillate flowers of watermelon. Bee visitation had a strong positive influence on seed set. All flowers which were bagged to prevent insect visitation aborted suggesting the need for active pollen transfer between staminate and pistillate watermelon flowers. When compared at equal bee visitation levels, flowers visited by *Bombus impatiens* consistently contained more seeds than those visited by *A. mellifera*.

Williams (1987) observed that mean total weight of melon from plots caged to exclude bees was 13.4 kg which was significantly lower than the plots caged with honey bee (mean 28.5 kg) or from plots in open fields provided with 1 honey bee colony/acre (29.4 kg) and total number of melons obtained were 20, 26 and 27, respectively. Mean fruit weights were 0.68, 1.11 and 1.10 kg.

Hernandez and Lemus (1999) observed higher pumpkin yield (weight /ha) in plots nearest to hives, which gradually decreased as the distance from the hive increased.

Shrivastava (1990) showed that the experimental crops of bottle gourd (*Lagenaria siceraria*), ridge gourd (*Luffa acutangula*), wild bitter gourd (*Momordica dioica*) and wild pointed gourd (*Trichosanthes cucumerina*), were pollinated by the bug *Cyrtopeltis tenuis*, sphingid moths, the beetle *Epilachna punctata* and pyralid moths, respectively.

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted at the Research farm of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, 23°41′N latitude and 90°22′E longitude with an elevation of 8.6 meter above sea level (Appendix-1). The experiment was carried out during December 2016 to May 2017. The material used and methodology adopted for these experiments are described as follow:

3.1 Treatments and Design

Bottle gourd (*Lagenaria siceraria* L.) was imposed with 3 treatments (Without bee pollination, Hand pollination, and Open pollination) in this study. The experiment was laid out in Randomized Complete Block Design (RCBD) during the year December 2016 to May 2017 maintained eight replications.

3.2 Details of experimental treatments

3.2.1 Without bee pollination

The bottle gourd plants in 3 selected plots were caged in 40 mesh nylon net and no bees were allowed to get in the net. (Plate 2)

3.2.2 Open pollination

The selected bottle gourd plots were left open for the access of insect pollinators. (Plate 3)

3.2.3 Hand pollination

The male and female flowers of selected plants were bagged with butter paper bag one day prior to anthesis. Afterwards, when anthesis took place, butter paper bags were opened and petals of male flower were removed. Pollen from bagged male flowers was dusted over the female flowers by gently rubbing the anthers on the stigma; then again the flowers were bagged for 2-3 days to avoid any contamination by foreign pollen. After 3-4 days of pollination, bags were removed. (Plate 4)

3.3 Raising of crop

The planting was done in a plot size of 3.75m X 2.74m at a distance of 0.5m x 0.5m with six plants of each treatment; three on each side of the plot. Three plants per treatment of each replication were checked. Healthy bottle gourd seeds were taken from the seed market and soaked in water for 24 hours. Seedlings were raised in polythene bags and planted as per spacing in the evening for reducing settlement stress. Cowdung , ash and water hyacinth were given in every pit at the rate of 5-6 kg and each pit was supplied with 100 gm TSP and 60-70 gm MP fertilizer with a fixed supplement of 50 gm urea after every 15 days. Irrigation and weeding was maintained as per necessity.

Plate 1: Raising of seedling

Plate 2: Without bee pollination

Plate 3: Open pollination

3.4 Counting and identification of insect species

Insect visitors on bottle gourd flowers were collected by usual cone type hand net. Sweeps were made throughout the blooming of bottle gourd at 8.00am-9.00am, 11.00am-12.00pm and 2.00pm-3.00pm, 5.00pm-6.00pm, 8.00pm-9.00pm. Insects were then killed in pure alcohol and preserved as dry specimen.

Insect collection was started after three days of commencement of flowering and continued till 90 percent of flowering was over. Collected insects were identified by comparing them with the identified species maintained in the Department of Entomology, SAU.

3.5 Relative abundance of insect visitors

For relative abundance of insect visitors, plants were selected randomly in three different plots and observations were started 2-3 days after the flowering. These observations were taken at 8.00am-9.00am, 11.00am-12.00pm and 2.00pm-3.00pm, 5.00pm-6.00pm, 8.00pm-9.00pm , and were continued for 7 sunny days.

3.6 Foraging behavior of bees

Major bee visitors associated with pollination of bottle gourd with different foraging behavior were recorded in three different plots as follows: The number of *Apis mellifera* bees foraging for pollen or nectar was recorded in one meter square bloom area per ten minutes at 8.00am-9.00am, 11.00am-12.00pm and 2.00pm-3.00pm, 5.00pm-6.00pm, 8.00pm-9.00pm . In total 18 observations were made during 6 days of observation.

Plate 4: Hand pollination

Plate 5: Crooked fruit

Plate 6: Healthy fruits

3.7 Data recorded on fruit and seed characters

3.7.1 Total fruit set, crooked fruits and healthy fruits

To find out the effect of different modes of pollination on fruit set, crooked fruits and healthy fruits, six plants of each treatment, three on each side of the plot, three plants per treatment of each replication was checked. (Plate 5 and 6)

The percentage of fruit set, crooked fruit and healthy fruit was calculated by using the following formula,

$$\text{Fruit set \%} = \frac{\text{Total no. of fruit set/vine}}{\text{Total number of female flowers/vine}} \times 100$$

Similarly the percentage of crooked and healthy fruits was worked out.

$$\text{Crooked fruit \%} = \frac{\text{Total no. of crooked fruits/vine}}{\text{Total number of fruits/vine}} \times 100$$

$$\text{Healthy fruit \%} = \frac{\text{Total no. of healthy fruits/vine}}{\text{Total number of fruits/vine}} \times 100$$

3.7.2 Fruit weight

To calculate fruit weight in different modes of pollination 5 fruits were selected randomly from each treatment and replicated three times and average fruit weight was recorded.

