SURVEY AND MOLECULAR DETECTION OF FRUIT FLY SPECIES OF GUAVA IN BANGLADESH AND ITS INTEGRATED MANAGEMENT

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A DISSERTATION FOR

THE DEGREE OF
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IN
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SHER-E-BANGLA NAGAR, DHAKA-1207, BANGLADESH

CERTIFICATE

This is to certify that thesis entitled "***SURVEY AND DETECTION OF FRUIT FLY SPECIES/RACES OF GUAVA IN BANGLADESH AND ITS INTEGRATED MANAGEMENT***" submitted to the faculty of agriculture, Sher-e-Bangla Agricultural University, Dhaka-1207, in partial fulfilment of the requirements for the degree of ***DOCTOR OF PHILOSOPHY IN ENTOMOLOGY*** embodies the result of a piece of *bonafide* research work carried out by ***MD. HABIBUR RAHMAN,*** Registration, No.18-08323 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

Date: June, 2021
Place: Dhaka, Bangladesh

(Prof. Dr. Mohammed Sakhawat Hossain)
Supervisor and Chairman
Advisory Committee

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BIOGRAPHICAL SKETCH

The author was born in the district of Natore on 3rd August 1976. He is the youngest son of late Babar Ali Mollah and Mrs. Halima Begum.

He passed Secondary Education and Higher Secondary Education from Rajshahi Education Board in 1991 and 1993, respectively. In both the examination he got the first division.

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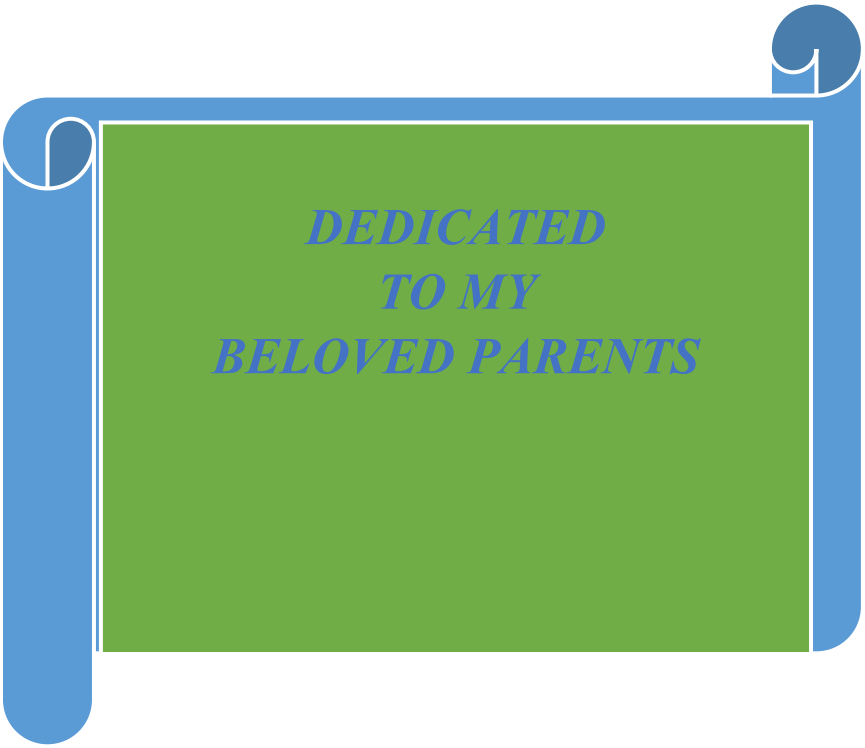
The Author


DEDICATED
TO MY
BELOVED PARENTS

SURVEY AND MOLECULAR DETECTION OF FRUIT FLY SPECIES OF GUAVA IN BANGLADESH AND ITS INTEGRATED MANAGEMENT

ABSTRACT

By

Md. Habibur Rahman

Fruit fly is one of the major insect pests of fruits and vegetables in the world. Fruit flies belong to the family Tephritidae (Order: Diptera) are considered as a very destructive group of insects that cause enormous economic losses in agriculture, especially in a wide variety of fruits, vegetables, and flowers. The identification and management of fruit flies in guava orchard is complex and complicated. The purpose of this study was morphological identification, molecular detection of the most invasive species of fruit flies attacking the vulnerable stage of guava fruit and their management with some promising control options. A survey was conducted at four intensive guava growing regions of Bangladesh (North, Central, South & Hill tracts) to evaluate the extent of infestation of fruit flies, losses due to its infestations, farmer's interest, and management of fruit fly. It revealed that more than 90 percent farmers were young, and 27 percent landless farmers were involved in guava farming at surveyed locations. However, female farmers involvement was comparatively low. Most popular guava variety was Thai payara and 31 percent area was covered by this promising variety. Other two popular local varieties were viz., Shorupkati and kanchannagar. Almost 20-25 percent yield loss was reported due to fruit fly attack. Fruit fly infestation was observed at ninety seven percent guava orchards in different surveyed locations. Guava farmers mainly used polybag to reduce the fruit fly infestation. Eighty-five percent guava farmer usually practiced trap with pheromone and polybag simultaneously. In morphometric study, examined the species identification of fruit flies infesting guava in Bangladesh. Oriental fruit fly (*Bactrocera dorsalis* Hendel) was found as the major species which covered seventy eight percent of total fruit fly population and other species were melon fly (*Zeugodacus cucurbitae*), pumpkin fruit fly (*Zeugodacus tau*), peach fruit fly (*Bactrocera zonata*) and. *Dacus longnicornis*. Data analyses based on five fruit fly taxa revealed moderate performance of this genetic marker COI. BLAST analysis revealed that the observed species showed 98-100% homology with the sequence of *Bactrocera dorsalis*, *Zeugodacus cucurbitae*, *Zeugodacus tau*, *Bactrocera zonata* and *Dacus longnicornis*, respectively. Present study investigated the population genetic diversity and structure of 35 populations and haplotype distribution of fruit fly in Bangladesh sampled throughout 12 geographical populations. The population genetic diversity and haplotype distribution study results revealed low genetic diversity of fruit fly of four different species in sampled areas. Significantly negative departures from zero for Tajima's D neutrality tests values also support population expansions. To find out the effective and sustainable management technique to improve the guava production by reducing the fruit fly infestation, a study was conducted at on-station and Farmers' field of Savar and Gazipur of Dhaka district and Sher-e-Bangla Agricultural University for testing and rearing the collected sample. Tretment T_4 (Wrapping of twig and fruits with micro nets) showed the higher level of mean number 129.67 of healthy fruit and weight 42.79 g of healthy fruits but the lowest number reduction over control per fruit was obtained from wrapping of twig and fruits with micro nets treated plot. The total fruit yield 34.23 t/ha was significantly higher in the plots treated with the components of T_4 which was statistically similar to Male Annihilation Technique (MAT).

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CONTENTS

LIST OF TABLES

LISTS OF FIGURES

LIST OF PLATES

LISTS OF APPENDICES

LIST OF ABBREVIATIONS

Abbreviation	Full Name
ACV	Apple cider vinegar
BAU	Bangladesh Agricultural University
BSMRAU	Bangabandhu Sheikh Mujibur Rahman Agricultural University
BAT	Bait application technique
DNA	Deoxyribonucleic Acid
DV	Daily value
DAA	Days After Application
ITS1	Internal Transcribed Spacer 1
IPM	Integrated Pest Management
MAT	Male annihilation technique
Mt COI	Mitochondrial cytochrome oxidase I
PCR	Polymerase Chain Reaction
RCBD	Randomized Complete Block Design
SAU	Sher-e-Bangla Agricultural University
TSS	Total soluble solid
12s rRNA	12s Ribosomal RNA
16s rRNA	16s Ribosomal RNA

Chapter-I

INTRODUCTION

CHAPTER I

INTRODUCTION

The production of fruit and vegetables in Bangladesh play important sources of income. These crops represent an important part of the gastronomic culture for Bangladeshi people. A constantly growing population, rising of income and urbanization levels increase the demand of fruits and vegetables. To fill up the gap of this demand, better farming strategies are necessary. The presences of pests such as fruit flies constitute an obstacle in their production. Fruit flies belonging to the family Tephritidae (Order: Diptera) are considered as a very destructive group of insects that cause enormous economic losses in agriculture, especially in a wide variety of fruits, vegetables and flowers (Diamantidis *et al*. 2008). The total number of species within this family exceeds 4,000. Approximately 10% of them are serious pests distributed around the world in temperate, subtropical and tropical areas (Christenson and Foote,1960, Singh, 2003). In particular, two species belonging to this family are of great importance in Bangladesh, namely the Melon fly (*Bactrocera cucurbitae* Coquillet)) and the Oriental fruit fly (*Bactrocera dorsalis* Hendel). In cucumber (*Cucumis sativus* Linnaeus) and bitter gourd (*Momordica charantia* Linnaeus) field infestation problems caused by *B. cucurbitae* are very common in Bangladesh (Ramadan and Messing, 2003). The losses due to infestation of fruit flies is surprisingly high. There are examples where losses have been up to 100% in cucurbit species, caused by Melon fly (*B. cucurbitae*) (Dhillon *et al.* 2005). Crop losses in mango (12-60%), guava (40-90%) and papaya (12-60%) have also been recorded by Allwood and Leblanc,1997.

The damages on crops consist on oviposition stings on the fruit surface, fruit that drops early and also destruction of the fruits due to internal feeding by fruit flies larvae (maggots). This results in unmarketable crop. In Bangladesh there are concerns about management of fruit flies in the most efficient way considering the growth stages of the target pest. Identification of fruit flies using conventional taxonomy/ morphometry has certain limitations due to homoplasmy on most morphological characters and difficulties in identification of egg and adult stages which has a significant relation in efficient management of fruit fly in the field.

The use of insecticides as the major way to control pests in fruit and vegetables causes environmental pollution and hygienic problems that represent a risk for people and animals. In the last four decades, the use of synthetic pesticides such as organophosphate and carbamates in an extensive way has led to the development of insecticide resistance in a number of pest species (Casida and Quistad, 1998) and in Bangladesh residues of organophosphate and organochloride and other compounds have been detected in soil, water and crops (Thapinta and Hudak, 1998). Insecticides in the form of pyrethroids, thriazophos have been used on cucurbits crops, but the results have not been satisfactory. Resistance problems due to the overuse of such insecticides and high residues in the sprayed vegetables aremajor concerns that necessitate some form of bio-rational management. Other approaches for fruit fly management, such as use of protein baits have been more or less ineffective because of our limited knowledge of the ecology of the fruit flies.

Many ecological studies have been carried out on these two species (Jang. 1997, Smith. 1989, Kuba *et al*. 1984, Kuba *et al*. 1982). Despite this, a lot of knowledge is still lacking, and it is indispensable to the understanding of these pests that this knowledge gap should be filled. It is urgent to find more effective and environmentally friendly control strategies that will be sustainable. Knowledge is needed that will increase the effectiveness (and safety) in the use of insecticides, and that gives farmers and pest advisors ideas about how to develop control schemes for this pest. Diurnal activity of fruit fly under field conditions has not been investigated in Bangladesh. Since insecticide application is the main control strategy used by farmers it is crucial to know when during the day fruit flies are most active and therefore most likely to come into contact with the insecticides. Furthermore, the setting of a pheromone trap for monitoring and control purposes by using material that can easily be acquired at a low cost that will not only be useful for pest control but also for recycling. The major advantage of using traps is that the farmers have the ability to monitor the species and number of guava fruit fly present in their fields. Information from farmers' field regarding the species/ race(s) of guava fruit fly and the insecticide use pattern during the management is indispensable to gather. That will give a better understanding about the severityof the problem. Related farmer surveys have been carried out by Lar Soe, 2007 at Nakhon Pathom in Thailand.

Such type of survey should be conducted to know the obstacles of guava cultivation among the guava cultivators of southern region in the country. Moreover, molecular detection of fruit flies' species/race(s) could offer the great opportunity as whole adoption of Integrated Pest Management (IMP) schedule in favor of farmers of guava cultivation of the country

Molecular genetics is a sub-field of genetics that applies an "investigative approach" to determine the structure and/or function of genes in an organism's genome using genetic screens. Researchers search for mutations in a gene or induce mutations in a gene to link a gene sequence to a specific phenotype. The molecular analysis helps identification of the biofilm composition to the genus level and to determine shifts in the community due to environmental changes. The main objective of this work is the standardization of the molecular detection protocols of different species and race(s) of fruit fly infesting guavas produced in Bangladesh.

DNA barcodes have been used successfully for the identification of fruit flies of the family Tephritidae in many geographic regions (Blacket *et al.* 2012, Virgilio *et al.* 2012). DNA barcode sequences were effective for species identification with >94% of the specimens being correctly identified (Kunprom and Pramual 2016).

Molecular detection augmented with morphometric is an efficient technique of insect detection which is not limited by sex and stage of development of the target species. Many kinds of molecular detection including microsatellites, internal transcribed spacer 1 (ITS1), amplified fragment length polymorphism, 16S rRNA, 12S rRNA, mitochondrial cytochrome oxidase I (mt COI), etc. have been employed as molecular detection approach and the (mt COI) used to standardize the detection of the fruit fly species infesting Bangladeshi guava. The variable region of the mitochondrial cytochrome oxidase subunit I (COI) gene was used to obtain better estimates of divergence for species of the fruit fly's complex. Many previous studies have been used the COI gene for obtaining the divergences among the species (Liu *et al*. 2011, Zhang *et al*. 2010).

The COI gene was used because it appeared to be among the most conservative protein-coding genes in the mitochondrial genome of animals (Brown. 1985). The COI gene was the slow-evolving gene in the mitochondrial protein-coding gene (Simon *et al*. 1994). The conserved sequence of the COI gene allows researchers to use it as a 'universal' primer, and it has been widely used to investigate multiple different taxa and for interspecific analysis. According to Hebert *et al*. 2003, in terms of the degree of variation, it was expected to be low in intraspecific variation such that through a given cluster analysis, the sequences from polymorphic species would cluster together in a genetic distance.

Considering the problems of fruit fly of guava, a research program with 5 (five) different experiment was undertaken for fullfiling the following objectives:

1. detection of the most destructive species of fruit flies attacking the vulnerable stage of guava fruit
2. identification of the different species of fruit flies attacking the guava fruit at different location of Bangladesh
3. to study the morphometric and phylogenetic relation and genetic diversity in the distribution of different species of fruit flies attacking the guava fruit
4. standardization of the molecular detection protocols of different species and race(s) of fruit fly infesting guavas produced in Bangladesh and
5. to find out the effective and sustainable management technique to improve the guava production by reducing the fruit fly infestation.

Chapter-II

REVIEW OF LITERATURE

CHAPTER II
REVIEW OF LITERATURE

2.1 Guava

Guava is a common tropical fruit cultivated in many tropical and subtropical regions (Morton 1987). *Psidium guajava* Linnaeus (common guava, lemon guava) is a small tree in the myrtle family (Myrtaceae), native to Mexico, Central America, the Caribbean and northern South America (Morton 1987). Although related species may also be called guavas, they belong to other species or genera, such as the pineapple guava, *Acca sellowiana* Linnaeus. Guava under the genus Psidium. This genus has about 150 species. 'The genus *Psidium guajava'* grown in our country.

The origin of guava is tropical America. It's extended rapidly from Peru to Mexico. Now a days guava grown all over the tropics and subtropics. The major producing countries are India, Mexico, Brazil, Pakistan, Sri Lanka, Bangladesh, Burma, Thailand, Malaysia, Indonesia, Hawaii, Philippine, Florida. Optimum growing temperature for guava is 23-28 °C., but the matures trees can tolerate up to 45°C (CABI,2017).

The most frequently eaten species, and the one often simply referred to as "The guava", is the apple guava (*Psidium guajava* Linnaeus). Guavas are typical Myrtoideae, with tough dark leaves that are opposite, simple, elliptic to ovate and 5–15 centimetres (2.0–5.9 in) long. The flowers are white, with five petals and numerous stamens. The fruits are many-seeded berries (CABI 2017).

2.1.1 Origin and distribution

Guavas originated from an area thought to extend from Mexico or Central America and were distributed throughout tropical America and the Caribbean region (Morton 1987). They were adopted as a crop in subtropical and tropical Asia, the southern United States (from Tennessee and North Carolina south, as well as the west and Hawaii), tropical Africa, South Asia, Southeast Asia, and Oceania (CABI 2017). Guavas are now cultivated in many tropical and subtropical countries (Morton 1987). Several species are grown commercially; apple guava and its cultivars are those most commonly traded internationally (Morton 1987). Guavas also grow in southwestern Europe, specifically the Costa del Sol on Málaga, (Spain) and Greece where guavas have been commercially grown since the middle of the 20th century and they proliferate as cultivars (CABI 2017).

Mature trees of most species are fairly cold-hardy and can survive temperatures slightly colder than 25 °F (−4 °C) for short periods of time, but younger plants will likely freeze to the ground (Sauls 1998). Guavas were introduced to Florida in the 19th century (Morton 1987) and are now grown in Florida as far north as Sarasota, Chipley, Waldo and Fort Pierce. However, they are a primary host of the Caribbean fruit fly and must be protected against infestation in areas of Florida where this pest is present (Boning and Charles 2006). Guavas are of interest to home growers in subtropical areas as one of the few tropical fruits that can grow to fruiting size in pots indoors. When grown from seed, guavas bear fruit as soon as two years and as long as 40 years (Morton 1987).

Guava fruits, usually 4 to 12 centimeters (1.6 to 4.7 in) long, are round or oval depending on the species (Morton 1987). They have a pronounced and typical fragrance, similar to lemon rind but less sharp. The outer skin may be rough, often with a bitter taste, or soft and sweet.

Varying between species, the skin can be any thickness, is usually green before maturity, but may be yellow, maroon, or green when ripe. The pulp inside may be sweet or sour and off-white ("white" guavas) to deep pink ("red" guavas). The seeds in the central pulp vary in number and hardness, depending on species (Anon.2010)

2.1.2 Production

In 2019, world production of guavas was 46.5 million tons, led by India with 41% of the total. Other major producers were China (10%) and Thailand (7%) (Tridge 2016).

2.1.3 Use

In Mexico and other Latin American countries, the guava-based beverage agua fresca is popular. The entire fruit is a key ingredient in punch, and the juice is often used in culinary sauces (hot or cold), ales, candies, dried snacks, fruit bars, and desserts, or dipped in chamoy. *Pulque de guava* is a popular alcoholic beverage in these regions.

In many countries, guava is eaten raw, typically cut into quarters or eaten like an apple, whereas in other countries it is eaten with a pinch of salt and pepper, cayenne powder or a mix of spices (masala). It is known as the winter national fruit of Pakistan. In the Philippines, ripe guava is used in cooking *sinigang*. Guava is a popular snack in Taiwan, sold on many street corners and night markets during hot weather, accompanied by packets of dried plum powder mixed with sugar and salt for dipping. In East Asia, guava is commonly eaten with sweet and sour dried plum powder mixtures (Tridge 2016).

Guava juice is popular in many countries. The fruit is also often included in fruit salads. Because of its high level of pectin, guavas are extensively used to make candies, preserves, jellies, jams and marmalades (such as Brazilian *goiabada* and Colombian and Venezuelan *bocadillo*), and as a marmalade jam served on toast (Morton 1987).

Red guavas can be used as the base of salted products such as sauces, substituting for tomatoes, especially to minimize acidity. A drink may be made from an infusion of guava fruits and leaves, which in Brazil is called *chá-de-goiabeira*, i.e., "tea" of guava tree leaves, considered medicinal (Anon.2010).

2.1.4 Nutrients

Guavas are rich in dietary fiber and vitamin C, with moderate levels of folic acid. Low in calories per typical serving, and with few essential nutrients, a single common guava (*P. guajava*) fruit contains 257% of the Daily Value (DV) for vit. C. Nutrient content varies across guava cultivars. Although the strawberry guava (*P. littorale* var. *cattleianum*) has only 39% of the vitamin C in common varieties, its content in a 100 gram serving (90 mg) still provides 100% of the DV (Anon. 2010).

Guava is the major source of vitamin C and Pectin. Guava contains moisture 80-83%; acid 2.45%; reducing sugar 3.5-4.45%, non-reducing sugar 3.97-5.23%, TSS 9.73-14.23; Potassium 0.48%, vitamin C 260 mg per 100 g of edible' potion (Akhter *et al*, 2005). However, nutrients contents depend on variety, season, maturity etc. Guava can be eaten both as in green and ripe stages. Fresh fruits used as salad, pudding etc. Jam, jelly, juice, pickles, ice cream can be made from guava through processing. Tea can be made from leaves of guava.

2.1.5 Varieties

There are lots of varieties in the world. Varietal variations like small, large, round, oblong, oval, pear shaped, white or red flesh also found in our country. Brief description of some of the varieties are as follows:

Kazi piyara: This variety was collected from Thailand in and around nineteen hundred eighties and named as Kazi Piyara. Fruits are oblong, flesh are crispy, light sour, tasty. Seeds are hard and profuse. Average fruit weight 500g. Quick growing and about 5-7m height (Akhter *et al,* 2005).

Sharupkathi: This variety grown extensively in Sawupkanti areas of Barisal district. Medium size tree, fruits oval to round, slightly rough surface. White flesh, very sweet and soft and less seeded (Akhter *et al,* 2005).

'Kanchannagar: This variety grown Kanchannagar areas of Chittagong district. Less seeds than other varieties. Pear shaped fruits, much pulp and highly tasted (Akhter *et al,* 2005).