3.7.3 Fruit length

The polar length of selected five fruits was recorded in cm and mean values were recorded.

3.7.4 Fruit width

The fruit width in the top, middle base parts were measured in cm on selected five fruits and mean values were recorded.

3.7.5 Number of seeds per fruit

Seeds were separated, cleaned from individual fruit and counted the number of seeds per fruit and mean values were recorded.

3.7.6 1000-seeds weight

Five representative samples of 1000-seeds were taken from each treatment to determine the average test weight in grams.

3.8 Statistical analysis

The data were statistically analyzed using randomized block design after proper transformation where ever needed (Gomez and Gomez, 1986). Graphical data representation, ANOVA, Multiple Range Test, Fisher's LSD and the P value calculated by STATGRAPHICS Centurion XV.I for better understanding.

CHAPTER IV

RESULT & DISCUSTION

Performance of insect pollination in bottle gourd was investigated and the findings of present study have been discussed under different characters. The results of the study showed a marked variation in different characters which are presented by following Tables and Figures.

The data pertaining to bottle gourd as well as yield and its contributing characters were computed and statistically analyzed and the results thus obtained are discussed below under the following headings:

4.1 Bloom foraging insects and their relative abundance on bottle gourd

4.1.1 Insect visitors of blooming bottle gourd

There were different types of insect species which visited the bottle gourd flowers during blooming, listed in Table 1. In total 12 insect species belonging to 11 families under 5 orders were recorded visiting the bottle gourd bloom during study. Out of these 5 to Hymenoptera, 3 to Coleoptera, 2 to Lepidoptera and each from Thysanoptera & Homoptera.

Among these, most frequent visitors were: *Therioaphis trifolii, A. mellifera L., Formica sp.*, *Halictus sp.* and *Bactrocera cucurbitae* whereas Pyralid moth, syrphid fly, spotted cucumber beetle, and Ladybird beetle were less frequent visitors.

4.1.2 Relative abundance of insect visitors on blooming bottle gourd

Data on the relative abundance of insect visitors during different hours of the day is presented in Table 2. During 8:00am-9:00am, *Formica sp.* were significantly most abundant (15.40ants/m^2/10min) followed by *Therioaphis trifolii* (3.82 bees/m^2/10min) and *Apis mellifera* (3.54 bees/m^2/10min). The latter two species showed similarity with each other in respect of abundance.

Syrphids were least abundant (0.56 flies/m^2/10min) followed by *Halictus sp.* (2.10 bees/m^2/10min). Other pollinators *Bactrocera cucurbitae* (2.41 insects/m^2/10min) and *Apis dorsata* (3.32 bees/m^2/10min) were statistically similar with each other. At 11.00am-12.00pm, *Formica sp.* were most abundant (13.8 ants/m^2/10min) followed by *Apis mellifera* (2.86 bees/m^2/10 min), the later two species were statistically at par with each other. But syrphids were least abundant (0.51 flies/m^2/10 min) followed by *Bactrocera cucurbitae* (1.50 insects/m^2/10 min), *Halictus sp.* (1.85 bees/m^2/10 min) and *Apis dorsata* (2.22 bees/m^2/10 min) which were statistically at par with each other.

At 2.00pm-3.00pm, *Formica sp.* were most abundant (9.96 ants/m^2/10min) followed by *Apis mellifera* (1.95 bees/m^2/10 min) and those were statistically at par with each other. Conversely Syrphids were least abundant (0.53 flies/m^2/10 min) and they were statistically similar with *Halictus sp.* (0.98 bees/m^2/10 min), and *Bactrocera cucurbitae* (1.02 insects/m^2/10 min) and *Apis dorsata* (1.91 bees/m^2/10 min).

At 5.00pm-6.00pm, *Formica sp.* was found the most abundant (7.80 ants/m^2/10 min) followed by *Therioaphis trifolii* (1.01 bees/m^2/10 min) and they were statistically same. But Syrphids were least abundant (0.36 flies/m^2/10 min) and were statistically at par with *Bactrocera cucurbitae* (0.58 bees/m^2/10 min) and other insects (0.85 insects/m^2/10 min) as well.

At 8.00pm-9.00pm, *Formica sp.* were mostly abundant (0.34 ants/m^2/10min) followed by *Therioaphis trifolii* (0.30 bees/m^2/10 min).

The data on the abundance of insect visitors irrespective of the day hours showed that *Formica sp*. were the most frequent visitors (9.46 ants/m^2/10 min). *Therioaphis trifolii* (1.92 bees/m^2/10 min) was statistically at par with each other followed by *Apis mellifera* (1.87 bees/m^2/10 min). But Syrphids were least abundant (0.39 flies/m^2/10 min) and were statistically at par with *Bactrocera cucurbitae* (1.10 bees/m^2/10 min) and other insects *Halictus sp.* (1.16 insects/m^2/10 min).