FTIP BAU Piyara 1 (Misti): This variety collected from BAU campus. All season regular bearing semi-dwarf variety. Fruits round, glossy, very sweet, soft seeded. No disease and pest noticed in the BAU Germplasm center (Akhter *et al* 2005).

FTIP BAU Piyara 2 (Ranga): Semi-dwarf regular bearing variety. Fruits are almost round to oval, yellowish green, red flesh, large size (300-600g), crispy, sweet, rough surface, seed medium hard. As the flesh is red, and high yielder this variety is highly acceptable to everyone (Akhter *et al,* 2005).

FTIP BAU Piyara 3 (Choudhury): Local collection. Fruits round, greenish yellow, flesh reddish pink, medium crispy, sweet, soft seeded, less seeds. This variety is attractive, sweet and tasty. No disease and pest are noticed (Akhter *et al.* 2005).

FTIP BAU Piyara 4 (Apple): Fruits round to oval, shiny green, smooth surface, attractive, soft, sweet flavored, seed medium hard. Infested by fruits fly. This variety is attractive, tasty and high yielder (Akhter *et al.* 2005).

FTIP BAU Piyara 5 (Oval): Collected from abroad. Fruits are round and sinus, white fleshed, soft seeded, crispy, highly sweet, flavored. Plants are spreads like umbrella. Average fruit weight 300.5g. No disease and pest noticed. Fruits produced in both the season (Akhter *et al.* 2005).

FTIP BAU Piyara 6 (Jelly): Collected from Hawaii. Pectin high, so jam and jelly prepared from this variety. Flesh bright red. Fruit size 120-250g. Fruits oblong, attractive, seed soft, crispy, heavy sour. No disease and pest noticed. Fruits produced in both the season (Akhter *et al.* 2005).

Apart from these, there are other varieties like Mukandapuri, Angur, IPSA, BARI Piyra 2 and 3, Syedi, Allahabad, L-49, Cherry, Kashi also grown in Bangladesh.

2.1.6 Insects and pests

Pests: Most of the guava plant affected by mealy bug. White insects in cluster attacked plants. They eat young leaves, twig and flowers. Infected fruit dry out. Infected twig may prune to control it. Malathion @ 20ml/10l water should be sprayed (Akhtaruzzaman *et, al.* 1999). White fly this is a serious pest. Spiraling cotton wool white fly look like blocks

of cotton. Sucking the leaves and finally the leaves die. Detergent powder @ 10 g/l water should be sprayed.

Diseases: Wilt, Anthracnose, canker leaf rust, die back, seedling blight are the major dieses of guava (Akhtaruzzaman *et al.* 1999).

Anthracnose: Leaves, branches fruits everything affected by anthracnose. Die back of twig is the symptom of the disease. Cool and hot both weathers favored the disease. Black spots are formed in infected fruits which become nonmarketable. Infected branches should be pruned. Fungicides like Bordeaux mixture (4:4:50) or Dithane M-45 should be sprayed (Akhtaruzzaman *et al.* 1999).

Wilt: Plants in acidic soil susceptible to this disease. Leaves in diseased plants become yellow and finally shredded. All branches wilted and finally the plants die. Only the remedy is to use wilt resistant rootstock. FTIP-BAU GPC succeed on controlling this disease through grafting on wilt resistant root stock (Akhter *et al.* 2005). Yield' Full bearing started from 3-4 years of planting. Fruits can be harvested after 4-5 months of flowering. Yield depends on variety, age, growing conditions etc. Full bearing trees may yield 400-800 fruits and 20-30t/ha.

4.2.1. Taxonomy and Nomenclature

Bactrocera dorsalis is a member of the Oriental fruit fly (*B. dorsalis* Hendel) species complex. This species complex forms a group within the subgenus *Bactrocera* and the name may therefore be cited as *Bactrocera* (*Bactrocera*) *dorsalis*. *B. dorsalis* was originally treated as a single species, widespread over Asia, until it was split into several species, with the description of *Bactrocera carambolae*, *B. papayae* and *B.*

philippinensis by Drew and Hancock (1994). Native range of true *B. dorsalis* became restricted primarily to continental Asian countries north of the Malay Peninsula. *Bactrocera invadens* was later described by Drew *et al.* (2005), when established populations were detected in East Africa (Lux *et al.* 2003) and in West Africa (Vayssières 2004). *Bactrocera philippinensis* was designated a synonym of *B. papayae* by Drew and Romig (2013).

The synonymization of *B. invadens* and *B. papayae* under *B. dorsalis* and leaving *B. carambolae* as a distinct species by Schutze *et al*. (2014), who summarized the extensive research and evidence supporting the synonymization. Records of *B. pedestris* (Bezzi) from outside of the Philippines are mostly based on misidentifications of *B. dorsalis*.

2.2 Fruit fly biology

Fruit flies can be commonly found in restaurants, homes, warehouses and food storage or processing plants, as well as grocery stores, wine cellars and anywhere else food is left to ferment and decay. Adult fruit flies typically have red eyes and measure 3 to 4 mm in length. The front of the fruit fly's body is tan or brown in color, while the rear portion has dark bands (Drew and Hancock 1994).

Female and male fruit flies engage in a series of dances prior to mating. The male approaches the female slowly, drumming his feet upon her head. Both flies then drag their feet from side to side while facing one another. Before consummation, the male spreads his wings, twisting the leading edges downward (Drew and Romig 2013).

Fruit flies breed rapidly. Females are capable of laying an average of 500 eggs, which can pass through the larval and pupal stages to become adults within a matter of days.

Fruit flies typically lay their eggs on moist, organic materials or near the surface of fermenting food. After fruit fly larvae emerge from eggs, they feed on the decaying materials in fermenting food (Drew and Romig 2013).

In the year 1930, fruit flies had already been recognized as indispensable to genetic study and research and they remain so today. Fruit fly populations are inexhaustible, simple to breed and are not costly subjects. Their genetic makeup is also incredibly simple.

2.2.1 Fruit fly metamorphosis

Fruit flies undergo three stages of development before emerging as adults: egg, larva and pupa. At room temperature, fruit flies can develop into adults within one to two weeks. The egg and larval stages span approximately eight days, while the pupal stage lasts six days. The adult fruit fly lives for several weeks. Twenty-four hours after a female fruit fly lays her eggs, larvae hatch. Fruit fly larvae undergo molting stages known as instars, during which the head, mouth, cuticle, spiracles, and hooks are shed. During the larva's third instar, it crawls to a drier area to pupate. The pupa case is formed from the larval skin as it darkens and develops a hard surface (Drew and Hancock 1994). Fruit fly adults develop in this pupal stage. Twenty-four hours before the adult emerges, the pigmentation of the eyes and the folded wings are already visible through the pupal case, called the puparium. The pupa darkens just before the adult fly emerges. When metamorphosis is complete, the adult fruit fly pushes its way through the anterior end of the puparium, known as the operculum. Initially, the fruit fly is light in coloration, with expanded wings and an elongated abdomen. Within a few hours, the fruit fly darkens, extends its wings and expands its abdomen (Drew and Hancock 1994). Approximately 48 hours after emerging from the puparia, female fruit flies are sexually mature and can

begin breeding and laying eggs. Adult fruit flies are fertile for the entirety of their life spans. Female fruit flies can store sperm from multiple inseminations for use in future egg productions (Drew and Hancock 1994).

Many species of fruit flies exist throughout the world. They vary in size, color and shape. Many fruit flies breed rapidly, although mating rituals range from intricate dances to territorial control or traditional breeding, wherein the male simply impregnates the female (Drew and Hancock 1994). Once impregnated, female fruit flies are capable of laying more than 500 eggs. Eggs are usually laid in fruit or other sugary, decaying organic material. Fruit flies choose such breeding sites in order to ensure a food source for their larvae after they have hatched, as well as protection from certain predatory species. Optimal temperatures for fly eggs range from 75 to 80 degree F, which is also the average temperature of a home's interior (Drew and Hancock 1994).

2.2.2 Fruit Fly Eggs

Fruit fly eggs measure only 1/2 mm in length. Under a microscope, they are yellow in color and appear to be the shape of a grain of rice. In optimal temperatures, fruit fly eggs hatch into larvae within 30 hours. These larvae ultimately mature into adults that feed, breed and lay their own generation of eggs. The life cycle of some fruit fly species is less than one week (Drew and Hancock 1994)

2.2.3 Fruit Fly Larvae - Fruit Fly Maggots

Like many other insects, fruit flies pass through egg, larva and pupa stages before emerging as sexually mature adults (Drew and Hancock 1994). Fertilized females lay their eggs in overripe fruit and other sources of soft, sweet, decaying matter. Depending

on the species, this behavior can be detrimental or beneficial to the local environment. For example, some species can act as scavengers and are beneficial; however, inside homes, this is not the case. Each female typically produces hundreds of eggs. Larvae are the small wormlike at early stages of fruit flies. These larvae prefer the wet fermenting areas near fruit or other sweet items. They can also be found commercially in baking areas where sweet fruit fillings may have fallen and have been moistened with wash water. Larvae prefer fermenting items, and if the fruit or other source becomes too well-fermented, the larvae will no longer feed, as fungi and bacteria may become too prevalent. After fruit fly eggs hatch, larvae begin to feed on the decaying materials within which they were laid. Larvae consume as much food as possible in order to store energy and nutrients for the upcoming pupal stage. After feeding, larvae find cooler, dryer locations within which to pupate. Inside the pupal case, the larva changes to an adult. The cycle continues as adult males breed with females to propagate the species (Drew and Hancock 1994).

Fruit fly larvae will eventually use the last-stage larval skin to form a pupal case, or shell, in which to morph into an adult. Many species of fruit flies exist throughout the world. They vary in size, color and shape. Many fruit flies breed rapidly, although mating rituals range from intricate dances to territorial control or traditional breeding, wherein the male simply impregnates the female. Once impregnated, female fruit flies are capable of laying more than 500 eggs. Eggs are usually laid in fruit or other sugary, decaying organic material. Fruit flies choose such breeding sites in order to ensure a food source for their larvae after they have hatched, as well as protection from certain predatory

species. Optimal temperatures for fly eggs range from 75 to 80 degrees Fahrenheit, which is also the average temperature of a home's interior (Drew and Hancock 1994).

2.2.4 Distribution

The revision by Drew and Hancock (1994) split the original *B. dorsalis* Hendel into *B. carambolae* Drew and Hancock and three species, *B. dorsalis* Hendel, *B. papaya* Drew and Hancock and *B. philippinensis* Drew and Hancock, with mutually exclusive geographic ranges. *B. dorsalis* sensu stricto became restricted to mainland Asia (and Taiwan) and its adventive populations in Hawaii and French Polynesia, and newly described *B. papayae* Drew and Hancock ranged from southern Thailand and Peninsular Malaysia through East Malaysia and all Indonesian islands to New Guinea Island, and *B. philippinensis* was restricted to the Philippines and introduced to Palau. When *B. dorsalis* invaded continental Africa, around 2003, it was described as *B. invadens*, the origin and native range of which was believed to be Sri Lanka (Drew *et al.* 2005).

With the exception of *B. carambolae,* all of these species are now treated together as *B. dorsalis* sensu lato, which is the most destructive, invasive and widespread of all Dacine fruit flies. The distribution and invasion history of *B. dorsalis* are summarized on a map published in (Vargas *et al.* 2015). EPPO (2014) includes California, USA, in the distribution because the fly is repeatedly trapped there in small numbers. Whether or not *B. dorsalis* is actually established in continental America is a hotly debated topic (Suckling *et al.* 2014, Papadopoulos *et al.* 2013).

2.2.5 History of Introduction and Spread

Oriental fruit fly has been established since about 1945 and quickly became widespread in the Hawaiian Islands (Pemberton, 1946). *B. dorsalis* (*B. papayae*) is believed to have been introduced accidentally into the eastern Indonesian province of Irian Jaya [Papua Barat] prior to 1992, when it was first detected in the border areas of Papua New Guinea (Sar *et al.* 2001). By 2000 it had spread throughout much of mainland Papua New Guinea (Sar *et al.* 2001). In March 1993, it was detected on several northern islands in Torres Strait, Queensland (Fay *et al.* 1997). This represents the first detection of this known invasive species in Australian territory and it was quickly eradicated. In October 1995, it was detected in the Cairns region of northern Queensland (Fay *et al.* 1997, Hancock *et al. 2000b*). This was probably the result of accidental introduction from Papua New Guinea.

The fly had spread throughout the Cairns-Mareeba-Mossman region and detections were made from Cooktown to Cardwell before it was eradicated during 1997-1998 (Cantrell *et al.* 2002, Hancock *et al.* 2000b). An isolated outbreak at Mount Isa in western Queensland was eradicated during 1997 (Cantrell *et al.* 2002, Hancock *et al.* 2000). Since then, there have been occasional incursions onto Torres Strait islands from Papua New Guinea. These have been eradicated whenever establishment occurred.

In 1991, the Ministry of Agriculture in Mauritius established a network of quarantine traps for exotic fruit flies. In June 1996, one Oriental fruit fly (*B. dorsalis*) was found in a trap near the airport in Mauritius (White 2006). The quarantine trap grid was immediately extended in the area surrounding the airport, and fruit in the area was inspected for larval infestations. The larvae were reared from infested fruit found near the airport and it was

clear that the oriental fruit fly had established in southern Mauritius. Morphological examination indicated that the flies had originated in southern India.

An eradication programme for *B. dorsalis* infesting various tree crops was conducted from July 1996 to April 1998, in the southern region of Mauritius, using bait application technique (BAT), male annihilation technique (MAT), cover spray of trees with ripening fruits, soil drenching under trees with ripening and fallen fruits, and fruit clean-up and disposal. The introduction was probably accidental, as the first flies were detected in the airport neighbourhood.

In 2003, an unknown *Bactrocera* species was found in Kenya. Taxonomic expertise showed that it could not be a native species of Africa, but that it proved to be a member of the *B. dorsalis* complex. Identical specimens were collected earlier during a survey in Sri Lanka in 1993 and initially classified as aberrant forms of *B. dorsalis*. It was decided that both populations belonged to the same, hitherto undescribed species: *B. invadens*, which was formally described in 2005 (Drew *et al.* 2005) and designated a synonym of *B. dorsalis* a decade later (Schutze *et al.* 2014). After its discovery in Kenya, it was recorded in a number of countries in eastern, central and western Africa in a relatively short time (Mwatawala *et al.* 2004, Drew *et al.* 2005, Meyer *et al.* 2007).

This threat has also been reported in 2004 Senegal (Vayssières 2004), Benin (Vayssières *et al.* 2005) and other West African countries. Recently, the species has also been reported from southern Africa (Meyer *et al.* 2007), southern India (Sithanantham *et al.* 2006) and Bhutan (Drew *et al.* 2007). The exact invasion pathway into Africa is unknown. From 1999 to 2004, an intensive sampling programme was conducted in Kenya (Copeland *et al.* 2004).

It was only in March 2003 that the first specimens were collected from the coastal region (Lux *et al.* 2003). Vayssières and Kalabane (2000) and Vayssières *et al.* (2004) conducted intensive fruit fly sampling in commercial mango (*Mangifera indica*) orchards in different localities in western Africa, in Coastal Guinea and Mali, from 1992 to 1995 and 2000, respectively. None of these yielded any specimens of *B. dorsalis.* The first specimens in that part of the African mainland were detected in June 2004 (*Drew et al.* 2005,Vayssières 2004). The presence of this species in those eastern or western African countries before the beginning of the twenty-first century is, therefore, very unlikely. Unfortunately, no similar studies were conducted at that time in other parts of the African continent where the fly is currently found. The fact that the first specimens were reported from the East African coast appears to indicate that the port of entry could have been an East African (coastal) locality, although there is no proof of this.

2.2.6 Risk of Introduction

The major risk is from the import of fruit containing larvae, either as part of cargo, or through the smuggling of fruit in airline passenger baggage or mail. For example, in New Zealand, Baker and Cowley (1991) recorded 7-33 interceptions of fruit flies per year in cargo and 10-28 per year in passenger baggage. Individuals who successfully smuggle fruit are likely to discard it when they discover that it is rotten. This method of introduction probably accounts for the discovery of at least one fly in a methyl eugenol trap in California, USA every year (Foote *et al.* 1993), although immediate implementation of eradication action plans has ensured that the fly has never been able to establish a proper breeding population, a view that has been challenged in recent years (Papadopoulos *et al.* 2013).

2.2.7 Hosts/Species Affected

With over 300 species of commercial/edible and wild hosts, *B. dorsalis* has the broadest host range of any species of *Bactrocera*. It is a serious pest of a wide range of fruit crops throughout its native range and wherever is has invaded. Due to the confusion between *B. dorsalis* and related species in Malaysia, the Philippines, Indonesia, southern India and Sri Lanka, there are very few published host records which definitely refer to *B. dorsalis*, as opposed to misidentifications of related species within the *B. dorsalis* species complex. Taking China as an example area where the pest populations are definitely the true *B. dorsalis*, the major hosts are apple, guava, mango, peach and pear (XJ Wang, unpublished data, 1988, as reported in White and Elson-Harris (1994)). Other recorded commercial and wild hosts are taken primarily from Allwood *et al.* (1999), Leblanc *et al.* (2013b, 2012) and Hancock *et al.* (2000a).

Records from Africa (as hosts of synonymous *B. invadens*) are mostly sourced from the website on invasive fruit fly pests in Africa.

2.2.8 Notes on Natural Enemies

Bactrocera spp. can be attacked as larvae either by parasitoids or by vertebrates eating fruit (either on the tree or as fallen fruit). Mortality due to vertebrate fruit consumption can be very high as can puparial mortality in the soil, either due to predation or environmental mortality. Parasitoids appear to have little effect on the populations of most fruit flies and Fletcher (1987) noted that 0-30% levels of parasitism are typical. To date there are only a few records of partial biological control success for any *Bactrocera* or *Dacus* spp. (Wharton 1989). Clausen (1978) noted that any benefit was almost entirely due to *Biosteres arisanus* (as *Opius oophilus*) and gave the example of guava fruit (*P. guajava*) attack being reduced from 100 to 22% as a result of reduction

in *B. dorsalis* populations through the effects of parasitism in Hawaii. More recently, *B. arisanus* introduction to French Polynesia has reduced infestations (larvae/kg) on guava, Tahitian chestnut and tropical almond by 70-95%, but reduction in percentage of infested fruits (by at least one larva) was not reduced substantially (Leblanc *et al.* 2013c). A number of parasitoids were also released in Guam against *B. dorsalis* and this programme was reviewed by Waterhouse (1993).

2.2.9 Means of Movement and Dispersal

B. dorsalis is known to have the potential to establish adventive populations in various tropical and subtropical areas. Using microsatellite markers, *Aketarawong et al.* (2007) investigated the population structure and genetic variability in 14 geographical populations across the four areas of the actual species range: Far East Asia; South Asia; South-East Asia; and the Pacific Area. Regarding the pattern of invasion, the overall genetic profile of the considered populations suggests a western-orientated migration route from China to the west. Adult flight and the transport of infected fruit are the major means of movement and dispersal to previously uninfected areas.

2.2.10 Detection and Inspection

Fruits (locally grown or samples of fruit imports) should be inspected for puncture marks and any associated necrosis. Suspect fruits should be cut open and checked for larvae. Larval identification is difficult, so if time allows, mature larvae should be transferred to saw dust (or similar dry medium) to allow pupation. Upon emergence, adult flies must be fed with sugar and water for several days to allow hardening and full color to develop, before they can be identified. Detection is described in the Prevention and Control section under Early Warning Systems (Leblanc *et al.* 2013c).

2.3 Economic Impact

B. dorsalis is a very serious pest of a wide variety of fruits and vegetables throughout its range and damage levels can be anything up to 100% of unprotected fruit. As a result of its widespread distribution, pest status, invasive ability and potential impact on market access, *B. dorsalis* is considered to be a major threat to many countries, requiring costly quarantine restrictions and eradication measures. In Mauritius, the total cost of the eradication operation was approximately US$1 million (Seewooruthun *et al.* 2000).

In Japan, eradication from the Ryukyu Islands has cost more than 200 million euros (Kiritani 1998).

In California, USA it has been estimated that the cost of not eradicating Oriental fruit fly would range from US$ 44 to 176 million in crop losses, additional pesticide use, and quarantine requirements. The cost for the eradication programme in northern Queensland (1995-1999) was AUS$ 33 million, but the estimated annual cost to control the pest, had it been left established, was estimated to be AUS$ 7-8 million (Cantrell *et al*. 2002). In Hawaii, annual losses in major fruit crops caused by *B. dorsalis* may exceed 13%, or US$ 3 million (Culliney 2002).

2.3.1 Environmental Impact

Due to the competition for food, oriental fruit flies would displace other less aggressive fruit fly species. Duyck *et al.* (2004) suggested that the r–K gradient could be used as a predictor of the potential invasive capacity of a species. Species with type K-demographic strategy traits, such as species of the genus *Bactrocera*, would be adapted for competition in saturated habitats. Duyck *et al.* (2004) reported that in all recorded

cases, species further along the r–K gradient, such as *B. dorsalis*; have invaded over r-selected species, such as *Ceratitis capitata*, never the reverse.

2.4 Molecular identification

Molecular genetics was a sub-field of genetics that applies an "investigative approach" to determine the structure and/or function of genes in an organism's genome using genetic screens. Researchers search for mutations in a gene or induce mutations in a gene to link a gene sequence to a specific phenotype. The molecular analysis helped to identify the biofilm composition to the genus level and to determine shifts in the community due to environmental changes (Kunprom and Pramual 2016).