Activity of insect visitors during different hours of the day showed that their abundance was significantly higher at 08.00am-09.00am, with an average of 4.45 insects/m^2/10 min, followed by 11.00am-12.00pm (3.60 insects/m^2/10 min) and 02.00pm-03.00pm (2.62 insects/m^2/10 min). But at 05.00pm-06.00pm (1.78 insects/m^2/10 min) and at night that rate were decreased ie. 08.00pm-09.00pm (0.09 insects/m^2/10 min) which were significantly different from each other.

Table 1. Insect visitors of bottle gourd bloom with their frequency of occurrence

Sl. No	Common Name	Scientific Name	Family	Order	Frequency of visits
1	Pyralid moth	*Arthoscista hilarialis Walker*	Pyralidae	Lepidoptera	*
2	Cabbage butterfly	*Pieris brassicae L.*	Pieridae		**
3	Thrips	*Magalurothrips usitatus*	Thripidae	Thysanoptera	**
4	Aphids	*Therioaphis trifolii*	Aphididae	Homoptera	***
5	Italian Honey bee	*A. mellifera* L.	Apidae	Hymenoptera	***
6	Ants	*Formica sp.*	Formicidae		***
7	Solitary bee	*Halictus sp.*	Halictidae		***
8	Syrphid fly	*Scaeva pyrastri* L.	Syrphidae		*
9	Fruit fly	*Bactrocera cucurbitae*	Tephritidae		***
10	Red pumpkin beetle	*Aulacophora foveicollis* L.	Chrysomelidae	Coleoptera	**
11	Spotted cucumber beetle	*Diabrotica undecimpunctata* Mann			*
12	Ladybird beetle	*Coccinella septempunctata* L.	Coccinellidae		*

***Most frequent
**Medium Frequent
*Less frequent

Table 2. Relative abundance of insect visitors/10 minutes/m^2

Sl. No	Species	08.00-09.00 hrs	11.00 - 12.00 hrs	14.00 - 15.00 hrs	17.00-18.00 hrs	20.00 - 21.00 hrs	Mean	Standard Deviation (SD)	CV (%)
1	*Bactrocera cucurbitae*	2.41	1.5	1.02	0.58	0	1.102	0.91	83.20
2	*Apis mellifera*	3.54	2.86	1.95	0.98	0	1.866	1.41	76.09
3	*Apis dorsata*	3.32	2.22	1.91	0.93	0	1.676	1.26	75.58
4	*Therioaphis trifolii*	3.82	2.46	2	1.01	0.3	1.918	1.35	70.73
5	*Formica sp.*	15.4	13.8	9.96	7.8	0.34	9.46	5.92	72.60
6	*Halictus sp.*	2.1	1.85	0.98	0.85	0	1.156	0.84	72.81
7	Syrphids	0.56	0.51	0.53	0.36	0	0.392	0.23	69.26
Mean		4.45	3.6	2.62	1.78	0.09	2.51	**F-Ratio**	**8.04**
								P-Value	**0.0000**

The ANOVA table decomposes the variance of the data into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 8.04 (Table 2), is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 7 variables at the 95.0% confidence level. Multiple range tests were applied to know the statistical significant difference between relative abundances of species. *Formica sp.* showed highest significant difference in relative abundance than all other species. The table applied a multiple comparison procedure to determine which means were significantly different from which others. The bottom half of the output shows the estimated difference between each pair of means. An asterisk had been placed next to 6 pairs, indicating that these pairs showed statistically significant differences at the 95.0% confidence level. The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0.

Illustration expressing the variations of relative abundance of different insect visitors on bottle gourd flower at the study period. *Formica sp.* showed the highest relative abundance in all five hours of study. (Figure 01)

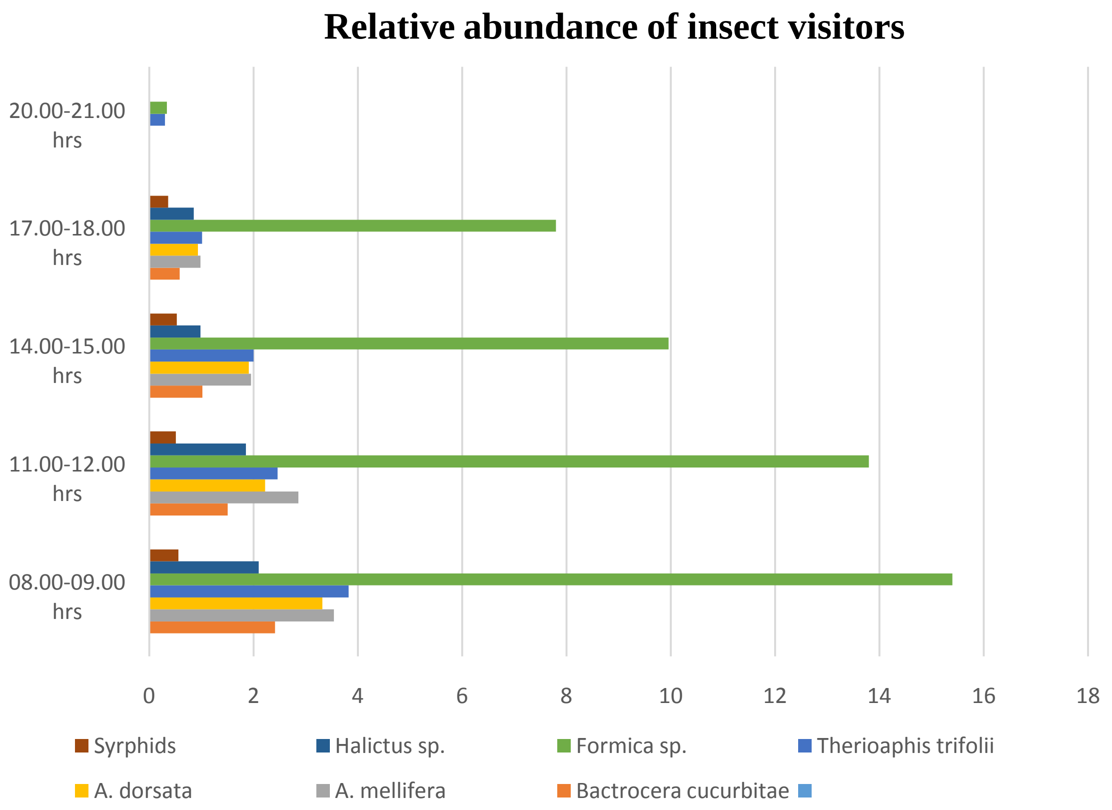


Figure 01. Relative abundance of insect visitors/10 minutes/m^2

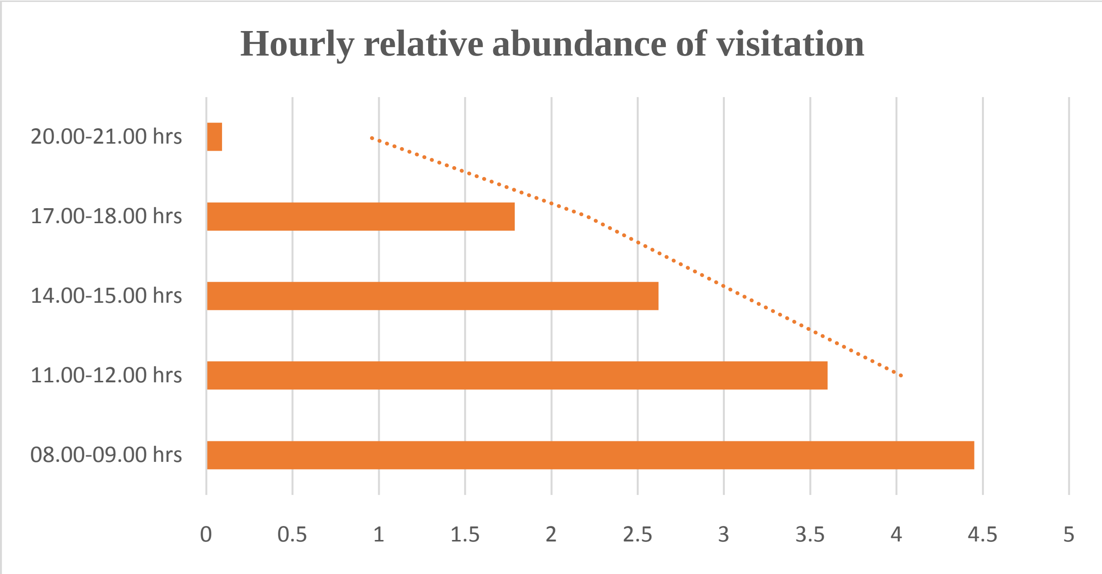


Figure 02. Hourly relative abundance of insect visitors/10 minutes/m^2 also shown in a graphical analysis below for clearer observation

Figure 02 represents highest abundance of visitation of all insects at 08.00am-09.00am.

4.2 Foraging behavior of bee pollinators

4.2.1 Proportion of pollen or nectar foragers of *Apis mellifera*

Data on the proportion of nectar or pollen foragers of *Apis mellifera* bees at different hours of the day on the bottle gourd bloom is presented in Table 3. It was revealed that irrespective of the day hours there were more number of nectar foragers with an average of 6.16 bees/m^2/10 min as compared to pollen foragers (5.27 bees/m^2/10 min) and the difference was statistically significant.

During 8.00am-9.00am, the average numbers of pollen foragers (6.75 bees/m^2/10 min) were significantly higher than nectar foragers (5.79 bees/m^2/10 min).

During 11.00am-12.00pm, the average numbers of nectar foragers (6.74 bees/m^2/10 min) were significantly higher than pollen foragers (4.98 bees/m^2/10 min).

During 2.00pm-3.00pm, the average numbers of nectar foragers were significantly higher (5.95 bees/m^2/10 min) than pollen foragers (4.1 bees/m^2/10 min) and statistically different from each other.

The data in Table 3 revealed that irrespective of the day hours, there were a significantly higher number of nectar foragers (6.16 bees/m^2/10 min) than pollen foragers (5.27 bees/m^2/10 min). Similar results had also been observed by several other workers (Kauffeld and Williams 1972; Collison and Martin 1975; Collision and Martin 1979).

The average numbers of pollen foragers were significantly higher during 08.00pm-09.00pm (6.75 bees/m²/10 min) whereas there were significantly more nectar foragers during 11.00am-12.00pm (6.74 bees/m²/10 min). Sajjanar *et al.* (2004), Bhambure (1958) and Rao and Suryanarayana (1988) also reported the pollen foraging to be maximum during morning hours and nectar foraging to be maximum during afternoon in bottle gourd flowers.