The main objective of this work is the Standardization of the molecular detection protocols of different species and race(s) of fruit fly infesting guavas produced in Bangladesh. DNA barcodes have been used successfully for the identification of fruit flies of the family Tephritidae in many geographic regions (Blacket *et al.* 2012, Virgilio *et al.* 2012). DNA barcode sequences were effective for species identification with >94% of the specimens being correctly identified (Kunprom and Pramual 2016).

Molecular detection augmented with morphometric was an efficient technique of insect detection which was not limited by sex and stage of development of the target species. Many kinds of molecular detection including microsatellites, internal transcribed spacer 1 (ITS1), amplified fragment length polymorphism, 16S rRNA, 12S rRNA, mitochondrial cytochrome oxidase I (mt COI), etc. had been employed as molecular detection approach and the (mt COI) used to standardize the detection of the fruit fly species infesting Bangladeshi guava.The variable region of the mitochondrial cytochrome oxidase subunit I (COI) gene was used to obtain better estimates of divergence for species of the fruit

flies complex. Many previous studies have been used the COI gene for obtaining the divergences among the species (Liu *et al*. 2011, Zhang *et al*. 2010). The COI gene was used because it appeared to be among the most conservative protein-coding genes in the mitochondrial genome of animals (Brown 1985). The COI gene was the slow-evolving gene in the mitochondrial protein-coding gene (Simon *et al*. 1994).

The conserved sequence of the COI gene allows researchers to use it as a 'universal' primer, and it has been widely used to investigate multiple different taxa and for interspecific analysis. According to Hebert *et al*. (2003), in terms of the degree of variation, it was expected to be low in intraspecific variation such that through a given cluster analysis, the sequences from polymorphic species would cluster together in a genetic distance.

2.4.1 FASTA formet

In bioinformatics and biochemistry, the FASTA format is a text-based format for representing either nucleotide sequences or amino acid (protein) sequences, in which nucleotides or amino acids are represented using single-letter codes. The format also allows for sequence names and comments to precede the sequences. The format originates from the FASTA software package but has now become a near universal standard in the field of bioinformatics (Hebert *et al*. 2003).

The simplicity of FASTA format makes it easy to manipulate and parse sequences using text-processing tools and scripting languages like the R programming language, Python, Ruby, Haskell, and Perl.

2.4.2 BLAST Formet

The BLAST program was developed by Stephen Altschul of NCBI in 1990 and has since become one of the most popular programs for sequence analysis. BLAST uses heuristics to align a query sequence with all sequences in a database. The objective is to find high scoring ungapped segments among related sequences.

The existence of such segments above a given threshold indicates pairwise similarity beyond random chance, which helps to discriminate related sequences from unrelated sequences in a database. BLAST is popular as a bioinformatics tool due to its ability to identify regions of local similarity between two sequences quickly. BLAST calculates an expectation value, which estimates the number of matches between two sequences. It uses the local alignment of sequences.

2.4.3 Tajima's D

Tajima's D is a population genetic test statistic created by and named after the Japanese researcher Fumio Tajima (Tajima 1989). Tajima's D is computed as the difference between two measures of genetic diversity: the mean number of pairwise differences and the number of segregating sites, each scaled so that they are expected to be the same in a neutrally evolving population of constant size.The purpose of Tajima's D test is to distinguish between a DNA sequence evolving randomly ("neutrally") and one evolving under a non-random process, including directional selection or balancing selection, demographic expansion or contraction, genetic hitchhiking, or introgression.

A randomly evolving DNA sequence contains mutations with no effect on the fitness and survival of an organism. The randomly evolving mutations are called "neutral", while

mutations under selection are "non-neutral". For example, a mutation that causes prenatal death or severe disease would be expected to be under selection. In the population, the frequency of a neutral mutation fluctuates randomly (i.e., the percentage of individuals in the population with the mutation changes from one generation to the next, and this percentage is equally likely to go up or down) through genetic drift.

The strength of genetic drift depends on population size. If a population is at a constant size with constant mutation rate, the population will reach an equilibrium of gene frequencies. This equilibrium has important properties, including the number of segregating sites and the number of nucleotide differences between pairs sampled (these are called pairwise differences). To standardize the pairwise differences, the mean or 'average' number of pairwise differences is used. This is simply the sum of the pairwise differences divided by the number of pairs and is often symbolized by pi (Tajima 1989).

The purpose of Tajima's test is to identify sequences which do not fit the neutral theory model at equilibrium between mutation and genetic drift. In order to perform the test on a DNA sequence or gene, you need to sequence homologous DNA for at least 3 individuals. Tajima's statistic computes a standardized measure of the total number of segregating sites (these are DNA sites that are polymorphic) in the sampled DNA and the average number of mutations between pairs in the sample. The two quantities whose values are compared are both method of moment’s estimates of the population genetic parameter theta, and so are expected to equal the same value. If these two numbers only differ by as much as one could reasonably expect by chance, then the null hypothesis of neutrality cannot be rejected. Otherwise, the null hypothesis of neutrality is rejected.

2.4.4 Seasonal Abandunce

Seasonal population dynamics of any insect pest provide knowledge on relationship between weather factors and insect abundance. It indicates the farmers of a particular area or region about management program of the pest. So, the growers can take proper control measures to prevent loss due to insect attack.

Insect pest species of guava, their occurrence and sseasonal dynamics have been studied in many countries (Clarke *et al.* 2001, Sarada *et al.* 2001, Mwatawala *et al.* 2006). But there is scarcity of information on insect pest species of guava in Bangladesh, their nature of occurrence and seasonal abundance. So, the aim of the study is to know the abundance of the sucking (mealy bug, white fly and scale insect) and chewing (fruit fly) insects which cause significant infestation on guava, and to find out the effect of the meteorological factors such as temperature and relative humidity on the abundance of the insects.

2.5 Management of fruit fly by Cultural & mechanical control

2.5.1 Cultural Control and Sanitary Methods

One of the most effective control techniques against fruit flies in general is to wrap fruit, either in newspaper, a paper bag, or in the case of long/thin fruits, a polythene sleeve. This is a simple physical barrier to oviposition, but it has to be applied well before the fruit is attacked. Little information is available on the attack time for most fruits but few *Bactrocera* spp. attack prior to ripening. Other control and sanitary methods include the removal and destruction of fallen fruits because they may harbour larvae that could form a next generation. Destruction can either be by burning, deep burrowing (at least 0.5

m below the surface), feeding them to pigs, or putting the fruits in dark-colored plastic bags and placing them in the sun (so that the inside temperature rises and kills the larvae). Another method is raking or disturbing the soil below the fruit trees using other means. This will expose the puparia, leading to desiccation or predation by other organisms. Several authors highly advocated hand picking of infested fruits to reduce fruit fly damage on cucurbit vegetables (Atwal 1999).

Nasiruddin and Karim (1992) recommended collection and destruction of infested fruits with larvae inside for reducing fruits fly population on snake gourd. Mitchell and Soul (1990) reported that this practice is widely used in USA for suppressing Mediterranean fruit fly *Ceratitis capitata*. Atwal (1999) suggested such mechanical control measures in farmer's fields as normal practice for effective control against this pest in India. Several authors recommended field sanitation for suppression of fruit fly population in many countries (Smith 1992, Mitchell and Saul 1990, Agarwal *et al*.1987).

2.5.2 Cultural control

The results of screening trials, for different cultural practices, revealed that plant to plant distance of 45 cm, manifested minimum fruit infestation, maximum marketable fruits/plant, minimum yield loss/plant and maximum marketable yield/plant, although, a plant-to-plant distance of 75 cm showed lower fruit and economic yields. Lower fruit infestation and yield loss/plant in plots at 45 cm is attributed to the behavior of cucurbit fruit fly, proper ventilation of the creepings and exposure of more fruit to sun rays due to more spacing among bitter gourd creepings. An experiment was conducted by Sultana *et al.* (2010) on effectiveness of some mechanical and cultural methods for suppressing fruit fly in cucumber. There was significant difference among mechanical control, field sanitation and untreated control at the different fruiting stages of cucumber by percent

fruit fly infestation by number. The mean percent of infested fruits of all stages from mechanical control (25.36%) showed the lower level of infestation compared with field sanitation (29.68%) and control (30.12%).

Sultana *et al.* (2010) also reported on effectiveness of some mechanical & cultural methods in case of infested fruit weight by fruit fly in cucumber. There was significant difference among mechanical control, field sanitation and untreated control at the different fruiting stages of cucumber by percent infested fruit weight by fruit fly infestation. The mean percent of infested fruits weight of all stages from mechanical control (21.81%) showed the lower level of infestation compared with field sanitation (24.89%) and control (25.12%).

The percent weight reduction per fruit due to fruit fly infestation at different reproductive stages. The mean value of weight reduction per fruit ranged from 34.36 to 76.87%. Significantly the lowest weight reduction per fruit was obtained in cucumber harvested from mechanically controlled plots which was statistically similar to that from Malathion treated plots. The highest weight reduction was observed in untreated plots which was similar to those from cultural control plots.

2.5.3 Ploughing of Soils

The dacine fruit flies not only pupate in soil but also over winter there in this stage. If during winter months, the soil in the field is turned over or given a light ploughing, the pupae underneath is exposed to sunlight and killed. Besides, they also become a prey to predators like ants, birds, lizards etc. and parasitoids. A large number of these are killed due to mechanical injury during ploughing (Agarwal *et al.* 1987, Chatlapadhyay 1991, Nasiruddin and Karim 1992, Kapoor 1993).

2.5.4 Physical Control

Sometimes attempts were made to control insects by the physical manipulation of environment or employment of physical sources.

2.5.5 Bagging of Fruits

Sometimes each and every fruit is covered by a paper or cloth bag to block the contact of flies with the fruit thereby protecting from oviposition by the fruit fly. This is quite useful when the fruits are within the reach and the number of fruits to be covered is less. This is a tedious task for big commercial orchards (Kapoor 1993). Simple bagging procedures without considering the days after anthesis and period of retaining the bags were conducted by few authors. Bagging bitter gourd in Taiwan at the stage of 3-4cm fruit length and sponge gourd at 5-6cm length with two layers of paper bags every after 2-3 days against *B. cucitrbitae* increased yield by 40-58 per cent compared to control. Amin (1995) obtained significantly lowest fruit fly infestation (4.61%) in bagged cucumber compared to other chemical and botanical control measures. Covering of teasel gourd by polythene bag reduced the fruit fly infestation substantially (Anon 1988).

An experiment was conducted by Akhtaruzzaman (1999) to evaluate the efficacy of bagging to suppress the cucurbit fruit fly infestation and revealed that the mean of all stages of fruit fly infestation was significantly lower (5.53%) in treatment (T_2) where bagging of fruits at 3 days after anthesis was made and retained for 5 days. An experiment was carried out by Hossain *et al.* (2002) to evaluate three packages viz., perforated polyethylene bagging, covering fruits by PVC pipe and poison bait trap against fruit fly on cucumber. Bagging of cucumber with perforated polythene bags at

immature stage significantly reduced the fruit infestation. The level of fruit fly infestation under PVC pipe was higher compared with bagged ones but it was lower than bait trap used. This method has been tried with the use of colorless polythene bags having a few holes made with an ordinary pin (Narayer and Batra 1960).

An experiment was conducted by Aktaruzzamn *et al.* (1999) on suppressing fruit fly infestation by bagging cucumber at different days after anthesis. They observed the following results: -

The mean of all stages of fruit fly infestation was significantly lower (5.53%) in T_2 where bagging of fruits at 3 days after anthesis was made and retained for 5 days. Fruit fly infestation under T_1, T_3, and T_4 comprising bagging of fruits at 1, 5, and 7 days after anthesis and retained for 3, 7 and 7 days, respectively were statistically similar. These infestations were also statistically comparable to those of untreated control. Here the best performance in suppressing fruit fly was obtained from treatment involving bagging of fruits at 3 days after anthesis and retaining the bag for 5 days. Table 4 also shows that treatment T_2 comprising fruit bagging at 3 days after anthesis and left for 5 days made statistically significant suppression of fruit fly thorough the initial, early, mid and late fruiting stages.

Significantly the lowest percent of fruit infestation by weight was obtained in fruits harvested from T_2 treatment comprising bagging of fruits at 3 days after anthesis and continued for 5 days. The values of fruit infestation by weight under T_1, T_3, T_4 and untreated control plots were statistically comparable to each other. Similarly, trend of fruit infestation by weight was observed in case of fruits harvested at early fruiting stage. At fruit initiation stage significantly minimum fruit infestation by weight was obtained

under T_2 treatment. At this stage there was no significant difference in fruit infestation by weight under T_1, T_3 and T_5. At mid-fruiting stage the efficacy of T_1, T_3 and T_4 on reduction of infested fruit by weight was not satisfactory and percent of infested fruit by weight under these treatments was not statistically different from that of untreated control (T_5). At late fruiting stage the percent of infested fruits by weight under T_2, T_4, and T_5 were statistically similar as compared with T_1 and T_3.

Therefore, this study indicated that the treatment T_2 where bagging of fruits was done at 3 days after anthesis and maintained for 5 days reduced the fruit infestation by weight significantly throughout the whole reproductive stages. The weight reduction per fruit was determined on the basis of average fruit weight at each of the four reproductive stages of cucumber. The mean value of weight reduction per fruit (%) recorded under T_2 (bagging of fruits at 3 days after anthesis and kept for 5 days) was statistically significant. The differences in reduction of fruit fly infested fruits under T_1, T_3, T_4 and T_5 were not significant.

Weight reduction per fruit recorded at fruit initiation, early fruiting and late fruiting stages under T_2 were also minimal and the trend of reduction was similar to mean values of all stages. This study indicates that bagging of fruits at 3 days after anthesis and left for 5 days produced the lowest value of weight reduction per fruit. Fine wire netting may sometimes be used to cover small orchards. Though it is a costly method, but this can effectively reduce the fruit fly infestation and protect the fruit from injury and deformation. It also protects the fruit crops against vertebrate pest like birds (Kapoor 1993).

2.5.6 Botanical insecticide control

Botanical insecticides are plant derivatives which have insecticidal properties against pest. Neem oil, Mahogany oil, Allamanda leaf extract were used as botanical in the experiment. Neem oil is a vegetable oil pressed from the fruits and seeds of the neem (*Azadirachta indica*), an evergreen tree which is endemic to the Indian subcontinent and has been introduced to many other areas in the tropics. It is the most important of the commercially available products of neem for organic farming and medicines. Neem oil is generally red as blood and has a rather strong odour that is said to combine the odours of peanut and garlic (Singh and Srivastava 1983).

It is composed mainly of triglycerides and contains many triterpenoid compounds, which are responsible for the bitter taste. It is hydrophobic in nature and in order to emulsify it in water for application purposes, it must be formulated with appropriate surfactants. Neembecidine is such an insecticide derived from seed kernel mixed with other preservatives. Besides this fresh neem seed kernel could be used for this purpose. Neem derivatives have been demonstrated as repellents, antifeedants, and growth inhibitors. Singh and Srivastava (1983) found that alcohol extract of neem oil, *A. indica* (5%) reduced oviposition of *B. cucurbitae* on bittergourd completely and its 20% concentration was highly effective to inhibit oviposition of *B. zonata* on guava.

The Mahogany tree, *Swietenia mahogany* linn is a tree of the Neem family. After long research it has been found that Mahogany tree has various insecticidal properties in addition it is well known for its good quality wood and its green color. After extensive research it has been found that the Mahogany trees have many fold pharmaceutical and medical properties, usefulness as insecticides without destroying the worms and insects

not harmful to crops. The present investigation relates to Mahogany oil and extracts use as a biological and botanical pest control. Furniture made up of Mahogany is very decent and long lasting (Singh and Srivastava 1983).

After long time research it has been found that its fruits are very effective in repelling pests in crops. All parts of the Mahogany tree should be considered dangerous if eaten. Its leaves are a natural insecticide and will repel insects from stored fruits and grains. An experiment was conducted by Amin (1995), in BSMRAU on cucurbit fruit fly and he found in his experiment that mahogany oil reduces 64.32% infestation of fruit over control.

Large yellow flowers that look like fat trumpets adorn the stem tips on allamandas from the spring through the fall. Native to tropical parts of Central and South America, 12 species of allamanda exist. Among the most ornamental and widely grown in American gardens species is *Allamanda cathartica*, also called the golden trumpet. It has some insecticidal effect on some insects like fruit fly, white fly etc. (Amin 1985).

2.5.7 Control with different traps

Apple cider vinegar (ACV), otherwise known as cider vinegar or ACV, is a type of vinegar made from cider or apple must and has a pale to medium amber color. Unpasteurized or organic ACV contains mother of vinegar, which has a cobweb like appearance and can make the vinegar look slightly congealed. ACV is used in salad dressings, marinades, vinaigrettes, food preservatives, and chutneys, among other things. It is made by crushing apples and squeezing out the liquid. Bacteria and Yeast are added to the liquid to start the alcoholic fermentation process, and the sugars are turned into alcohol (Anon 2006).

In a second fermentation process, the alcohol is converted into vinegar by acetic acid-forming bacteria (acetobacter).

Acetic acid and malic acid give vinegar its sour taste (Anonymous 2006). They took two small plastic party cups, put about a cup and a half of apple cider vinegar in each, added three drops of dish soap to break the surface tension and cause the flies to sink and then set them right where the peaches had been. They covered one cup with plastic wrap and punched holes in it like the original post suggested and left the other one uncovered like commenters had suggested as an alternative and more effective method. Within less than minute fruit flies were flying over to investigate the sickly-sweet smell of the apple cider vinegar. They left the two traps out for 24 hours (roughly how long it took for the fruit fly horde to vanish). The covered cup had only a single fly in it. The uncovered cup had dozens of flies in it, blanketing the entire bottom of the cup.

Pheromones are a class of semio-chemicals that insects and other animals release to communicate with other individuals of the same species. The key to these entire behavioral chemicals is that they leave from the body of the first organism, pass through the air (or water) and reach the second organism, where they are detected by the receiver. In insects, these pheromones are detected by the antennae. The signals can be effective in attracting far away mates, and in some cases, can be very persistent, remaining in place and active for days. Long-lasting pheromones allow marking of territorial boundaries or food sources. Other signals are very short lived, and are intended to provide an immediate message, such as a short-term warning of danger or a brief period of reproductive readiness. Pheromones can be of many different chemical types, to serve different functions. Pheromones can range from small hydrophobic molecules to water soluble peptides (Anon 2006).

Since pheromone is naturally occurring biological products, they are environmentally safe, non-target organisms are not affected, insect is less likely to develop resistance and moreover they are effective at incredibly low concentrations. They are active (e.g. attractive) in extremely low doses (one millionth of an ounce). Sex pheromones have been utilized in the insect pest control program through population monitoring, survey, mass-trapping, mating disruption and killing the target pest in the trap. Hossain (2007) in an investigation reported that IPM approaches consisting of pheromone traps with detergent showed the lowest infestation of fruit fly by number (5.11%) in sweet gourd compare with overall infestation by number (30.32%) and by weight (23.07%) was obtained from the control plot. Rahman (2005) observed that the infestation of brinjal shoot, and fruit borer was comparatively lower (19.22%) in pheromone trap used plot than that of untreated control and the reduction of infestation over control was 37.66%. Rahman (2005) also tested some IPM packages and the lowest rate of infestation (6.27%) and infestation reduction over control was 79.65% which was recorded in the package consisting of Marshal 20 EC at 3 days' interval + Mechanical control + Pheromone trap placed at plant canopy at the center of the plot. The principal potential applications of pheromones especially sex pheromones in a sophisticated and environment friendly IPM system are monitoring, mass trapping, attract-and kill and disruption of communication (also known as confusion) (Anonymous 1998). A field experiment was conducted at Sher-e-Bangla Agricultural University farm to find out the effectiveness of different pheromone-trap design for management of cucurbit fruit fly during January to July 2012.

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Among the treatments the Pheromone trap with funnel + Bait trap (T_5) showed the best performance in controlling cucurbit fruit fly. Consequently, highest yield (38.44 t ha-1), highest healthy fruit (35.23 t ha-^{1}) and lowest infested fruit (3.21 t ha^{-1}) were achieved from the treatment. Also, the highest number of fruit fly was trapped in T_5 at early, mid and late fruiting stage. Pheromone trap with funnel (T_3) was superior to other treatments but significantly lower than funnel + Bait trap (T_5) treatment. The experiment revealed that pheromone trap with funnel could be effectively utilized in fruit fly management. Cue-lure traps have been used for monitoring and mass trapping of the melon fruit flies in bitter gourd (Seewooruthun *et al*. (2000). Jaiswal *et al*. (1997) reported that integrated control with pheromone traps, field sanitation and bagging of individual fruits proved very effective against *Bactrocera cucurbitae* in Nepal. Methyl eugenol and cue-lure traps have been reported to attract *B. cucurbitae* males from mid-July to mid – November (Ramsamy *et al*. 1987).

A leaf extract of *Ocimum sanctum,* which contains eugenol (53.4%), beta–caryophyllene (31.7%) and beta–elemene (6.2%) as the major volatiles, when placed on cotton pads (0.3 mg) attract flies from a distance of 0.8 Km. The sex attractant cue-lure traps are more effective than the food attractant tephrit lure traps for monitoring the *B. cucurbitae* in bitter gourd (Ramsamy *et al*. 1987).