The average numbers of nectar or pollen foragers were significantly higher at 08.00am-09.00am (6.27 bees/m²/10 min) and 11.00am-12.00pm (5.86 bees/m²/10 min) followed by 02.00pm-03.00pm (5.03 bees/m²/10 min). This might be correlated with the abundance of insect visitors which was higher during morning hours.

Table 3. Proportion of *Apis mellifera* bees foraging for pollen or nectar per 10 minutes per m^2 blooming bottle gourd at different hours

Day hours	Pollen	Nectar	Mean
08am-09am	6.75	5.79	6.27
11pm-12pm	4.98	6.74	5.86
02pm-03pm	4.1	5.95	5.025
Mean+SE	5.27±0.4	6.16±0.9	5.71±0.6

$CD_{0.05}$
Foragers 0.32
Day hours 0.39
Day hours x foragers 0.56

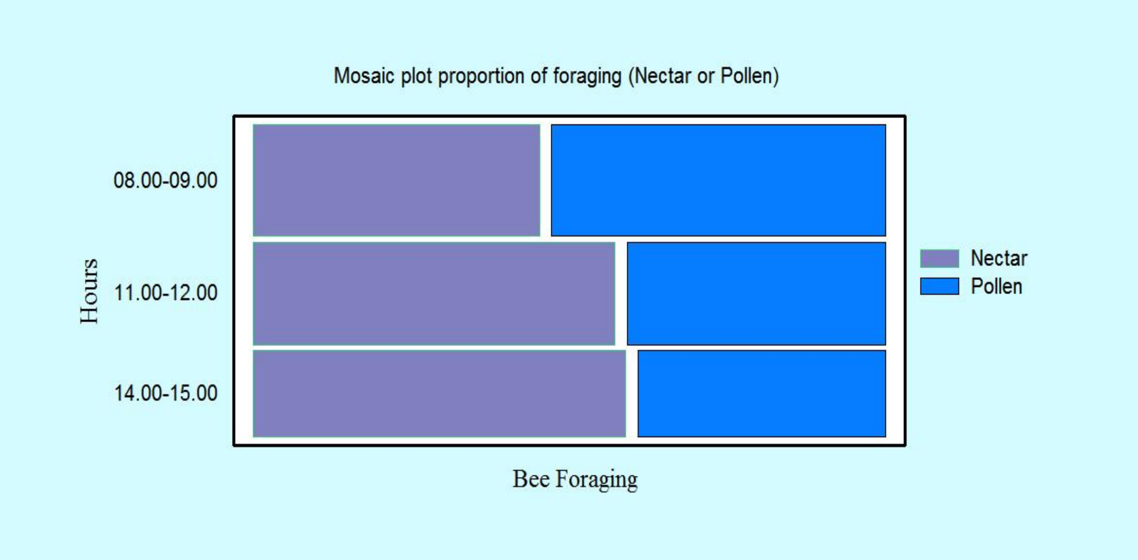


Figure 03. Mosaic plot showing proportion of *Apis mellifera* bees foraging for pollen or nectar/10 minutes/m^2 bottle gourd bloom at different hours

Mosaic plot expressing that in the morning 8.00am-9.00am was efficient for pollen collection and 11.00am-12.00pm and 2.00pm-3.00pm was peak for nectar collection.

4.3 Effect of different modes of pollination on fruit set, crooked fruits and healthy fruits

Data on percentage of fruit set, crooked fruits and healthy fruits were presented in Table 4, 5 and 6, respectively.

4.3.1 Fruit set

The data presented in Table 4 revealed that the percent of fruit set was significant in hand pollination (71.52%) and open pollination (60.85%) and higher than without honey bee pollination (45.65%).

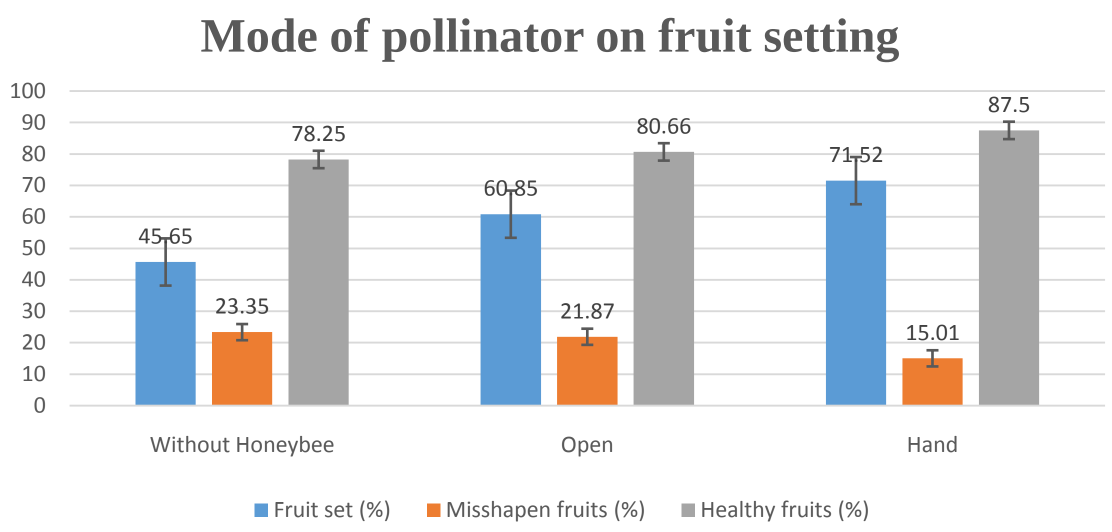
4.3.2 Healthy fruits

The data (Table 4, Figure 4) showed that irrespective of lines, the percentage of healthy fruits was maximum in hand pollination (87.50%) followed by open pollination (80.66 %) and minimum percentage of healthy fruits was observed in without honey bee pollination (78.25%) and all of them were statistically different from each other.

Among different modes of pollination the fruit set (Table 4, Figure 4) was observed to be significantly higher in hand polination (71.52%) and open pollination (60.85%) as compared to without bee pollination (45.65%). This might be due to the reason that in hand pollination pollen is applied generously to whole stigmatic surface. Mouzin *et al.* (1980), Lemasson (1987), Cervancia and Bergonia (1991) and Rafiq (1992) also obtained that higher percentage of fruit set in bee pollination as compared to open pollination. So in absence of bee there were lack of pollination. The fruits obtained from open pollination (21.87%) and hand pollination (15.1%) was also found to be better with respect to shape since the percentage of misshapen fruits was highest in without bee pollination (23.35%). Therefore, the percentage of well-formed healthy fruits was highest in hand pollination (87.5%) followed by open pollination (80.66%) and without bee pollination (78.25%). The result of the present study are in accordance with the results of Cervancia and Bergonia (1991), Hernandez *et al.* (1999), and Kato and Couto (2002), which might be attributed to sufficient amount of pollen being received by the flowers in hand and bee pollination treatments.

Table 4. Effect of different modes of pollination on percent fruit set, crooked and healthy fruits

Mode of pollination	Fruit set (%)	Misshapen fruits (%)	Healthy fruits (%)
Without honeybee	45.65	23.35	78.25
Open	60.85	21.87	80.66
Hand	71.52	15.01	87.5
$CD_{0.05}$	0.89	0.78	0.74



There are no significant pair wise differences among the means.

F-ratio: 0.05; P value: 0.94

Figure 04: Effect of different modes of pollination on percent fruit set, crooked and healthy fruits

The F-ratio, which in this case equals 0.05, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is greater than or equal to 0.05, there is not a statistically significant difference between the means of the 3 variables at the 95.0% confidence level.

4.4 Effect of different modes of pollination on fruit weight, number of seeds/fruit and fruit size in bottle gourd at the time of seed harvesting

4.4.1 Fruit weight

Data presented in Table 5 showed that the weight of fruit was maximum in hand pollination (2200.54 g) which was statistically similar with open pollination (1700.56 g) and minimum in without bee pollination (1500.4 g) the latter being statistically similar with hand pollination.

4.4.2 Number of seeds/fruit

The maximum number of seeds per fruit were observed in hand pollination (185 seeds/fruit) but minimum in without bee pollination (152.6 seeds/fruit), and statistically at par with open pollination (172.54 seeds/fruit) (Table 5).

4.4.3 Fruit diameter

The data presented in Table 6 showed that the fruit diameter was found maximum in hand pollination with an average of (60.8 cm/fruit) followed by open pollination (55.6 cm/fruit) which is statistically non-significant whereas minimum fruit diameter was found in without bee pollination (46.4 cm/fruit).

4.4.4 Fruit length

The fruit length was found maximum in hand pollination (89.7 cm/fruit) followed by open pollination (80.9 cm/fruit) while minimum fruit diameter was found inwithout bee polllination (62.7 cm/fruit).

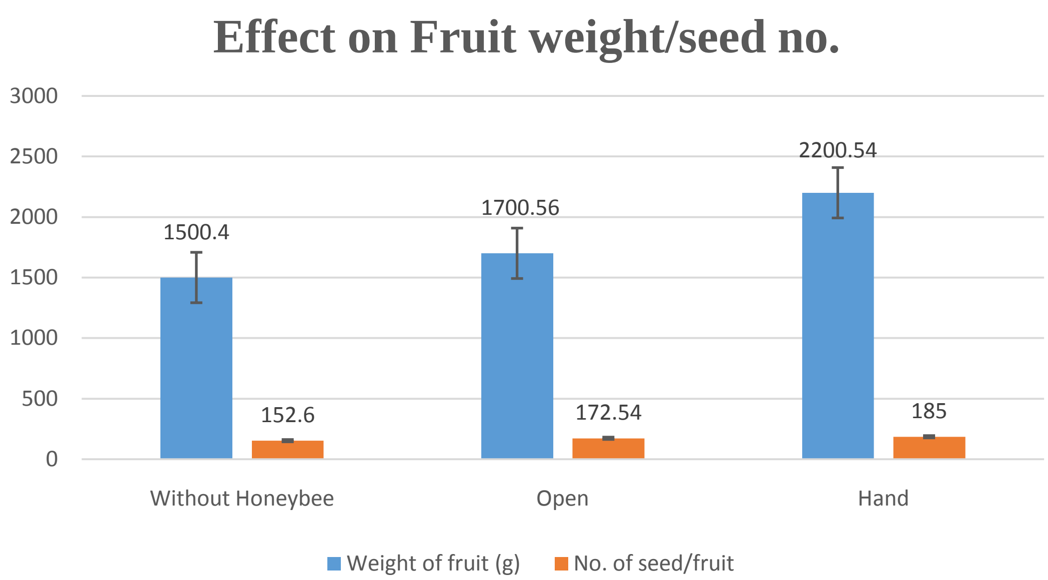
4.5 Weight of 1000-seeds

Data presented in Table 6 showed that the mean weight of 1000-seeds was significantly higher in hand pollination (88.3 g) followed by open pollination (86.56 g) and without bee pollination (83.54 g). The fruit characteristics such as fruit weight (2200.54.g), number of seeds per fruit (185), fruit diameter (46.4 cm) and fruit length (89.7 cm) was found to be significantly higher in hand pollinated plants whereas minimum fruit weight (1500.4 g), number of seeds per fruit (152.6), fruit diameter (46.4 cm), fruit length (62.7 cm) and