2.5.8 Management of fruit fly by pheromone and indigenous bait traps

A poisoned bait gave good control of fruit flies (Steiner *et al.*, 1988). An experiment was conducted by Sultana *et al.* (2010) on the evaluation of potential control measure for fruit fly, *Bactrocera* (Dacus) *cucurbitae*, in bitter gourd. They observed that fruit fly infestation rates in bitter gourd fruits in bait trap treatment plot were 21.51%, 21.29%, 24.04% against 30.57%, 28.53% and 31.25% consecutively in early, mid and late fruiting stage, infested fruits in the control plot which differed significantly. An experiment was conducted on comparative effectiveness of various sex pheromone dispensers and mashed sweet gourd bait traps for fruit fly control. The following results were observed:

Fruit fly capture in pheromone dispensers and the bait trap differed significantly. Cuelure +methyl eugenol + naled captured significantly more fruit flies (269) than any other treatment. It was followed by cuelure liquid +5% dibrom (185) and 92% cuelure +8% naled (172). Catches in mashed sweet gourd and methyl eugenol + naled were the lowest, 86 and 18, respectively. The noteworthy feature of the mashed sweet gourd trap was that it captured both male (25) and female (61) fruit flies, indicating its biological impact in the management of cucurbit fruit fly. On the contrary, all the pheromone traps captured only males. The fruit fly capture can create a negative impact on fruit infestation. The higher the fruit fly capture the lesser was the fruit infestation and higher was the yield (Seewooruthun *et al*. (2000).

The pheromone traps captured the highest number of flies, more than 20 times higher than that captured in indigenous mashed sweet gourd traps, and effected 5 times less fruit infestation than the untreated fields. The mashed sweet gourd baits, although captured lower number of fruit flies than the pheromone traps, significantly lessened fruit

infestation and produced 35% more yield than the untreated control plot. Cucumber yields in pheromone and sweet gourd baited fields were comparable. The results suggested that pheromone and indigenous bait traps have great potential for use as control techniques for fruit fly IPM (Seewooruthun *et al*. (2000).

2.5.9 Management of fruit fly by IPM Package(s)

The effective control of fruit fly in cucurbit demands some new approaches which don't rely only on chemicals, reduce the use of chemicals, safeguard the environment and ensure economic and social acceptance. This might lead to develop the IPM package(s) against this pest.

An effectiveness of various IPM packages for the management of fruit fly on sweet gourd was reported by Rahman (2005). He observed the following result: The mean values of infestation at all stages of reproduction under IPM package (Cypermethrin (@ 0.5 ml/litre of water) applied at 10 days interval + bagging fruits at 3 DAA for 5 days) was lower (23.66%) but statistically similar to that of package (Cypermethrin (@ 0.5 ml/litre of water) applied at10 days interval + bait spray with Malathion and molasses) and package (Hand picking of infested fruit + bagging of fruits at 3 days after anthesis (DAA) for 5 days). The rates of infestation of fruits harvested from plots subjected to package (Hand picking of infested fruits+ bait spray with Malathion and molasses) was significantly higher. Effect of different IPM packages on yield was evaluated in terms of total, healthy and infested fruit yield obtained during the entire reproductive period of the crop.

The results thus obtained including the percent increase/ decrease of yield over control (Akhtaruzzaman *et al.* 1999). The total fruit yield was significantly higher (31.64 t/ha) in the plots treated with the components of IPM package (Cypermethrin (@ 0.5 ml/litre of water) applied at10 days interval + bait spray with Malathion and molasses) which was statistically similar with package (Cypermethrin (@ 0.5 ml/litre of water) applied at10 days interval + bagging fruits at 3 DAA for 5 days) (Akhtaruzzaman *et al.* 1999).

2.5.10 Management of fruit fly by color ribbon

Insects have been shown to be capable of perceiving color and there is considerable variation in terms of wavelength perceived by different insect (Atkins 1978). An experiment was conducted by Rahman *et al.* (2005) on effectiveness of different color ribbons for suppressing fruit fly (*Bactrocera cucurbitae*) infestation on bitter gourd in net house. They observed the following results: The percent fruit infestation was statistically higher (50.96%) in untreated control plot and statistically lower (27.67%) in silver color ribbon treated plot. The higher and lower percent reduction over control by number was obtained from silver color ribbon and yellow color ribbon treated plots, respectively. In net house the effects of yellow, red, indigo and silver color ribbons on fruit fly infestation by weight on bitter gourd were presented. Statistically higher rate of infestation (%) was observed in untreated control plot. The lowest fruit infestation by weight was obtained from the silver color ribbon treated plot. Percent weight reduction per fruit due to fruit fly infestation calculated for the entire reproductive stages and its reduction over control were presented.

2.5.11 Bait Sprays

The dacine fruit flies have long been recognized to be susceptible to attractants. The breakthrough to this principle was achieved around the fifties when protein lures were discovered. Protein hydrolysate insecticide formulations are now used against various dacine fruit fly species (Kapoor 1993).

The poison baits used for various *Dacus* species are : 20g Malatllion 50 per cent or 50ml of Diazinon plus 200g of molasses in 2 litre of water kept in Hat containers or applying the bait spray containing Malatllion 0.05 per cent plus 1 per cent sugar/molasses or 0.025 per cent of protein hydrolysate (20 nil of Malatllion 50EC and 200g of sugar/molasses in 20 litre of water) or spraying slants with 500g molasses plus 50g Malatllion in 50 litre of water or 0.025 per cent Fenitrothion plus 0.5 per cent molasses. This is repeated at weekly intervals where the fruit fly infestation is serious (Kapoor 1993). In 1987, Agarwal *et al.* achieved very good results for fruit fly *(D. cucurbitae)* management by spraying the plants with 500g molasses and 50g Maladiion in 50 litre waters at 7 days intervals. In Hawaii, poisoned bait containing Malatllion and protein hydrolysate gave better results in fruit fly management program (Steiner *et al.* 1988). Baiting (with Malatllion in protein bait sprays) is a good method for the control of *B. aquilonis* and *B. jarvisi* on fruits and vegetables in home gardens in the northern territory of Australia (Smith 1992).

Bait spray (1.0 g Dipterex 80SP and l00.0 g of molasses per litre of water) on snake gourd against fruit fly *(B. cucurbitae)* showed 8.50 per cent infestation compared to 22.48 per cent in control (Nasiruddin and Karim 1990). It is advisable to spray the lower surface of leaves as these flies have the habit of resting there. The flies are attracted to

sugar solution and are killed while trying to feed on them. The time of repeated applications is adjusted in such a way that it is less than the required time for the sexual maturation of newly emerged adult Hies. This is useful for efficient destruction of the population as a whole, rather than only the individuals (Kapoor 1993).

An experiment was conducted by Akhtaruzzaman (1999) to evaluate the efficacy of different bait sprays for suppressing fruit fly infestation on cucumber and revealed that the significant level of mean fruit fly infestation values of all reproduction stages in treated plots was almost comparable with that of fruit initiation stage. The fruit fly infestation at that stage was lowest under having Malathion cover sprayed alone (4.92%).

2.5.12 Regulatory Control

Many countries, such as the mainland USA, forbid the import of susceptible fruit without strict post-harvest treatment having been applied by the exporter. This may involve fumigation, heat treatment (hot vapour or hot water), cold treatments, insecticidal dipping, or irradiation (Armstrong and Couey 1989). Irradiation is not accepted in most countries, and many have now banned methyl bromide fumigation. Heat treatment tends to reduce the shelf life of most fruits and so the most effective method of regulatory control is preferentially to restrict imports of a given fruit to areas free of fruit fly attack.

2.5.13 Chemical Control

Although cover sprays of entire crops are sometimes used, the use of bait sprays are more economical and more environmentally acceptable. A bait spray consists of a suitable insecticide (e.g. Malathion, Spinosad, Fipronil) mixed with a protein bait. Both males and females of fruit flies are attracted to protein sources emanating ammonia, and so insecticides can be applied to just a few spots in an orchard and the flies will be attracted

to these spots. The protein most widely used is hydrolysed protein, but some supplies of this are acid hydrolysed and so highly phytotoxic. Smith and Nannan (1988) have developed a system using autolysed protein. In Malaysia, this has been developed into a very effective commercial product derived from brewery waste. Light-activated xanthene dye is an effective alternative (McQuate *et al.* 2005).

2.5.14 Sterile Insect Technique

Sterile insect technique was used successfully to eradicate *B. dorsalis* from Okinawa and neighbouring islands in the Ryukyu Archipelago, Japan (FFEPO 1987).

2.5.15 Male Suppression

The males of *B. dorsalis* are attracted to methyl eugenol (4-allyl-1,2-dimethoxybenzene), sometimes in very large numbers. On a small scale, many farmers use male suppression as a control technique; however, with flies attracted over a few hundred metres the traps may be responsible for increasing the fly level (at least of males) on a crop as much as for reducing it. However, the technique has been used as an eradication technique (male annihilation), in combination with bait (Bateman 1982).

2.5.16 Early Warning Systems

Many countries that are free of *Bactrocera* spp., e.g., the USA (California and Florida) and New Zealand, maintain a grid of methyl eugenol and cue lure traps, at least in high-risk areas (ports and airports) if not around the entire climatically suitable area. The trap used will usually be modelled on the Steiner trap (White and Elson-Harris 1994) or Jackson trap.

Chapter-III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

Five experiments were conducted in the field and laboratory to achieve the objectives of the study: to detect the destructive species of fruit flies attacking the vulnerable stage of guava and their management, to identify different species of fruit flies attacking the guava at different locations of Bangladesh through their taxonomic studies, its morphometric and phylogenetic relations and distribution of different species attacking the guava fruit, standardize of the molecular detection protocols of different species and race(s) of fruit fly infesting guavas produced in Bangladesh and finally to find out the effective and sustainable management technique to improve the guava production by reducing their infestation.

The materials and methods include a short description of the experiments, materials used for the experiment, design and layout of the experiment, data collection and statistical analysis. The materials and methods adopted for different experiments are discussed here in:

Experiment 3.1 Survey on different fruit flies infested locations of Bangladesh and collection of fruit fly sample

Fruit fly is a major problem in Bangladesh and some species causes huge damage of guava. Inforamation on fruit fly infestation were collected directly from the guava growers and sampling from their orchard.

3.1.1 Collection of farmers' information

The survey was conducted to collect farmers' information on the pest status of fruit fly at four major guava growing regions such as Southern region (Mukundupur, Pirojpur, Jhalokathi), Northern region (Natore, Rajshahi, Chapai Nawabgang and Naogaon), central region (Greater Dhaka, Narshingdi, Gazipur) and Hilly areas (Khagrachari, Rangamati, Bandarbun and Kanchannagar and Patya of Chittagong) in Bangladesh (Table 3.1.1). The selected districts for survey are shown in Figure 3.1.1.

3.1.1.1 Selection of Farmers and Their Characteristics

Guava growers were selected from the four guava growing regions viz., Southern region, Northern region, central region (Greater Dhaka) and Hilly areas of Bangladesh.Three Upazilla were selected from each district of the regions and three villages under each Upazilla were randomly selected for the survey and field inspection. From each listed village, 10 farmers were randomly selected by applying the statistical random chart. So, for each region a total of 12 upazilla, 36 villages and 360 farmers were selected for the study. For this purpose, a list of guava growers of the selected villages was made with the help of the Sub-Assistant Agricultural Officer (SAAO) of the respective areas. Thus, the basic demographic data of each of the selected farmer were collected by administering a simple checklist.

Table 3.2.1: Fruit fly survey locations in Bangladesh with selected districts along with their respective latitude, longitude and date of collection

Survey locations for sample collection	District	Latitude	Longitude	Date of collection
Pirojpur sadar	Pirojpur	22.5841° N	89.9720° E	July, 2018
Jhalokati sadar	Jhalokati	22.5721° N	90.1870° E	July, 2018
Patiya	Chittagong	22.3569° N	91.7832° E	April, 2019
Bhandarban sadar	Bhandarbhan	21.8311° N	92.3686° E	May, 2019
Mukundopur	Brahman Baria	23.9675° N	91.1119° E	October, 2018
Savar	Dhaka	23.8479° N	90.2576° E	April, 2019
Gazipur sadar	Gazipur	23.9999° N	90.4203° E	April, 2019
Rajshahi sadar	Rajshahi	24.3745° N	88.6042° E	April, 2018
Chapainawabgonj sadar	Chapainawabgonj	24.7413° N	88.2912° E	April, 2018
Natore sadar	Natore	24.4079° N	88.9749° E	April, 2018
Rangamati sadar	Rangamati	22.3760° N	92.120° E	May, 2019
Khagrachari sadar	Khagrachari	23.0417° N	91.9944° E	May, 2019

3.1.1.2 Data Collection

All 10 farmers of a village of respective upazilla and district were interviewed using survey questionnaire and direct data recording by trained personnel. Farmers' practices (FPs) related data were collected at two levels: directly from the sample farmers by administering pre-designed and pre-tested questionnaires (Instrument I) and recording of data in pre-formatted register (Instrument II) at 15 days interval from the sample farmer's crop fields through field and crop inspection. In questionnaire survey (Appendix IV), the researcher was directly interviewing the sample farmers and collected recalled data on overall cultivation practices of guava with specific emphasis on pest management practices including all aspects of insecticides use, pheromone trap use, mechanical

control measures and their advantages and disadvantages. In case of field data collection through field inspection, the researcher directly inspected the practice (s) including insecticide usage, use of pheromone traps, mechanical control, field sanitation and other control measures, pest incidence, insect incidence, crop condition, crop damage, healthy and infested fruits and ultimately healthy and infested yield at harvest and sales of harvested produces. Such field data collection activities were assisted by the technical manpower.

Technical manpowers were divided into small groups and each group was asked to take field walks to observe and collect 100 samples per group (or as many as possible from early damage to late or completely damaged and/or rotten fruits/vegetables by fruit fly). List of all observations were related to fruit fly concern, crops or weeds infested rate and nature of damage, among others.

3.1.2 Field data collection

Data on fruit fly infestation were collected from one farmer's orchard in each region. For this, 10 plants were selected from each farmer's orchard and fruits were observed visullay at harvesting stage. Fruits with characteristic of damage symptoms of fruit fly were observed and recorded from each plant. Suspected fruit fly damaged fruits were separated from the undamaged fruits and dissected to confirm the presence of fruit fly eggs or larvae. Number of healthy and infested fruits was recorded for each plant and percent fruit infestation was calculated using the following formula:

$$\% \text{ Fruit infestation by number} = \frac{\text{Number of infested fruits}}{\text{Total number of fruits}} \times 1\,00$$

$$\% \text{ Fruit infestation by weight} = \frac{\text{Weight of infested fruit}}{\text{Total weight of fruit}} \times 100$$

3.1.3 Data Processing and Analysis

All the collected data were coded, tabulated, checked, and analyzed by using descriptive statistical methods including the computer-based statistical package SPSS suitable for survey data analysis (Rashid *et al*. 2003). For effectiveness and/or impact assessment, different farmers' practices were considered as treatments and three Upazilla were considered as replications for output of all the four regions together while three villages under each Upazilla will be considered as replications for the respective district data analysis

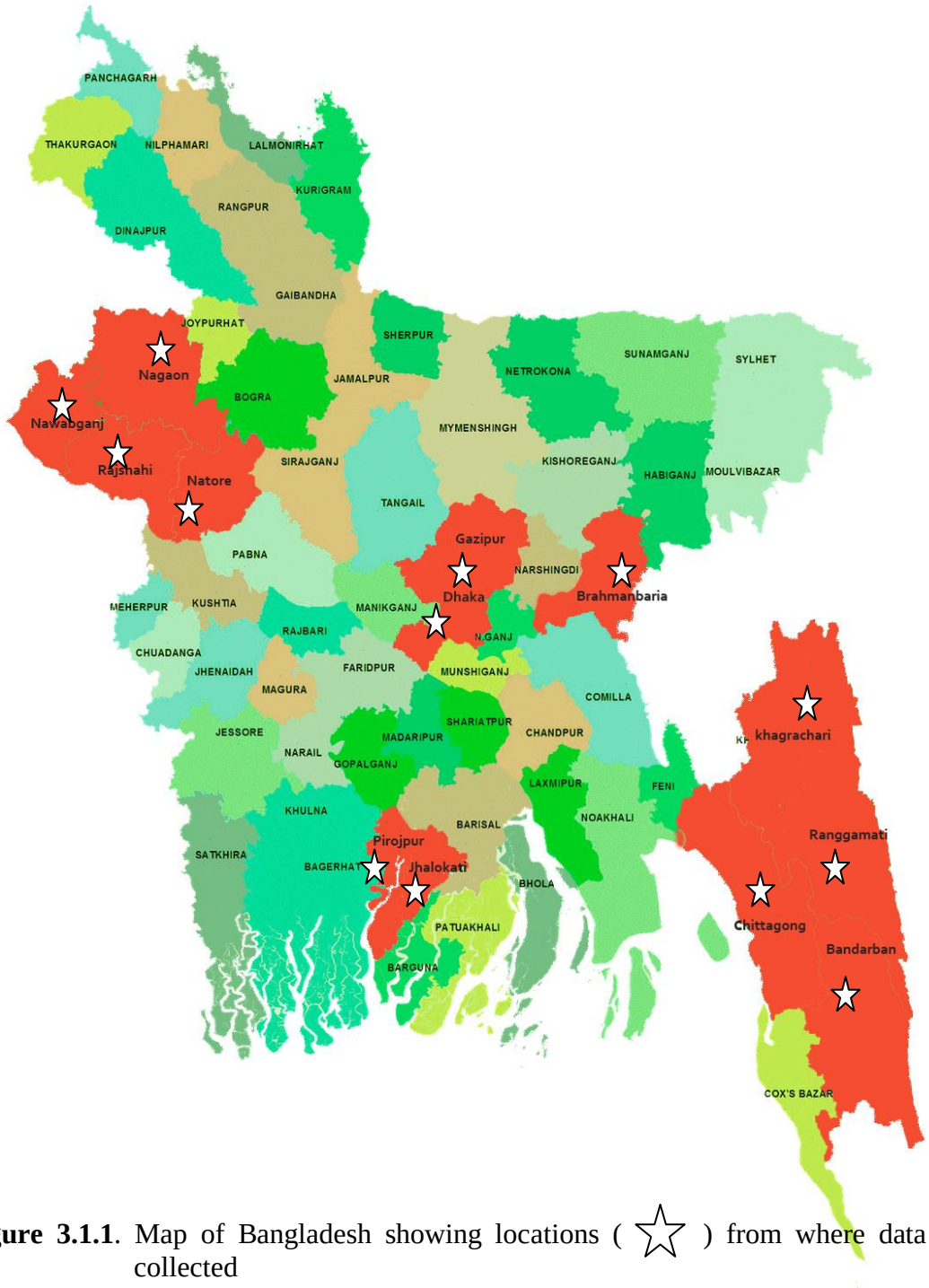

Figure 3.1.1. Map of Bangladesh showing locations (☆) from where data were collected

Experiment 2. Study on the biology of oriental fruit fly (*Bactrocera dorsalis*) and its seasonal abundance for guava variety in Bangladesh

3.2.1 Rearing of Fruit Flies

The infested sample of guava fruits were collected from different orchards of four regions and brought to the laboratory of the Department of Entomolgy at Sher-e-Bangla Agricultural University (SAU), Dhaka. Infested fruits were placed in rearing cage (45 cm × 30 cm) made with wood and net containing sand at the bottom. Rearing cages were kept on the experimental table at prevailing environmental conditions. The temperature and relative humidity of the room during rearing period were recorded. Full grown maggot (larvae) moved out from infested fruit and jumped on the sand for pupation. After 15 days of pupation, pupa took another 12 days for adult development. After emergence, adults were collected and stored for further study. The full procedure of biological study are as follows:

Collection of adult fruit fly

The infested sample of guava fruits were collected from different orchards of four regions and brought to the laboratory of the Department of Entomolgy at Sher-e-Bangla Agricultural University (SAU), Dhaka. Infested fruits were placed in rearing cage for obtaining adult male and female fruit flies. The size of insect rearing cage was (45 cm × 30 cm) made with wood and net containing sand on the bottom. Rearing cages were kept on the experimental table at prevailing environmental conditions. The temperature and relative humidity of the room during rearing period were recorded.

The emerged adult fruit flies were collected with the help of small vial. After identification of male and female adults with the help of morphological characteristics, they were paired for further investigation

Preparation of oviposition boxes

Six rearing boxes containing two healthy infestations free almost ripened guava, and each were maintained for oviposition following CRD method. Two pairs of adult fruit flies were introduced in each cage for oviposition. Eggs laid by female fruit fly on guava fruit were observed every day to know the further development. Full grown maggot (larvae) moved out from infested fruit and jumped on the sand for pupation. After 15 days of pupation, pupa took another 12 days for adult emergence. After emergence adults were collected and stored for further study. During investigation the observations were also continued on different larval instars and subsequent developmental stages till the death of adult flies.

Egg period

Guava fruits having eggs were collected for examining under microscope and after examining, guava fruits were placed in a cage again for further development. The rearing cage were observed at every 24 hours till hatching to record the incubation period.

Maggots (Larval) period

After hatching the fruit fly larvae was allowed to feed the guava fruit flesh. To record the period of full-grown maggots the fruits were observed daily. The duration from egg hatching to pupation was considered as larval period.

Pupal period

To know the pupal period of the fruit fly species the same maggot was observed daily and was noted. This full-grown maggot (larvae) moved out from infested fruit and jumped on the sand for pupation. Observation was continued till adult emergence. The duration from pupal formation to adult emergence was considered as pupal period.