1000-seeds weight (83.54 g) was found in without bee pollination (Tables 5, Figure 5). But Brewer (1974), Garcia *et al.* (1998) and Prakash *et al.* (2004) had found that t he number of seeds per fruit and larger fruit size in bee pollinated plants might be attributed to the sufficient number of pollen grains received by the flowers which were provided best by honey bees in caged conditions as compared to open pollination and hand pollination. This also might be due to the adequate pollination done by honey bees inside the cage whereas this study obtained the lowest value in yield in case of without bee pollination.

Table 5. Effect of different modes of pollination on fruit weight and number of seeds/fruit at the time of seed harvesting

Mode of pollination	Weight of fruit (g)	No. of seeds/fruit
Without honeybee	1500.4	152.6
Open	1700.56	172.54
Hand	2200.54	185
$CD_{0.05}$	86.65	49.84



F-ratio: 0.049; P value: 0.95

Figure 05: Effect of different modes of pollination on fruit weight and number of seeds/fruit at the time of seed harvesting

The F-ratio, which in this case equals 0.049, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is greater than or equal to 0.05, there is not a statistically significant difference between the means of the 3 variables at the 95.0% confidence level.

Table 6. Effect of different modes of pollination on fruit size (cm) and 1000-seeds weight

Mode of pollination	Fruit diameter (cm)	Fruit length (cm)	1000-seeds weight (g)
Without honeybee	46.4	62.7	83.54
Open	55.6	80.9	86.56
Hand	60.8	89.7	88.3
$CD_{0.05}$	5.86	8.25	3.62

There are no significant pair wise differences among the means.
F-ratio: 0.274
P value: 0.775

The F-ratio, which in this case equals 0.274, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is greater than 0.05, there is a statistically significant difference between the means of the 3 variables at the 95.0% confidence level.

CHAPTER V

SUMMARY AND CONCLUSION

To evaluate the comparative study of pollination method on bottle gourd yield, an experiment was conducted in RCBD with eight replication during the period from December 2016 to May 2017 in the experimental area of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka. From the investigation, the effect of different modes of pollination on fruit and seed characters, foraging behavior of major bee pollinators, various insect visitors and their relative abundance in bottle gourd were studied. A total of twelve insect species were recorded that visited the bottle gourd bloom. Among them *Formica sp*. (9.46) were most abundant visitor followed by *Therioaphis trifolii* (1.918) *Apis mellifera* (1.866), *Halictus sp*. (0.95), and *B. cucurbitae* (1.102). But Syrphids (0.392) were least abundant. Activity of insect visitors was at peak in between morning (4.45) followed by noon (3.6) and evening (2.62), while least activity was recorded at night 08.00pm-09.00pm (0.09).

From the experiment the pollen or nectar foragers proportion of *Apis mellifera* revealed that there were significantly more number of nectar foragers (6.16/m^2/10 min) as compared to pollen foragers (5.27/m^2/10 min). The pollen foragers were highest during morning hours (6.75/m^2/10 min) whereas the nectar foragers were maximum during noon hours (6.74/m^2/10 min).

Findings of the present investigation indicated that the maximum value of the fruit set was found in hand pollination (71.52%) while minimum in without bee pollination (45.65%) among the different approach of pollination. The percentages of crooked fruits were the highest in without bee pollination (23.35%) but minimum in hand pollination (15.01%). The percentages of healthy fruits were maximum in hand pollination (87.5%) and minimum in without bee pollination (78.25%). The fruit weight (2200.54 g), number of seeds per fruit (185), fruit diameter (60.8cm) and fruit length (89.7cm) were maximum in hand pollination as compared to open and without bee pollination. The weights of 1000-seeds were also found maximum in hand pollination (88.3 g) as compared to other modes of pollination.

In Bangladesh, among winter vegetables bottle gourd is important because of their dietary values and sources of income. In that sense pollination might be a keystone process in bottle gourd production and directly links wild ecosystems with agricultural production systems. There exists many ways of pollination in the nature. But this experiment was about the effect of bee pollination, hand pollination, and open pollination on bottle gourd. The experiment revealed that the bee pollination *(Apis millifera)* resulted in higher fruit set, healthy fruits, fruit weight, fruit size, number of seeds per fruit and weight while without bee pollination exhibited the lowest fruit set, healthy fruits, fruit weight, fruit size, number of seeds per fruit and weight. Thus from the results, it can be concluded that in absence of bee pollination, hand pollination could be considered as the best followed by open pollination.

CHAPTER VI

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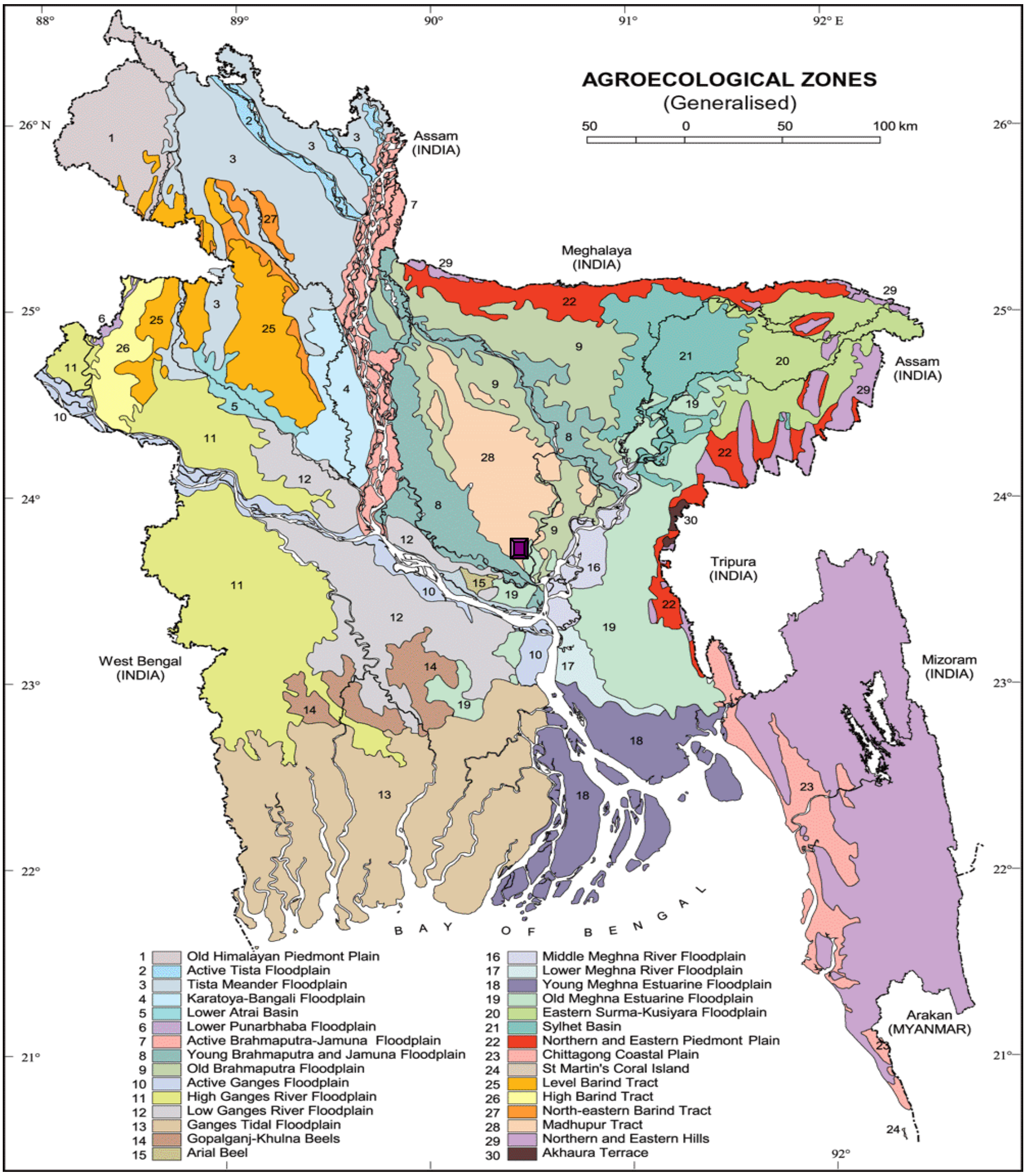
ANNEXURE

Appendix I: Map showing the experimental sites under study



Experimental site

Appendix II: Characteristics of soil of experimental is analyzed by Soil Resources Development Institute (SRDI), Khamarbari, Farmgate, Dhaka-1207

A. Morphological characteristics of the experimental field

Morphological features	Characteristics
Location	Research Field laboratory, SAU, Dhaka
AEZ	Madhupur Tract (28)
General Soil Type	Shallow red brown terrace soil
Land type	Medium hHigh land
Soil series	Tejgaon
Topography	Fairly leveled
Flood level	Above flood level
Drainage	Well drained

Appendix III. Multiple Range Test for relative abundance of insect visitors/10 minutes/m^2

Method: 95.0 percent LSD

Contrast	Sig.	Difference
A_ cerana - *A_ dorsata*		-0.764
A_ cerana - *A_ florea*		-0.574
A_ cerana - *A_ mellifera*		-0.816
A_ cerana - *Formica sp_*	*	**-8.358**
A_ cerana - *Halictus sp_*		-0.054
A_ cerana - Syrphids		0.71
A_ dorsata - *A_ florea*		0.19
A_ dorsata - *A_ mellifera*		-0.052
A_ dorsata - *Formica sp_*	*****	**-7.594**
A_ dorsata - *Halictus sp_*		0.71
A_ dorsata - Syrphids		1.474
A_ florea - *A_ mellifera*		-0.242
A_ florea - *Formica sp_*	*	**-7.784**
A_ florea - *Halictus sp_*		0.52
A_ florea - Syrphids		1.284
A_ mellifera - *Formica sp_*	*	**-7.542**
A_ mellifera - *Halictus sp_*		0.762
A_ mellifera - Syrphids		1.526
*Formica sp_* - *Halictus sp_*	*	**8.304**
*Formica sp_* - Syrphids	*	**9.068**
*Halictus sp_* - Syrphids		0.764

* denotes a statistically significant difference.