Adult longevity

To study the adult longevity, emerged adults were introduced indivually in another cage with food supplement. As a food supplement fresh ripen guava were provided and bservations were continued till death of the individual adult. The period between adult emergence and adult death was recored as adult longevity.

3.2.2 Identification of Fruit Flies

Adult fruit flies reared from infested fruit of four regions were primarily observed under stereomicroscope and identified with the help of taxonomic keys described by Kapoor (1993). Then the adult samples were sent to Molecular Laboratory for further confirmation.

3.2.3. Data Collection

The study was conducted over a year (January 2017 to December 2017) at the Dhaka (central zone), hill tract (Bhandarban), north zone (Rajshahi) and south zone (Pirojpur) where a guava orchard and vegetables (including cucurbitaceous and solanaceous hosts) are grown outside the orchard. Specimens requiring further species confirmation were collected and preserved in 95% ethanol. Information on the monthly availability of host plants at the fruiting stage was recorded during of the study. Daily temperature and rainfall data for Dhaka were provided by the Bangladesh Meteorological Department, Agargaon, Dhaka 1207, Bangladesh. Weekly trap capture data was recorded on a Microsoft Excel spreadsheet, and numbers of captured flies for each species were divided by the actual number of days past the previous trap installation to generate standardized flies-per-trap-per-day (FTD) data and used for analysis. For each species, mean (and SE) monthly FTD was calculated using all weekly FTDs for all traps. Likewise, mean daily rainfall and temperature were calculated based on the actual day intervals used to generate monthly FTD data. Fruit fly population fluctuations in relation to abiotic factors (rainfall, temperature) and host fruit availability (number of fruiting host species available each month) were analyzed using the Pearson correlation coefficient, based on FTD data for the three trapping sites continuously monitored over year.

Experiment 3. Morphometric characterization of fruit fly species infesting guava in Bangladesh

Identification of fruit flies using conventional taxonomy on most morphological characters in the egg and adult stages was considered as the most important stage of detection of any pest for successful management.

3.3.1 Sample Collection

Fruit fly samples were collected from different locations of Bangladesh using pheromone trap (methyl-eugenol). The pheromone trap was setup in the guava field for 48 hours. After 48 hours the fruit fly sample were collected from the trap and wash for preservation in ethanol. The sample was studied under stereomicroscope.

3.3.2 Data Collection

Head, thorax, abdomen, and wing were observed under stereomicroscope and took photograph of individual sample. The lengths, width of each fruit fly sample (head, thorax, abdomen and wing noted below) was measured (Appendix III). The fruit flies' samples were catagoriezed according to their region and each region's samples were measured separately and finally the statistical analysis were made.

i. Head length
ii. Head width
iii. Thorax length
iv. Thorax width
v. Abdomen length
vi. Abdomen width
vii. Wing length
viii. Wing width

The data obtained for different characters were statistically analyzed to find out the significance of parameters. The mean values of all the characters were evaluated, and analysis of variance (ANOVA) performed by the 'F' (variance ratio) test using STATISTICS 10 program. The significance of the difference among the various combinations for separate characters were estimated by the Duncan's Multiple Range Test (DMRT) at 5% level of probability (Gomez and Gomez 1984).

Experiment 4. Molecular Detection of guava infesting Fruit Fly Species in Bangladesh

3.4.1 Chemical Used

Ethanol, PCR Master Mix, Primer, Agarose, Ethidium Bromide and other reagents were utilized according to the different kit used in different steps.

3.4.2 Material Used

Biosafety cabinet class II (A), Applied Biosystem 2720 Thermal Cycler (B), Eppendorf Centrifuge 5415R (C) and Quantus™ Fluorometer (D), 3500 Dx Genetic Analyzer (E) shown below. Heat block, microcentrifuge tubes, PCR tubes, Falcon tubes, Electronic Balance & Vortex mixer were also used.

A. Biosafety cabinet Class II

B. Applied Biosystem 2720 Thermal cycler

C: Appendorf Centrifuge 5415

D: Quantus Fluorometer

E: 3500 Dx GeneticAnalyzer

3.4.3. Sample collection and preparation

According to Wu *et al*. (2011), the specimens were preserved in 95% ethanol and then stored at -20 ^{0} C until the process for DNA extraction. The specimen bottles were used to keep the specimens. For labeling, the locality, the collector's name, date, the specimen's name, and also the host plants were required. The voucher specimens of the species were kept for the experiment.

3.4.4 Cytochrome Oxidase I (COI)

In this study, the variable region of the mitochondrial cytochrome oxidase subunit I (COI) gene was used to obtain better estimates of divergence for species of the fruit flies complex. Many previous studies have been used the COI gene (Table.3.4.1) for obtaining the divergences among the species (Liu *et al*. 2011, Zhang *et al*. 2010). The COI gene was used in this study because it appeared to be among the most conservative protein-coding genes in the mitochondrial genome of animals (Brown 1985). The COI gene was the slow-evolving gene in the mitochondrial protein-coding gene (Simon *et al*. 1994). The conserved sequence of the COI gene allows researchers to use it as a 'universal' primer, and it has been widely used to investigate multiple different taxa and for interspecific analysis (Hebert *et al*. 2003). In terms of the degree of variation, it was expected to be low in intraspecific variation such that through a given cluster analysis, the sequences from polymorphic species would cluster together in a genetic distance.

Table 3.4.1: Name of the COI gene primer with their base pair along with primer length
.

Primer	5'->3'	Primer length	Product Length bp
UEA-7	TACAGTTGGAATAGACGTTGATAC	24	689
UEA-10	TCCAATGCACTAATCTGCCATATTA	25	
COI_F	TYACAGTAGGAATAGAYGTAGAYAC	25	691
COI_R	TCCATTGCACTAATCTGCCATATTA	25	

3.4.5. DNA Extraction and COI gene Amplification

DNA was extracted from the whole part of the adult using the Favor Prep Tissue Genomic DNA Extraction Mini Kit (Cat No. FATGK001, Favorgen, Taiwan). After DNA Extraction, QuantiFlour® dsDNA Dye, (Promega, USA) was used for measuring DNA concentration by Quantus™ Fluorometer (Promega, USA) (Table 3.4.2). A fragment of approximately 691 bp of the cytochrome c oxidase subunit I (COI) gene was amplified by using primers (Table 3.4.3). Polymerase chain reactions (PCR) for specimens in the fruit fly genera were performed in a total volume of 26 ll containing 2 µl of DNA template, 2 µl of each primer, 12.5 µl of GoTaq® G2 hot start a colorless master mix, and 7.5 µl sterile ddH2O. The temperature profile was as follows: initial denaturation at 95^{o}C for 3 min followed by 35 cycles of denaturation at 95^{o}C for 60 s, annealing at 50^{o}C for 60 s, and extension at 72^{o}C for 60 sec followed by a final extension at 72^{o}C for 10 min (Table 3.4.4).

Table 3.4.2. Fruit flies' DNA concentration.

Sample ID	Concentration
201910 a	48 ng/µl
201910 b	15 ng/µl
201910 c	4.25 ng/µl
201911 a	9.0 ng/µl
201911 b	47 ng/µl
201911 c	20 ng/µl
201912 a	49 ng/µl
201912 b	46 ng/µl
201912 c	58 ng/µl
201913	50 ng/µl
201914	53 ng/µl
201921 a	73 ng/µl
201921 b	43 ng/µl
201921 c	68 ng/µl
201922 a	43 ng/µl
201922 b	28 ng/µl
201922 c	15 ng/µl
201923 a	47 ng/µl
201923 b	34 ng/µl

Table 3.4.3. The preparation of the master mix for the PCR

GoTaq® G2 Hot Start Colorless Master Mix	**12.5 ul**
COI_F	2
COI_R	2
H2O	7.5
DNA (20 ng)	2

Table 3.4.4: The reaction condition used in the polymerase chain reaction

1	**95^{o}C**	**3 min**	
2	95^{o}C	1 min	35 Cycle
	50^{o}C	1 min	
	72^{o}C	1 min	
3	72^{o}C	10 min	
4	4^{o}C	∞	

3.4.6 Gel Electrophoresis

By using 1% of agarose gel, the electrophoresis was done to visualize the product of the DNA extraction (Figure: 3.3.1). For the 0.5 g of agarose powder was weighed and 50 ml of 1X TAE buffer solution was added into it. They were mixed well before heating in the microwave oven for 2 minutes. The casting tray was set on with the comb inserted in it. Then, 1 μL of ethidium bromide was added to the agarose. After the solution was mixed, poured them into the gel tray. The gel got cooled about 30 minutes before taking out the comb. The gel was inserted into the tank which consists of TAE buffer. Now 2 μL of the sample was pipetted

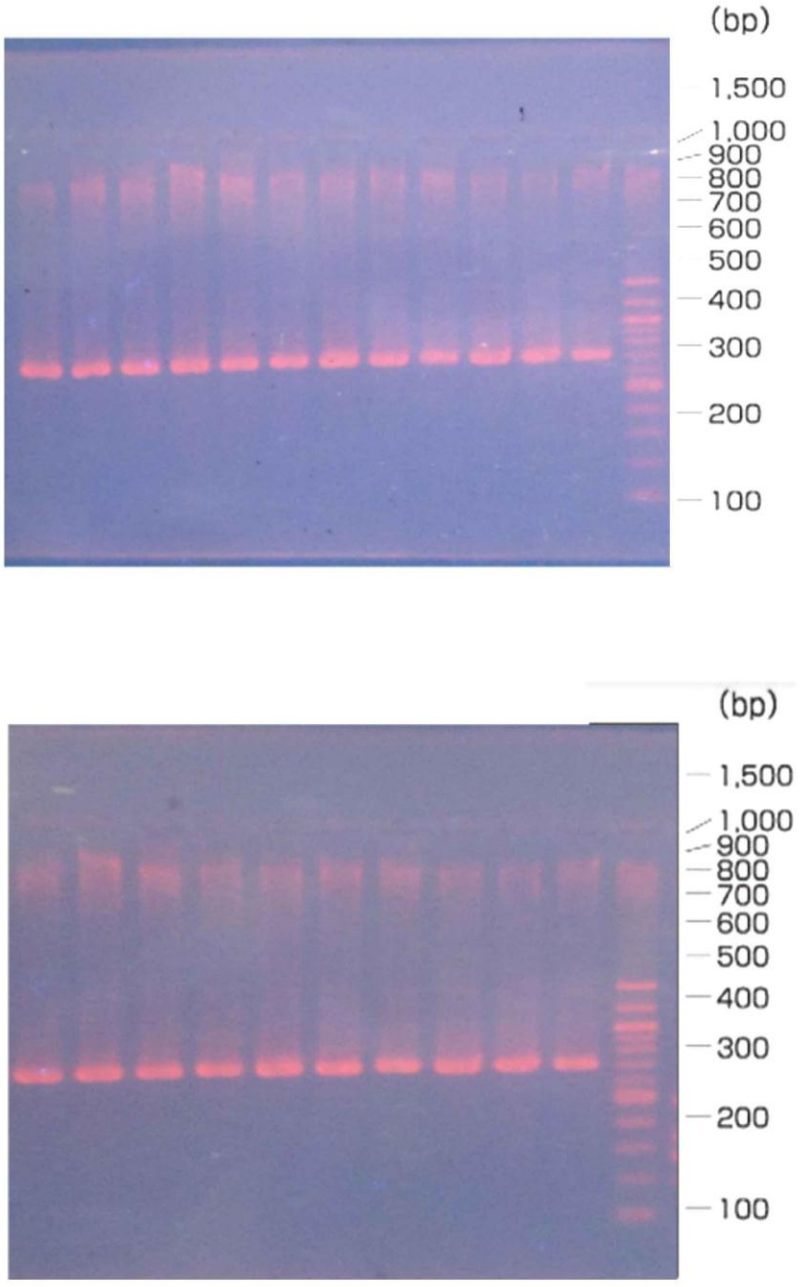

Figure 3.3.1. Figure showed DNA fragments migrated through agarose gel during electrophoresis. The graph to the right showed the nonlinear relationship between the size of the fragment and the distance migrated.

DNA isolation was performed to obtain the DNA template in the amplification process. The PCR process was successfully amplified as shown through electrophoresis results in Figure 3.3.1. The electrophoresis results showed the presence of clear and thick DNA bands as evidence of successful amplification. The PCR results were sequenced to determine the sequence of nucleotides.

3.4.7. Data analysis for genetic divergence and haplotype distribution

Mitochondrial COI gene sequences were edited and aligned using Clustal W program in MEGA ver. X software (Kumar *et al.* 2018). Descriptive statistics number of haplotypes (H), haplotype diversity (Hd), variance and standard deviation of haplotype diversity were calculated using DnaSP ver. 5.10.01 software (Librado and Rozas 2009). To depict the evolutionary and geographical relationships among haplotypes, a median-joining (MJ) haplotype network was constructed with Network V4.61 by Fluxus Technology (http://www.Fluxus-engineering.com). Genetic distances among zones were calculated based on pairwise matrix of sequence divergences using Kimura's two parameter methods implemented in MEGA X software (Tamura *et al.* 2004).

3.4.8. Phylogenetic analysis

The phylogenetic analysis based on the maximum likelihood (ML) method was performed using MEGA ver. X software for investigating the degree of consistency of mutation patterns in different regions of Bangladesh (South, central and north region). The reliability of branches was assessed by 1000 bootstrap replications (Felsenstein, 1985).

3.4.9. Neutrality test and genetic differentiation

Tajima's D tests of neutrality index and genetic differentiation was useful for demographic history information, with demographic expansion related to negative values and subdivided populations at equilibrium leading to positive values (Tajima 1989) and were also performed using DnaSP ver. 5.10.01 software for detecting the range of population expansions.

The genetic differentiation (FST) between different district populations in range referring to the criterion by (Wright, 1984) defined genetic differentiation as low for FST<0.05, moderate for 0.05<FST<0.15, high for 0.15<FST<0.25 and very high for FST>0.25.

Experiment 5. Development of management approaches of guava attacking fruit fly species in Bangladesh

To develop sustainable management approach, efficacy of different selected pesticides (biopesticides and chemicals) was evaluated in the laboratory and pot/ net house for suggesting in the field trials as sole and/(or) IPM (Integrated Pest Management) component.

3.5.1 Location

The experiments were conducted at on-station (SAU Horticulture farm) and Farmers' field of Savar (Birulia) of Dhaka district and Salna, Gazipur district.

3.5.2 Duration

The study was conducted over a year (April 2019 to March 2020) at on-station (SAU Horticulture farm) and Farmers' field of Savar (Birulia) of Dhaka district and Salna, Gazipur district.

3.5.3 Treatments

There were eight treatments including an untreated control. The treatments were as follows:

T_1: Setting of Pheromone trap at plant canopy + spraying of Neem oil 5 m1/L of water + trix 5.0 g at 7 days' interval

T_2: Spraying of Neem oil 5.0 m1/L of water plus trix 5.0 g at 7 days' interval

T_3: Spraying of carbosulfan 20 EC @ 2.0 ml/L of water at 7 days' interval + Setting of Pheromone trap at plant canopy

T_4: Wrapping of twig and fruits with micro nets (Prabhat Kumar *et al*. 2011)

T_5: Setting of Pheromone trap at plant canopy

T_6: Bait Application Technique (BAT), in which food baits were mixed with a small amount of insecticide to attract and kill adults; and

T_7: Male Annihilation Technique (MAT), in which synthetic pheromones were mixed with insecticide, applied to a suitable substrate to allow slow release, and were used selectively to attract and kill male flies (Ravi kumar *et al*. 2007).

T_8: Untreated control

3.5.4 Design

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The treatments were randomly allotted in each block.

3.5.5 Layout

Farmers guava orchards where plant to plant distance was 8 meters and line to line distance was 8 meters were used. Equal number of plants of similar age and variety were considered in each guava fruiting season at on-station and farmer field. A single plant was selected as a treatment randomly and three replications were considered accordingly, so in an experimental field total 24 plants were treated on random basis in farmer's orchard. Studies were conducted in three locations viz., SAU horticulture farm, Dhaka, Birulia, Savar, Dhaka and Salna, Gazipur.

3.5.6 Data collection

3.5.6.1 Number of species of guava fruit fly per pheromone trap

The number of adult fruit fly trapped in pheromone and bait traps was counted and recorded separately for each treatment at 12 hours' interval (Appendix V).

3.5.6.2 Number of fruits per plant

Data were collected on the basis of the number of harvested fruits per tree for each treatment. The marketable fruits were harvested at 7 days' interval at early- mid- and late- fruiting stages.

3.5.6.3 Number of healthy and infested fruits

Data were recorded on the basis of the number of the healthy fruits (HF) and infested fruits (IF) harvested at early, mid and late fruiting stages of the season. There were 4, 4 and 3 harvest at early, mid and late fruiting stage, respectively. Infestation rate (by number and weight) of guava fruits caused by fruit fly at early- mid- and late- fruiting stage in different treatments and percent infestation reduction over control were calculated.

3.5.6.4 Weight of healthy and infested fruit

The weight of healthy and infested fruits at early- mid- and late- fruiting stage of guava were taken separately per plot for each treatment.

3.5.6.5 Fruit infestation percent

The overall percent fruit infestations and those at 3 different fruiting stages were calculated using the following formulae:

$$\% \text{ Fruit infestation by number} = \frac{\text{Number of infested fruits}}{\text{Total number of fruits}} \times 1\,00$$

$$\% \text{ Fruit infestation by weight} = \frac{\text{Weight of infested fruit}}{\text{Total weight of fruit}} \times 100$$

$$\%\text{Reduction in infestation Over control} = \frac{\%\text{ Infestation of untreated control - \% infestation of treatment all ones}}{\%\text{ infestation of untreated control}} \times 100$$

The stage-wise percent fruit infestation was calculated on the basis of the infestation occurred at each fruiting stage of the crop. The overall or accumulated infestation rate (both by number and weight) were derived at early, mid and late fruiting stages for different treatments and percent infestation reduction over control was also calculated.

3.5.6.6 Yield

The healthy and total yields of guava for each treatment were calculated in tons/ha from the cumulative fruit production in an orchard. Effect of different treatments on the increase and decrease of guava yield over control were also calculated.

3.5.6.7 Benefit/Cost Analysis

For benefit cost analysis, record of costs incurred in each treatment and that of control were maintained. Similarly, the price of the harvested fruits of each treatment and that of untreated control were calculated at market rate. Benefit-Cost analyses were expressed in terms of Benefit Cost ratio (BCR).

The economic analysis or Benefit Cost Ratio (BCR) was analyzed on the basis of total expenditure of the respective treatment along with the total return from that particular treatment. In this study BCR was analyzed for a hectare of land. For this analysis following parameters were considered:

Treatmentwise management cost: This was calculated by adding the costs incurred for labors and inputs for each treatment including untreated control. Yield of guava: The total yield after each harvest was calculated separately for every treatment.

The total yield of each treatment was converted tor determining yield (t ha^{-1}). This yield was utilized to calculate the gross return.

Gross return: This was measured by multiplying the total yield by the unit price of guava at that time.

Finally, the benefit cost ratio (BCR) was calculated by utilizing the formula:

$$\text{Benefit Cost Ratio (BCR)} = \frac{\text{Gross return}}{\text{Total treatment management cost}}$$

3.5.7 Data analysis

The data were analyzed statistically for important parameters like percent fruit infestation, healthy and infested fruit yield, and extent of damage, fruit bearing capabilities, intensity of attack by male and female fruit fly. The analyses of variance (ANOVA) of different parameters were performed and the range tests of the means were done by using Statistics 10.

Chapter-IV

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

The results obtained from different experiments have been presented sequentially, interpreted and discussed as revealed in the findings systematically in the line of targeted objectives of the study.

Experiment 1. Survey on fruit fly infestation in major guava growing regions in Bangladesh

4.1.1 Demography of farmers

Results on demography of farmers of four major guava growing regions viz., Southern region (Pirojpur, Jhalokati), Northern region (Natore, Rajshahi, Chapai Nawabgonj and Naogaon), Central region of greater Dhaka (Mukundupur of Bramhonbaria, Savar of Dhaka and Salna of Gazipur district), Hilly areas (Bandarban, Rangamati, Khagrachari) and Patyia of Chittagong of Bangladesh have been presented and discussed herein. Farmers were categorized into five different groups on the basis of their age. The guava growing farmers' age ranged from 15 to 65 years and the highest numbers of guava growers were in 26-35 age group and the lowest in 15-25 age group. Thirty-four percent guava growers came from 26-35 age group and the number of these young farmers were the highest 34 percent. (Table 4.1.1).

The results indicated that most of the farmers were middle aged group. Since guava production recently has become a profitable farming and considered as one of the promising high value crop in Bangladesh so young farmers are involved in guava cultivation to change their economic condition through guava production. 28% middle-aged farmers (36-45 years) were involved in guava production.(Table; 4.1.1).

Around 7 percent of 56-65 age group farmers were involved in guava production. So, more than 90 percent farmers were young and involved in guava production.

Table 4.1.1. Five different categories of guava growing farmers' ages range at different surveyed locations of Bangladesh

Farmer age range	Percent
15-25	6.0
26-35	34.0
36-45	28.0
46-55	25.0
56-65	7.0
Total	100.0

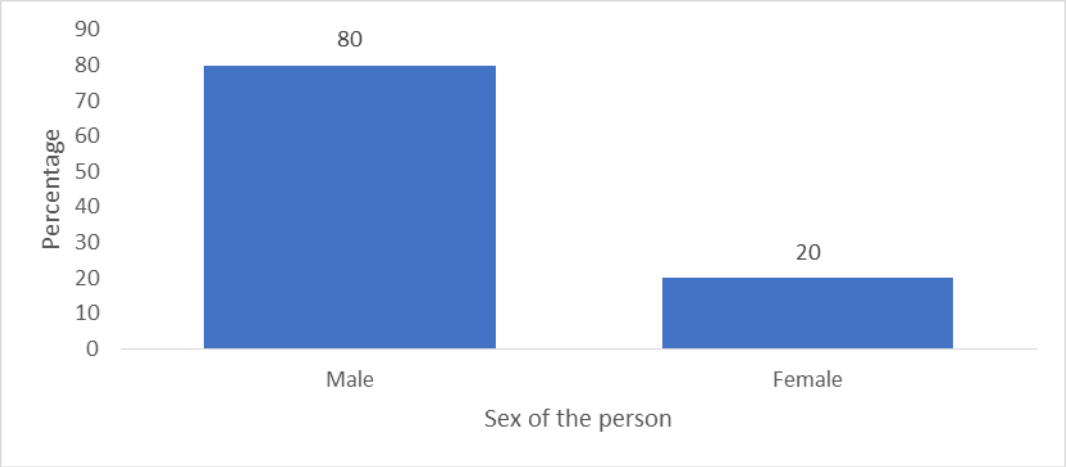

Figure 4.1.1. Diagram revealed gender of the person categories of guava growing farmers at different surveyed locations of Bangladesh.

Mostly male growers were involved than female in guava farming. Almost 4 times higher male were involved in guava production on compared to female. 80 percent male people were active in guava production in Bangladesh at surveyed area (Fig. 4.1.1). On the other hand, only 20 percent female farmers were involved in guava production (Fig; 4.1.1). Therefore, more than 90 percent farmers were young and most of them were male.

According to (BBS 2013) farmers were categorised into four different groups on the basis of their farm size. Landless farmers have less than 0.02 ha of land, but 27 percent landless farmer were involved in guava cultivation because of guava production recently has become most profitable in Bangladesh and they want to change their economic condition through guava production. Most small farmers having (more than 1ha of land) were involved in guava production, ie., about 43 percent and that was the highest at surveyed area (Fig; 4.1.2). Around 22 percent large farmers having (more than 2 ha of land) were involved in guava production.

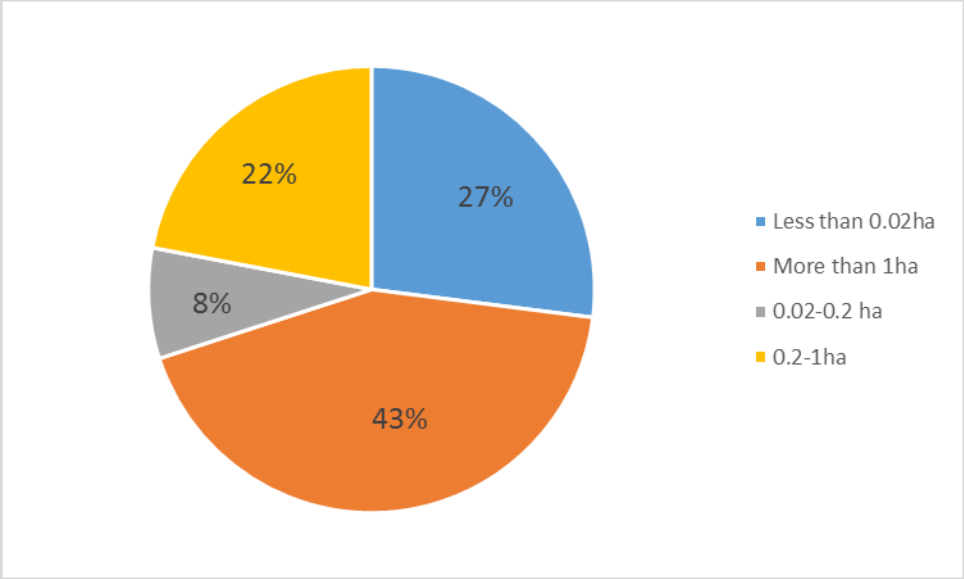

Figure. 4.1.2. Diagram revealed Guava grower's category according to their farm size at surveyed locations of Bangladesh.

The guava growing farmers level of education ranged from primary to graduation level and categorized into four different educational levels. One to five classes were categorized as primary education, 6-10 classes were categorized as secondary education, 11-12 classes were categorized as higher secondary education, and 12 class and above were categorized as graduation level of education. The highest numbers of guava growers were primary (34%) and secondary level categories (52%) and the numbers of higher secondary education level guava growers were low (11%). On the other hand, only 3 percent growers were of graduation level of education. So, it can be said that 100 percent farmers were literate and involved in guava production (Table 4.1.2).

Table 4.1.2. Guava growing farmer's category according to their level of education at different surveyed locations of Bangladesh

Level of education	**Percent (%)**
Primary (1-5)	34.0
Secondary (6-10)	52.0
Higher Secondary (11-12)	11.0
Graduation (12 & above)	3.0
Total	100

In Bangladesh, some promising and popular varieties of guava are cultivated commercially at different regions. Photographs of some popular guava varieties are shown in plates A-G .

Plate A. Shorupkathi variety familiar at Shorupkathi, Pirojpur.

Plate B. Mukundapuri variety familiar at Mukundapur, Akhaura, Bramhonbaria.

Plate C. Kanchannagar variety familiar at Knachannagar Patiya, Chittagong.

Plate D. Thai peara variety familiar at northern and middle regions of Bangladesh.

Plate E. Kazipeara variety familiar at middle and northern region of the country.

Plate F. BARI Peara -3 variety familiar at Salna, Gazipur

Plate G. BAU Peara -3 variety familiar at Savar, Dhaka

Most popular guava variety was Thai payara (Plate D) and 31 percent area were covered with that variety at surveyed locations (Fig.4.1.3). Thai payara were cultivated mainly in northern area of Bangladesh but other area like central region also cultivated the same variety.

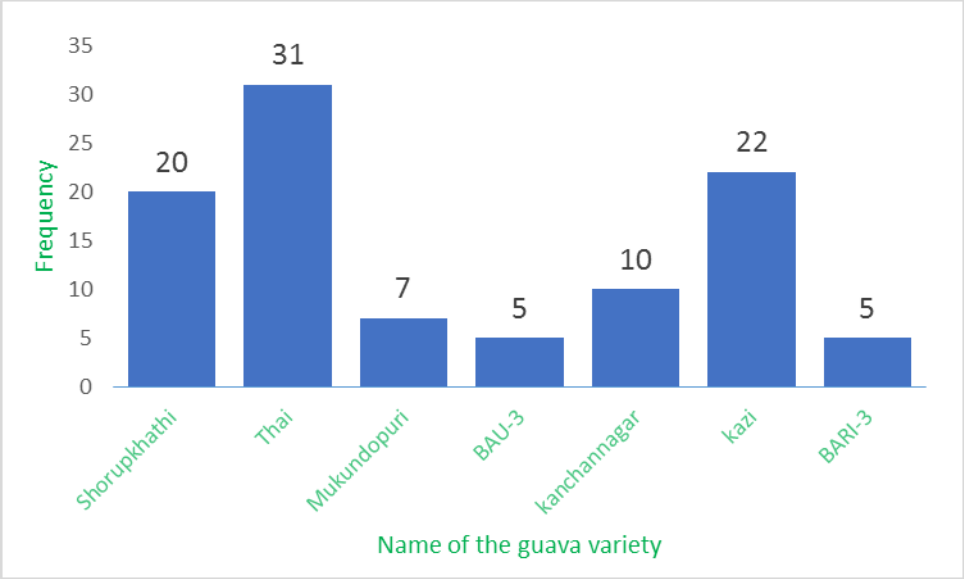

Figure 4.1.3. Diagram showed the percentage of guava varieties cultivated at different surveyed locations of Bangladesh.

The second most popular variety was kazipayara and mostly cultivated at southern area, hilly area and middle part of Bangladesh. Moreover, the other two popular local variety viz. Shorupkati and kanchannagar were also popular at southern part namely Pirojpur, jhalokathi and Patiya of Chittagong district in Bangladesh respectively. Mukundapury, the very special and popular local variety have been cultivated in Akhaura, Brahmonbaria district of Bangladesh.

Table 4.1.3. Average guava varieties saplings' age cultivated at different surveyed locations of Bangladesh

Guava varieties sapling age (year)	Percent
0-5	15.0
6-10	37.0
11-15	40.0
16-20	7.0
26-30	1.0
Total	100.0

The highest average age of guava plants (40%) were 11-15 years and 40 percent of area were covered with that age of saplings (Table 4.1.3). But old aged plants were less available at the surveyed areas. Second most aged plants were 6-10 years of age groups and around 37 percent area were covered with those saplings. In Shorupkathi, Pirojpur maximum orchard were covered with old, aged plants of 16-30 years.

The highest number of guava harvesting was done at the month of July-October and generally, 64 percent of guava were harvested at that time and rest of the guava were harvested at July-September (36%) at surveyed area (Table 4.1.4).

Moreover, the harvesting was done at different day's interval. Fifty percent harvesting was done at one-day interval and 25 percent were harvested at every day and three days' interval (Table 4.1.5).

Table 4.1.4. Harvesting time of guava fruits at different surveyed locations in Bangladesh

The harvesting time	Percent (%)
July - September	36.0
July - October	64.0
Total	100.0

Table 4.1.5. Fruits harvesting interval at different surveyed locations in Bangladesh

Harvest interval	Percent (%)
Everyday (0 interval)	25.0
1day	50.0
3 days	25.0
Total	100.0

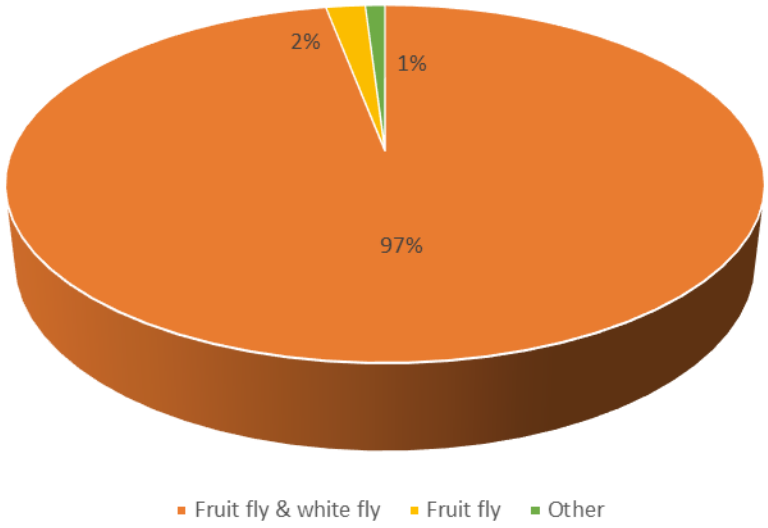

Figure 4.1.4. Farmer's response on major insect pests of guava at their orchard in different surveyed locations of Bangladesh.

Most of the farmers (97%) opined that the fruit fly and whitefly were the major insect pests of guava in their orchard, two (2%) percent farmers reported fruit fly as single major insect pest and only 1% farmers reported mite, white fly and others as major insect pest. So all the farmers at surveyed area were acquanted about fruit fly.

The variations of fruit flies' infestation at different locations might be due to the variations in the local environmental conditions and relative susceptibility of the crop varieties. In this survey, the availability of fruit fly and white fly at surveyed area were 97% and both fruit fly and white fly were destructively affected the production of guava (Fig. 4.1.4). The highest fruit flies' infestation was recorded at Palampur (80.00%) in Kangra district India according to Sood *et al.* (2010).

Table 4.1.6. Farmer's information on Percentage of yield loss due to effect of insect pests' attack at different surveyed locations of Bangladesh

Fruit yield loss (%)	Percent response
01-5	20.0
06-10	4.0
11-15	14.0
16-20	35.0
21-25	27.0
Total	100.0

Percent yield loss due to fruit fly infestation at different surveyed locations of Bangladesh ranged from 1-25 percent (Table 4.1.6). The Maximum yield loss (21-25%) was reported by 27 percent farmers, 35 percent farmers reported 16-20 percent yield loss and 20 percent farmers reported 1-5 percent yield loss due to fruit fly infestation (Table 4.1.6). Due to increase of insect pests' infestation the cumulative yield loss also increased. In Himachal Pradesh average fruit fly's infestation was 65.88 percent, the highest being in Kangra at Palampur (80.00%) and lowest in Chamba at Banikhet (44.44%) (Prabhakar 2011).

Table 4.1.7. Farmer's response on different control measure against guava fruit fly management at different surveyed locations of Bangladesh

Control measure	Percent
Biological	1.0
Chemical	10.0
Mechanical	85.0
Physical	4.0
Total	100.0

Management is an important practice to reduce the yield loss. In Bangladesh most popular practice was mechanical control measure (85%). Guava growers basically used polybag to reduce the fruit fly infestation. Eghty five percent guava growers usually practiced trap with pheromone and polybag simultaneously (Table 4.1.7). Only 4 percent farmers used sweeping net and hand picking and 10 percent farmers used chemical insecticides.

Table 4.1.8 Farmer's response on vulnerable stage and percent of inestation of attacking fruit fly on guava at different surveyed locations of Bangladesh

Sl. No	Name of the variety	Stage of fruit development	Percent of infestation
1	Shorupkathi	Earrly	00
		Middle	00
		Ripening	00
2	Kanchannagar	Early	23
		Middle	18
		Ripening	59
3	Mukandapuri	Early	19
		Middle	15
		Ripening	67
4	Thai payara	Early	31
		Middle	22
		Ripening	47
5	BARI Payara-3	Early	26
		Middle	19
		Ripening	55
6	BAU Payara-3	Early	29
		Middle	25
		Ripening	46

To know the vulnerable stage of fruit at which the severe fruit fly infestation occur is important for finding proper management technique against this devastating pest. The sorupkathi guava variety was cultivated at Pirojpur and Jhalokathi and covered almost 80% of the total guava growing area. This variety was locally improved and resistant against fruit flies. So there are zero infestation at any stage of guava development. Kanchannagar and mukundopuri also locally improved variety and cultivated at Chittagang and Brahmanbaria. The percent of infestation was high at ripening stage of guava and 59 and 67 percent damage were occurred at ripening stage, respectively. Thai guava variety was most susceptible compared to grafted guava variety and mostly cultivated at Rajshahi, natore and Noagoan. Total of 47 percent infestation occurred at ripening stage of thai payara and same trend were followed in case of BAU payara-3 and BARI payara-3 (4.1.8).

Young people were more engaged in guava cultivation compared to older farmers. In field, almost every farmer was aware about the fruit fly and its severity. Fruit fly problem in guava production has become a major barrier to get the profitable yield. Thai payara is the first choice of farmers and some other local variety also popular in local market because of low infestation of fruit fly. Most of the farmers used to apply mechanical approaches. IPM technology is getting popular now a days.

Experiment 2. Study on biology of oriental fruit fly (*Bactrocera dorsalis*) and seasonal abundance of fruit flies on different guava varieties in Bangladesh

Oriental fruit fly, *B. dorsalis* is a highly invasive species. Native to Asia, it is now found in at least 65 countries, including parts of America and Oceania, and most of continental Africa (sub-Saharan countries). The potential risk of its introduction to a new area is facilitated by increasing international tourism and trade and is influenced by changes in climate and land use. After introduction, it can easily disperse as it has a high fecundity, high biotic potential (short life cycle, up to 10 generations offspring per year depending on temperature), a rapid dispersal ability and a broad host range. The economic impact would result primarily from the loss of the export markets and the costly requirement of quarantine restrictions and eradication measures. Furthermore, its establishment would have a serious impact on the environment, following the initiation of chemical and/or biological control programmes. Invasive *B. dorsalis* has been shown to be highly competitive with native fruit flies where it has established, quickly becoming the dominant fruit fly pest (Vayssières*et al.* 2015, Vargas *et al.* 2007, Duyck *et al.* 2004).

Table 4.2.1. Size (length x width) of developmental stages of reared oriental fruit fly (*B. dorsalis*) collected from the farmers' orchards at different zones of Bangladesh with ±SE value.

Developmental stages		Length (mm)	Width (mm)
Egg		1.89 ±0.03	0.38 ±0.01
Larva		2.51 ±0.07	0.49 ±0.06
Pupa		6.40 ±0.1	2.90 ±0.1
Adult	Male	10.94±0.53	3.80 ±0.33
	Female	11.17±0.56	4.96±0.38

4.2.2. Description

Table 4.2.2. Duration of different development stages of reared Oriental fruit fly *(B. dorsalis*)

Particulars	Days		Average	±SE
	Maximum	Minimum		
Egg	9.67	5.67	7.67	±2.83
Larvae	9	3.67	6.33	±3.77
Pupae	24	10.33	17.17	±9.66
Adult	9.67	7.67	8.67	±1.41

4.2.2.1. Eggs

The eggs of *Bactrocera dorsalis* were 1.9 mm long and 0.4 mm wide (Table 4.2.1) with slightly curved on one side and narrow at both ends and white to yellowish in color (Fig 4.2.1). According to Margaritis (1985) the eggs of *Bactrocera oleae* are 0.8 mm long and 0.2 mm wide, with the micropyle protruding slightly at the anterior end, and white to yellow-white. The chorion is reticulate (requires scanning electron microscope examination). Duration of egg (7.67 days), maggot (larvae) (6.33 days), pupae 17.17 days and that of the adult (8.67 days) are indicated in table 4.2.2.

Figure 4.2.1: Eggs of the oriental fruit fly, *Bactrocera dorsalis* (Hendel).

4.2.2.2. Maggots (Larvae)

Since maggots are internal feeders so it is very difficult to study its different instars. Therefore, the investigation was carried out by studying the first instar (newly emerged) and fully grown maggots. The freshly emerged first instars are apodous and white and slightly yellowish in color whereas full grown maggots are creamy white or yellowish in color (Figure4.2.2). The larvae of *Bactrocera dorsalis* were 2.5 mm long and 0.5 mm wide with medium sized end and white to yellowish in color (Fig 4.2.2). It can be varied depending on hosts.

According to White and Elson-Harris (1994), *B. dorsalis* the third-instar larva: medium-sized: 7.5-10.0 mm long and 1.5-2.0 mm wide.

Figure 4.2.2: larva of the oriental fruit fly, *Bactrocera dorsalis* (Hendel).

4.2.2.3. Puparium

Pupation of *B. dorsalis* took place at a depth of 0.5 to 5.0 cm in soil. The pupae were barrel shaped and having eleven distinct segment and last abdominal being little more prominent. It is light brown or golden brown in color Figure (4.2.3). The length and width of of pupa was found 6.40 mm. and 2.90 mm. respectively (Table 4.2.1).

Figure 4.2.3: Pupa of the oriental fruit fly, *Bactrocera dorsalis* (Hendel).

4.2.2.4. Adults

The *B. dorsalis* adults were brown to black, brown in color with hyaline wings, legs are yellow, and thorax is brownish black in color. In thoracic region, pair of yellow colored lateral were prominent. Wings of adult consists of continuous black marking on coastal margin. In male abdominal end was blunt, on the other hand it was developed into pointed ovipositor (Fig.4.2.4) in case of female. During study, female was larger in size 11.17 mm in length and 4.96 mm in width than the male 10.96 mm in length and 3.80 mm in width (Table 4.2.1)

Figure 4.2.4: Adult of the oriental female fruit fly, *Bactrocera dorsalis* (Hendel).

According to Drew and Hancock (1994), *Bactrocera* spp. with a clear wing membrane, except for a narrow costal band (not reaching R4+5); cells bc and c colourless (except in a few non-pests with a very pale tint) with microtrichia restricted to outer corner of cell c. Scutum generally black with lateral vittae present and medial vitta absent; yellow scutellum, except for basal band which is usually very narrow; abdomen with a medial dark stripe on T3-T5; dark laterally (but form of marking varies from species to species). *B. dorsalis* belongs to a subgroup that has yellow postpronotal lobes, parallel lateral vittae, and femora not extensively marked. Within this group it is distinguished by its short to long aculeus/aedeagus; tomentum with no gap; narrow costal band; generally narrow but sometimes extensive abdominal markings. It is noteworthy that colour of scutum varies in *B. dorsalis* from generally black to black with an extensive lanceolate red-brown pattern to almost entirely red brown. Populations from the Indian subcontinent and Africa have extensive pale markings (Leblanc *et al.* 2013), whereas specimens from Asia east of Myanmar mostly have dark scutum.

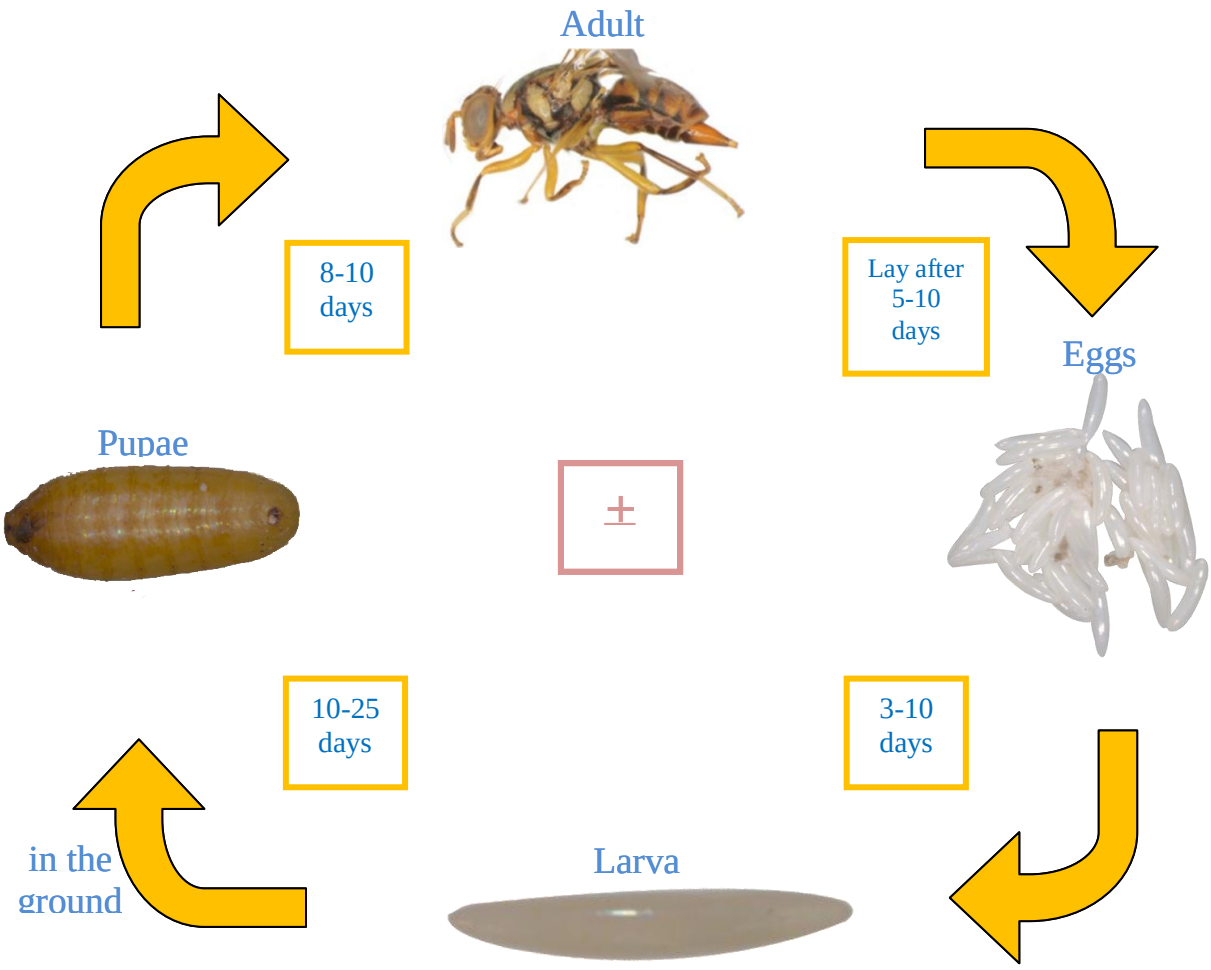

Figure 4.2.5. Life cycle of Oriental fruit fly (*Bactrocera dorsalis*) with different stages.

Bactrocera dorsalis undergo three stages of development before emerging as adults: egg, larva, and pupa. At room temperature, *Bactrocera dorsalis* can develop into adults within one to two weeks. The egg and larval stages span approximately eight to fifteen days, while the pupal stage lasts ten to twenty-five day (Fig. 4.2.5). The adult fruit flies' life span was found 37 days. The average natural life span of fruit fly adults in optimal temperatures is 40 to 50 days. Female fruit flies are capable of mating and laying several batches of eggs in that time. The life span of the fruit fly is heavily influenced by temperature (Drew *et al.* 1984).

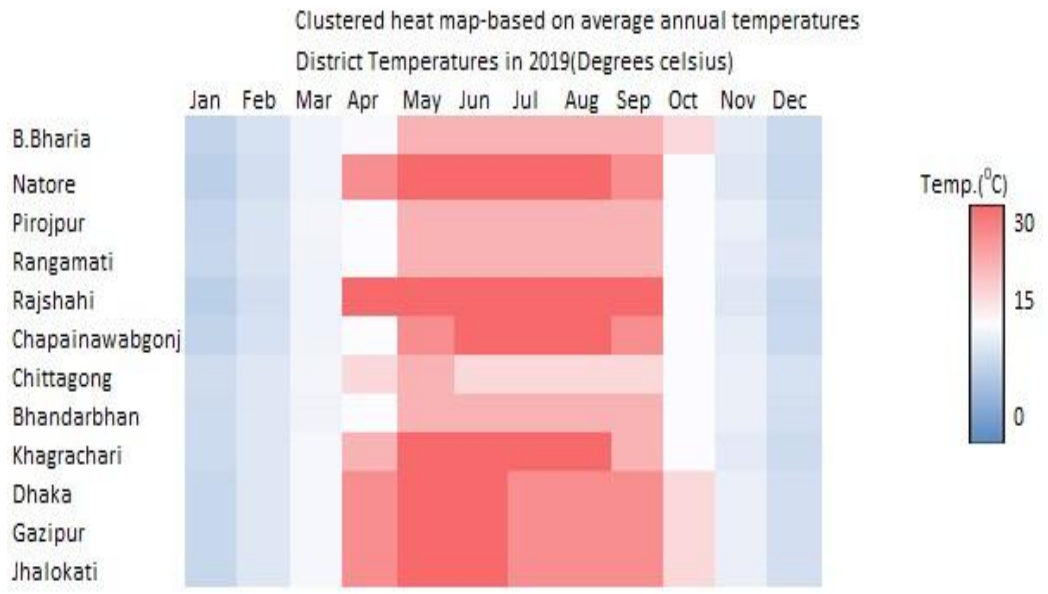

Figure 4.2.6. Head map of fruit fly surveyed location in Bangladesh with selected district along with their average mean temperature from January 2019 to December 2019.

Host fruit availability is well known to be the main driver of seasonal abundance of fruit flies (Drew and Hooper 1983, Vargas *et al.* 1983a, b, Drew *et al.* 1984, Leblanc *et al.* 2014a). The variations of fruit flies' infestation in guava at different locations might be due to the variations in the local environmental conditions and relative susceptibility of the crop varieties (Figure: 4.2.6). Huge quantity of fruits from cultivated trees were available in largest quantities during the hot rainy season (May–August) and lower during the cooler, drier season (November–February) Moreover, wild hosts were mainly observed in fruiting during the hot summer months. For example, highest fruit flies'number was recorded at August month (65) in guava growing district, where Sood *et al.* (2010) reported high fruit flies' activity in Himachal Pradesh might have been facilitated by congenial climatic conditions like high rain fall (1251.90 mm annual

rainfall) and humidity, with majority rains being received during active cucurbits growing season (May-Sept). The same trend was observed in Bangladesh (Fig. 4.2.6). This is supported by faulty insect-pest control practices adopted by the farmers, as they are not using IPM (Integrated Pest Management) approach like field sanitation, MAT (Male Annihilation Technique) and BAT (Bait Application Technique) techniques as observed during the year.

On the other hand, most of the agricultural land have bushy hedges and is surrounded by forest and pastures consisting of many wild cucurbits which could facilitate the fruit flies to rest and pick the resources during and after insecticide application. High fruit flies' infestation in cucurbits recorded in the Himachal Pradesh accordance with Gupta *et al.* (1992) who had observed 60.00-80.00 per cent fruit flies' infestation on different cucurbits in Himachal Pradesh.

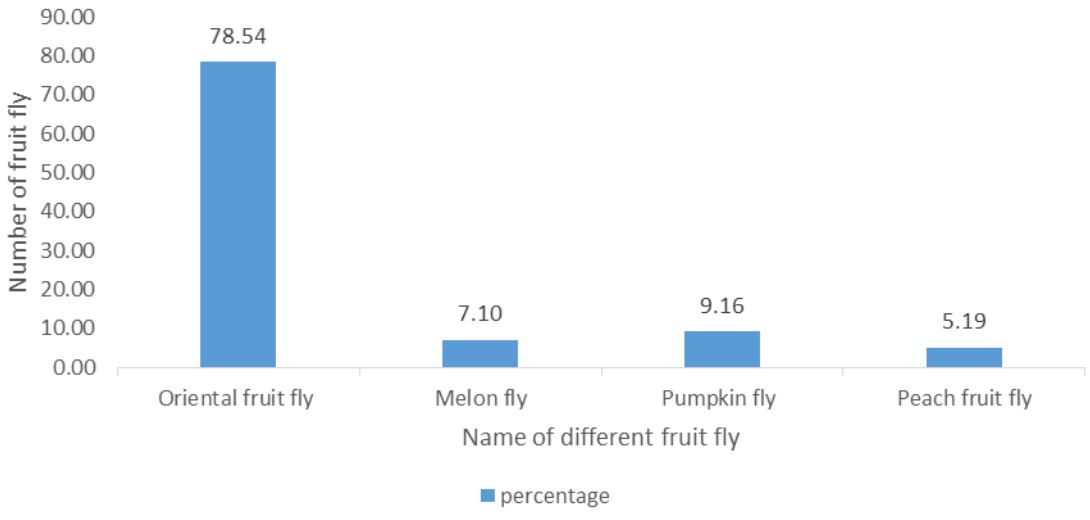

Figure 4.2.7. Abundance of different fruit fly species collected from pheromone traps at different surveyed locations in Bangladesh.

The four dominant polyphagous fruit pest *B. dorsalis* (78.54 % of all trapped flies), followed by cucurbit pests *Z. cucurbitae* (7.10 %) and *Z. tau* (9.16 %), and *B. zonata* (5.19 %) were collected from guava orchard using methyl eugenol trap. Cucurbit pest *D. longicornis* were collected in much smaller numbers (Fig.4.2.7). During the year of trapping, showed a diversity of host fruits and vegetables were commonly available at the fruiting stage throughout the year in Bangladesh (trapping were done at southern, middle, northern and hilly areas of Bangladesh). Seasonal abundance was positively correlated with average temperature with four different surveyed zones (North zone 0.76, south zone 0.78, middle zone 0.77 and hill tract 0.78). It also correlated with rain (North zone 0.98, south zone 0.99, and middle zone 0.99 and hill tract 0.98) and relative humidity (North zone 0.70, south zone 0.69, and middle zone 0.70 and hill tract 0.68) (Table 4.2.3).

Table 4.2.3. Correlation (Pearson) between monthly captures of fruit flies on the basis of the region and mean monthly rainfall, relative humidity, temperature, and number of known hosts available at the fruiting stage for each fly species.

	AV.Temp.	**Rainfall**	**R.H**	**Middle Zone**	**Hill Tract**	**North Zone**	**South Zone**
AV.Temp.	1.00						
Rainfall	0.77	1.00					
R.H.	0.43	0.72	1.00				
Middle Zone	0.77	0.99	0.70	1.00			
Hill Tract	0.78	0.98	0.68	0.99	1.00		
North Zone	0.76	0.98	0.70	0.99	1.00	1.00	
South Zone	0.78	0.99	0.69	0.99	0.99	0.99	1.00

Seasonal abundance was positively correlated with rainfall, temperature, and host availability for most of the fruit infesting species, and especially for *B. dorsalis* in Bangladesh (Hossein*et al.* 2017). The very high captures (almost 100%) of fruit flies (*B. dorsalis*) in methyl eugenol traps at hilly tract and its consistent peaks of abundance

during the wet season or summer months were consistent with those documented in studies in ChapaiNawabganj, Bangladesh (Uddin*et al.* 2016), Hawaii (Vargas *et al.* 1983b, 1989, 1990, Leblanc *et al*. 2014a), Kunming, China (Ye and Liu 2005), and India (Gupta and Bhatia 2000).

At hilly tract most famous guava variety was kazipayara and at Chittagong kanchonnagar guava variety and popular locally. By April in Bangladesh, most of the guava fruit plant varieties started flowering, and fly populations started increasing. Between June and August, at the peak of the monsoon season, most of the guavas get ready for harvesting..Oriental fruit flies were correspondingly most abundant. However, populations declined and remained low until March of the following year (Fig. 4.2.8; 4.2.9).

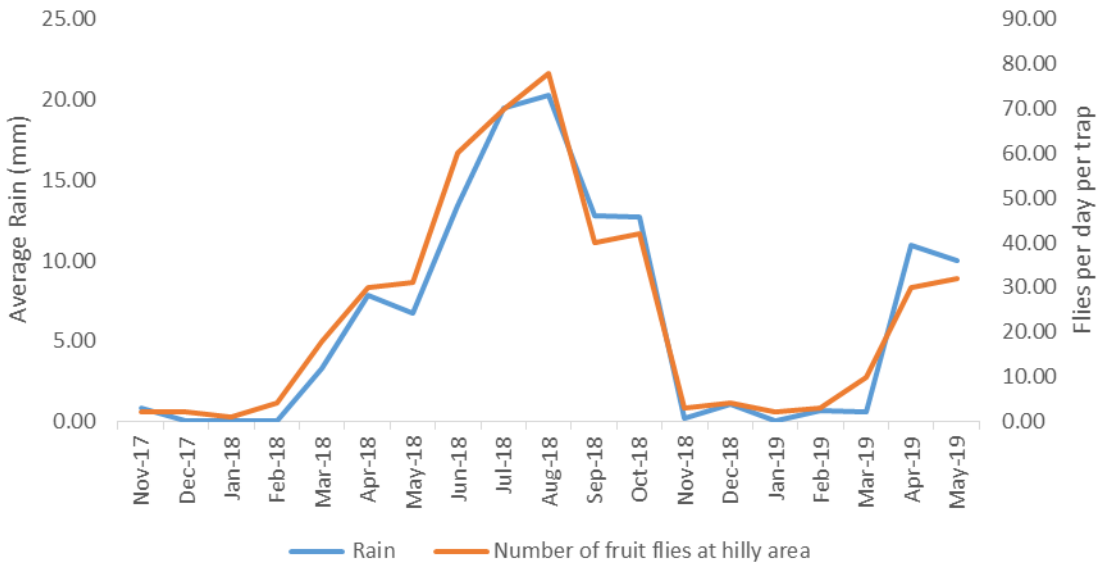

Figure 4.2.8. Distribution and mean monthly trap capture of number of fruit flies in relation to abiotic factor (mean daily rain) and host fruit availability at hill tract and Chittagong region of Bangladesh.

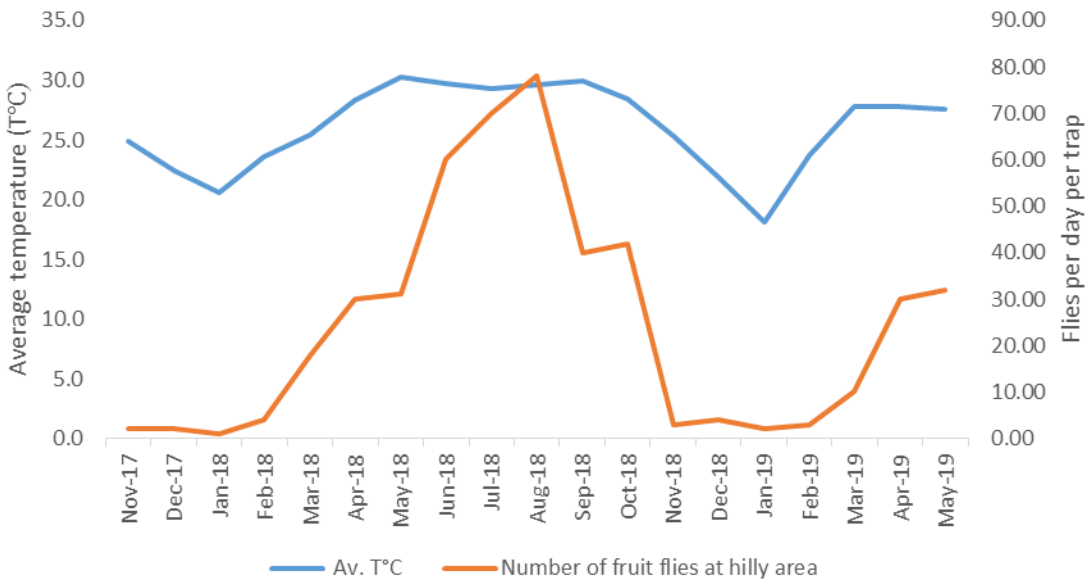

Figure 4.2.9. Distribution and mean monthly trap captures of number of fruit flies in relation to abiotic factor (mean temperature) and host fruit availability at hill tract and Chittagong region of Bangladesh.

At central region (Dhaka, Gazipur, B. Bharia, Norsinghdi) of Bangladesh, the most famous guava variety was kazipayara. By April in Bangladesh, most of the guava fruit plant varieties started flowereing, and fly populations started increasing. Between June and August, at the peak of the monsoon season, most of the guavas become ready for harvesting, oriental fruit flies were correspondingly most abundant in the month of April to September. However, populations declined and remained low until March the following year (Fig. 4.2.10, 4.2.11) .

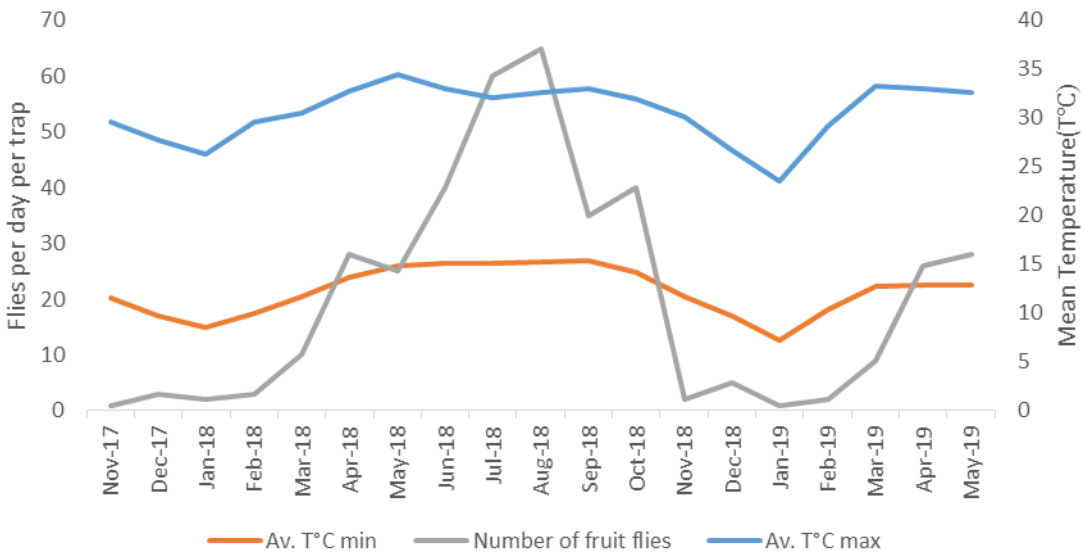

Figure 4.2.10. Distribution and mean monthly trap capture of number of fruit flies in relation to abiotic factor (temperature) and host fruit availability at middle (Dhaka, Gazipur, B. Bharia, Norsinghdi) region of Bangladesh.

At northern part (Chapainawabgong, Natore, Rajshahi, Noagoan) of Bangladesh the number of oriental fruit fly reduced almost 50 percent compared to hilly tract but the total number of fruit flies almost same. The number of other fruit flies were high in number in that part. This might be lots of vegetable production in that particular area. The most famous and cultivated guava variety was Thai payara in the north part which had soft surface and soft inside. The same seasonal abundance trend was observed at the north part as hilly area. However, the seasonal abundance at southern part differs from north, middle and hilly area. Southern part was very close to the sea and tidal water come into the inner cultivated land and salinity was high at that part.

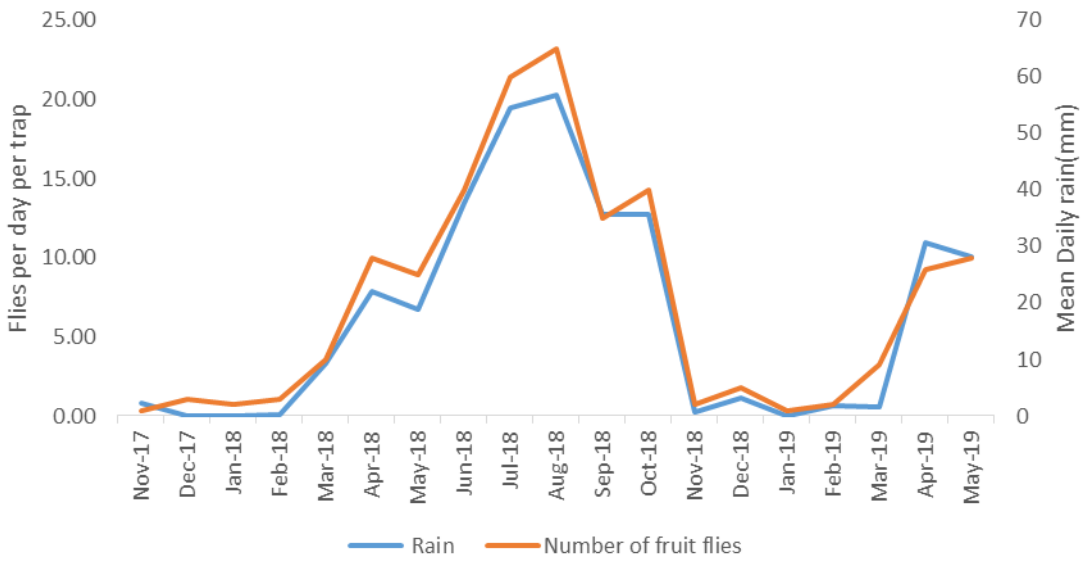

Figure 4.2.11. Distribution and mean monthly trap capture of number of fruit flies in relation to abiotic factor (mean daily rain) and host fruit availability at middle (Dhaka, Gazipur, B. Bharia, Norsinghdi) region of Bangladesh.

The cultivated guava varieties in Pirijpur were sorupkathi and other local variety but sorupkathi was resistant against fruit fly due to the varietal hardness. Outer surface was hard at early stage of sorupkathi guava and produce bitter like mucus that might be differ from other variety.

High fruit infestation in cucurbits at Haryana, might be due to the micro climatic conditions like irrigated farming system (canal irrigation) followed by warm climate during crop season supported by low to moderate rainfall (617 mm annual rainfall) facilitating the rapid fruit fly growth and development (Dhillon*et al.* 2005). Whereas, at Patna and Bihar Sharif (Bihar) which are in the east of the Indo-Gangetic plain, the holy river Ganga flow round the year making local climate warm and humid with onset of monsoon, in the vicinity of Tropics of Cancer helped rapid expansion of fruit flies and consequently heavy fruit damage (Dhillon*et al.* 2005). Higher infestation rate (fruit damage) of fruit flies in Hilly area as well as in other part of Bangladesh on the crops

necessitates large scale adoption of integrated pest management program with wide-area management program as an essential component of IPM for fruit fly management with firm cohesion between farmers-government agriculture departments and educational & research institutions.

Experiment 3. Morphometric characterization of guava infesting fruit fly species in Bangladesh

The study of morphology was a common means of biological grouping and classification. The different species of fruit flies were identified attacking the guava fruit at different location of Bangladesh according to their taxonomy. The morphometric analysis was done at Entomology lab of central laboratory in Sher-e-Bangla Agricultural University, Dhaka. The fruit flies were identified using conventional taxonomy on most morphological characters in the egg & adult stages which were the most important stage of detection of any pest for successful identification. The fruit fly sample was collected at different locations of Bangladesh using pheromone trap (methyl-eugenol). The pheromone trap was setup in the guava field for 48 hours. After 48 hours the fruit fly samples were collected from trap then washed and preserved in ethanol. The samples were then studied under stereomicroscope.

4.3.1 Results and discussion

The fruit fly samples were collected at different location of Bangladesh using pheromone trap (methyl-eugenol) at guava orchard. Five different species were identified using stereomicroscope. The name of the fruit flies were Oriental fruit fly (*Bactrocera dorsalis*), melon fly (*Zeugodacus cucurbitae*), pumpkin fruit fly (*Zeugodacus tau*), peach fruit fly (*Bactrocera zonata*) and. *Dacus longnicornis.* (Table 4.3.1). The abundance of fruit fly was high at Bhandarban followed by chapainawabgonj.

Table 4.3.1. Total number of different fruit flies collected from pheromone trap placed at different regions of Bangladesh with (%) percentage value

Area	Total fly/trap	*Bactrocera dorsalis*(%)	*Zeugoda cuscucurbitae* (%)	*Zeugodacus tau* (%)	*Bactrocera zonata* (%)	*Dacus longnicornis* (%)
Dhaka	162	59.88	19.75	6.79	13.58	--
B.Baria	122	86.06	13.93	--	--	2 samples
Norsingdi	112	81.25	18.75	--	--	--
Rangamati	275	100	--	--	--	--
Bhandorbon	515	99.61	0.38	--	--	--
Khagrachuri	51	100	--	--	--	--
Chittagang	43	100	--	--	--	--
Rajshahi	109	12.84	32.11	32.11	22.94	--
Naogoan	107	0.93	14.95	63.55	20.56	--
Chapainawabgong	487	81.72	0.21	13.55	4.52	--

Total 78.54 percent of oriental fruit flies were found in total number of pheromone trap which was the highest number of fruit fly in guava orchard. However, 5.19 percent of peach fruit fly were identified which was the lowest number of fruit fly. Moreover, 9.16 percent and 7.10 percent of pumpkin fly and melon fly were collected from pheromone trap respectively. Only 2 *Dacus longnicornis* samples were collected from pheromone trap (Fig.4.3.1). Therefore, numbers of oriental fruit fly were the highest compare to others fruit flies.

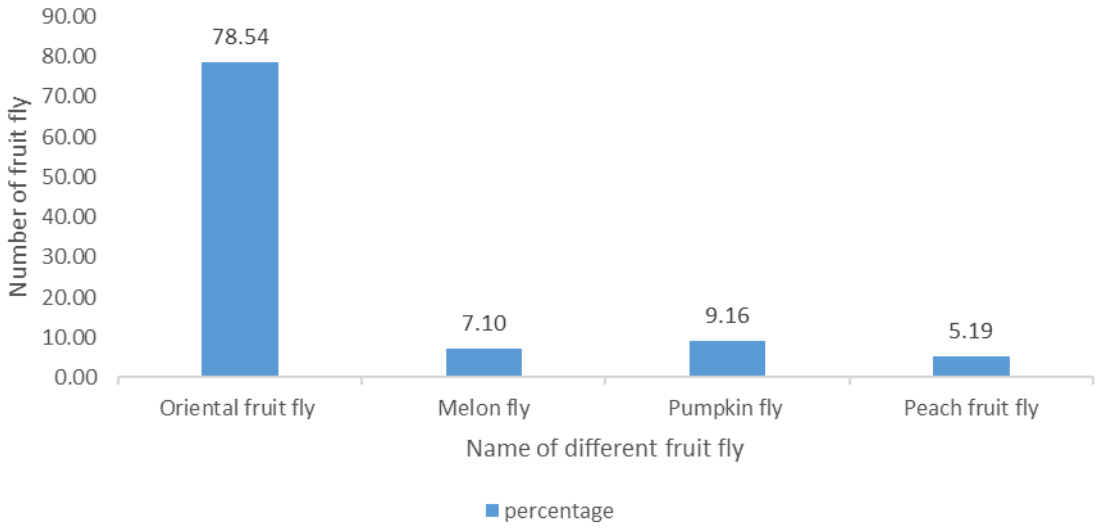

Figure 4.3.1. Percentage of different fruit fly species collected from pheromone trap at different surveyed locations of Bangladesh.

The hilly ecosystem covers around 12% of the country's land area. There is an occurrence of wide genetic variations in plants and insects, both in the wild and cultivated areas. Total 43.21 percent of fruit flies were found in hilly zone which was the highest number of fruit fly (Fig.4.3.2). So that, the number of infestations was higher at hilly tract part (Chittagong, Rangamathi, Bhandarban and khagrachari). On the other hand, 19.40 percent fruit flies were found in central zone which was the lowest number of fruit fly. Moreover, 37.38 percent of fruit flies were collected from north zone pheromone trap (Fig. 4.3.2). Therefore, the highest infestations were observed at hilly zone compared to other two zones of guava orchard. In southern zone, there were no fruit fly infestations found in the surveyed guava orchard. The sorjan method of orchard establishment, presence of water bodies, microclimatic and varietal factors might be the causes behind it.

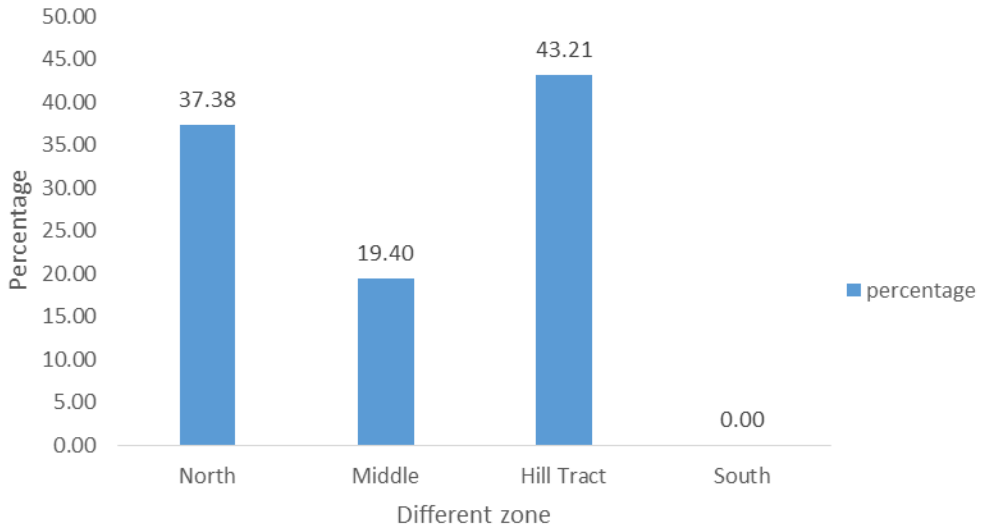

Figure 4.3.2. Percentage of different fruit fly species collected from pheromone trap according to different zones of Bangladesh.

According to Drew and Hancock (1994) distinguished the *B. dorsalis* species complex as follows: *Bactrocera (Bactrocera) spp*. with scutum generally black with lateral vittae present and medial vitta absent (Fig 4.3.3), yellow scutellum, except for basal band which is usually very narrow (Fig. 4.3.3. B). Abdomen with a medial dark stripe on T3-T5; dark laterally (but form of marking varies from species to species) (Fig. 4.3.3. C). A clear wing membrane, except for a narrow costal band (not reaching R4+5); cells bc and c colourless (except in a few non-pests with a very pale tint) with microtrichia restricted to outer corner of cell c (Fig. 4.3.3. D).

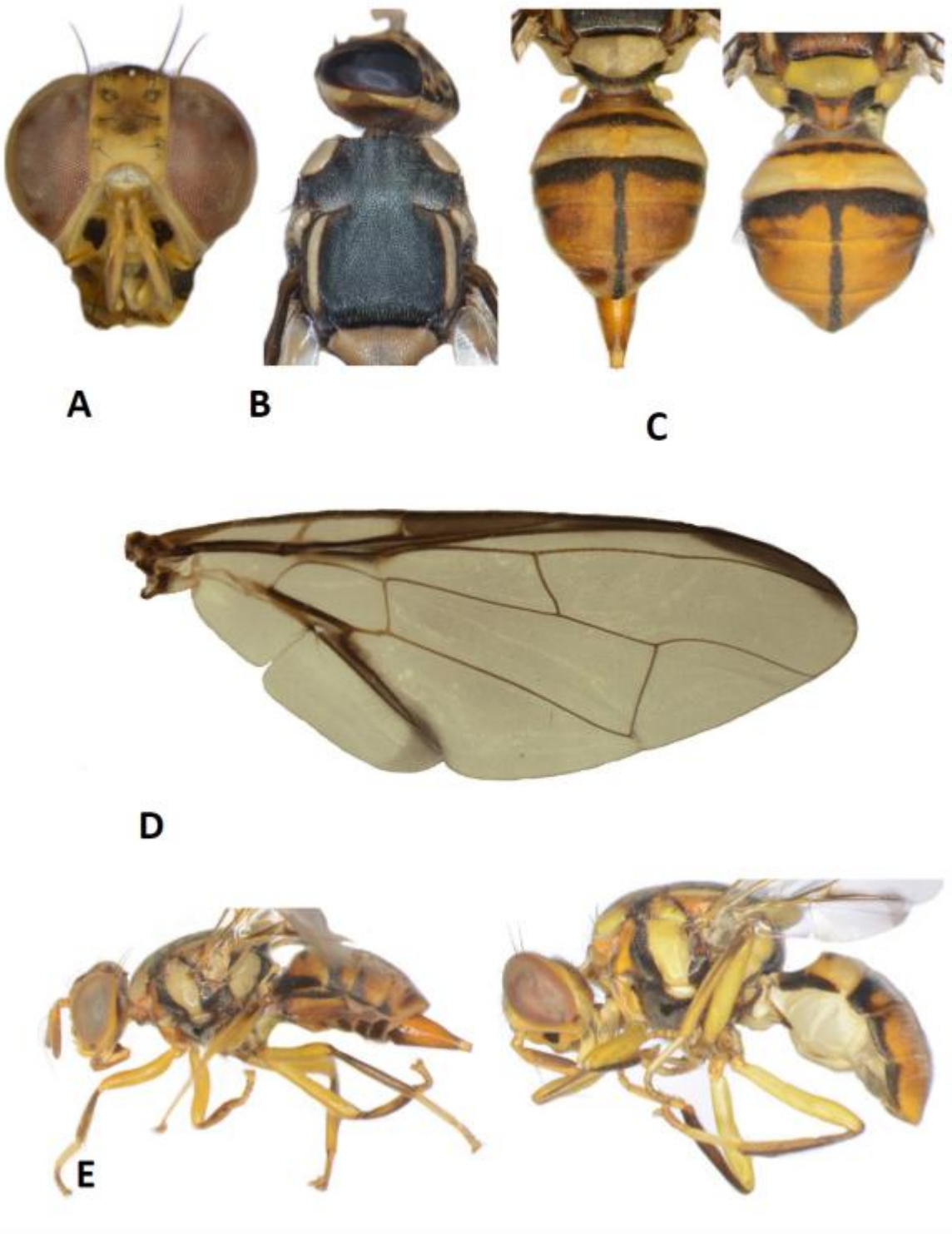

Figure 4.3.3. *Bactrocera dorsalis*, Habitus and body details. (A) Head with compound eyes (B) scutum with a medial postsuturalvitta (C) abdomen, (D) wing and (E) Legs.

Middle zone oriental fruit fly was larger at four different variables compared to other two different zones. 17.17 mm^2 areas were measured at abdominal part of the oriental fruit fly which was higher in contrast to north and hilly zone and were 15.04 mm^2 and 10.29 mm^2 respectively. According to thorax, 15.07 mm^2 areas were measured of the oriental fruit fly which was higher compared to north and hilly zone and were 14.60 mm^2 and 9.17 mm^2 respectively. Moreover, lowest 3.12 mm^2 head was measured at hilly zone compared to middle and north zone. Same observation was recorded in case of wing and the 35.08 mm^2 were measured which was highest at middle zone of Bangladesh (Table 4.3.2).

No significant differences were observed at three different zones of Bangladesh in case of oriental fruit flies. Therefore, middle zone oriental fruit fly was larger compared to other two zones and hilly zone oriental fruit fly were small (Table 4.3.2**).**

According to Drew and Hancock (1994) *Bactrocera* (*Bactrocera*) spp. with a clear wing membrane, except for a narrow costal band (not reaching R4+5); cells bc and c colourless (except in a few non-pests with a very pale tint) with microtrichia restricted to outer corner of cell c (Figure 4.3.3. A). Scutum generally black with lateral vittae present and medial vitta absent; yellow scutellum, except for basal band which is usually very narrow (Figure 4.3.3. B). Abdomen with a medial dark stripe on T3-T5; dark laterally (but form of marking varies from species to species) (Figure 4.3.3. C).

Table 4.3.2. Size (length x width) of oriental fruit fly (*Bactrocera dorsalis*) collected from pheromone trap at different zones of Bangladesh with CV and LSD (0.05%).

Name of fruit fly	Zone	Abdomen(mm^2)	Thorax (mm^2)	Head (mm^2)	Wing (mm^2)
Oriental Fruit fly	Middle	17.173 a	15.065 a	4.600 a	35.081 a
Oriental Fruit fly	North	15.042 ab	14.597 a	4.971 a	33.362 a
Oriental Fruit fly	Hilly tract	10.294 b	9.167 b	3.118 b	17.436 b
CV (%)		18.83	13.34	11.79	12.32
LSD (0.05%)		2.17	1.40	0.40	2.88

According to White and Hancock (1997) melon fly head was like Pedicel+1st flagellomere no longer than ptilinal suture. Face with a dark spot in each antennal furrow; facial spot round to elongate (Fig. 4.3.4. A). Thorax was predominant colour of scutum red brown. Scutum with parallel sided lateral postsutural vittae (yellow/orange stripes) which extended anterior to suture and posteriorly to level of the intra-alar setae. Medial vitta was present; not extended anterior to suture. Scutellum was yellow, except for narrow basal band (Fig. 4.3.4, B). Abdomen were predominant colour orange brown. Tergites were not fused. Abdomens were not wasp waisted. Pattern distinct; transverse band across tergite 3; tergite 4 dark laterally; medial longitudinal stripe on T3-5 (Fig. 4.3.4, C). Length of wing was 4.2-7.1 mm with a complete costal band; depth to below R2+3, sometimes reaching R4+5. Costal band expanded into a spot at apex, which extended about halfway to M with an anal streak. Cells bc and c were colorless (Fig. 4.3.4, D). Legs were all femora pale basally, red brown apically (Fig. 4.3.4, E).

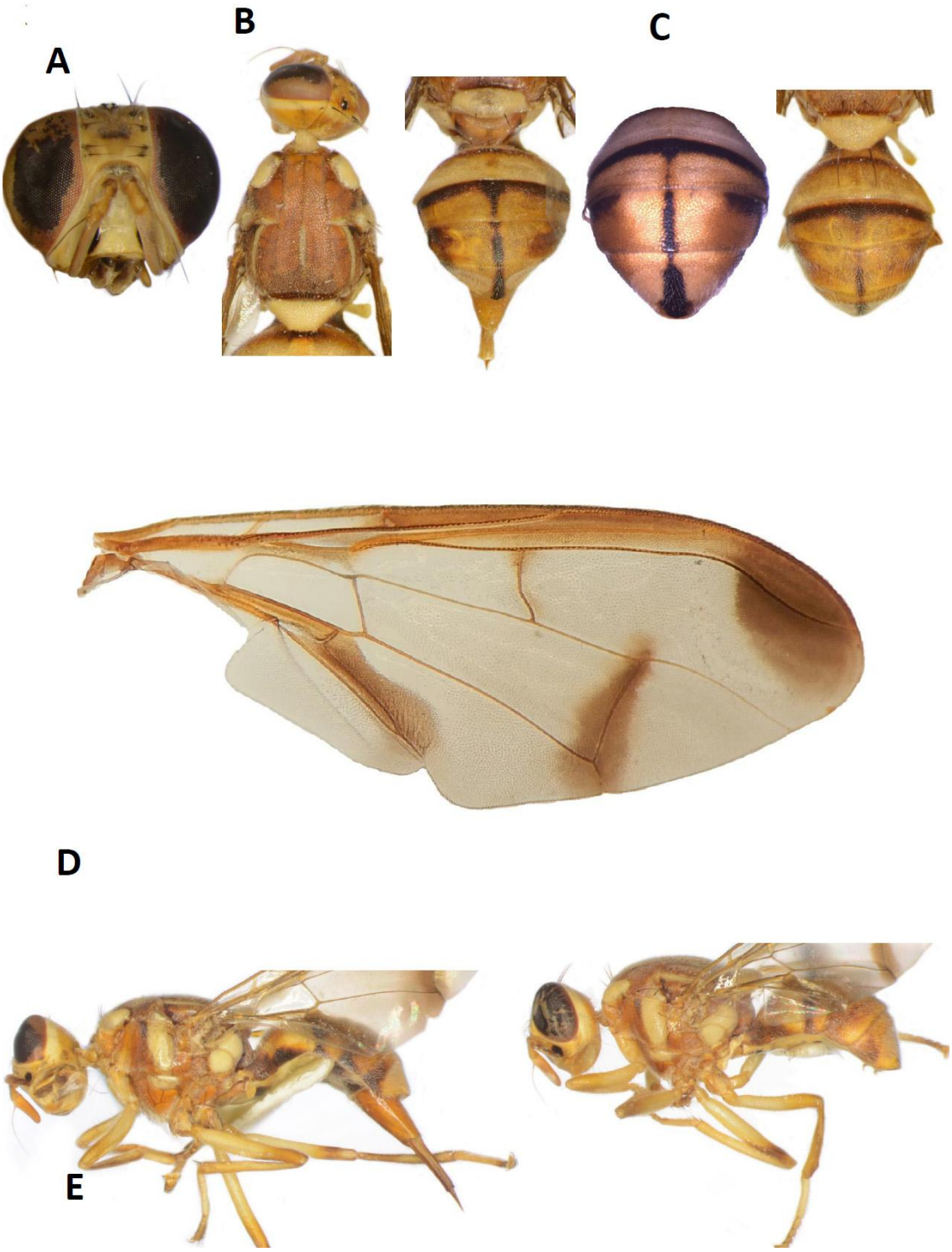

Figure 4.3.4. *Zeugodacus cucurbitae*, Habitus and body details. (A) head with compound eyes (B) scutum with a medial postsuturalvittae (C) abdomen, (D) wing and (E) Legs.

North zone melon fruit flies were larger at abdomen area compared to other two different zones. 15.17 mm^2 areas were measured at abdominal part of the melon fly which was higher in contrast to middle and hill tract zone and were 14.67 mm^2 and 12.42 mm^2 respectively. According to thorax, 14.50 mm^2 areas were measured of the melon fly which was higher compared to middle and hill tract zone and were 13.70 mm^2 and 13.39 mm^2 were respectively. Moreover, lowest 4.01 mm^2 head was measured at north zone compared to middle and north zone and highest 5.56 mm^2 area were observed at hill tract zone which is highest. Same observation was observed in case of wing and the 85.20 mm^2 were measured which was highest at hill tract zone of Bangladesh (Table 4.3.3).

No significant differences were observed at three different zone of Bangladesh melon fly. Therefore, middle zone melon fly was prominent compared to other two zones and melon fly were larger compared to oriental fruit fly.

Table 4.3.3. Size (length x width) of melon fly (*Zeugodacus cucurbitae*) collected from pheromone trap at different zones of Bangladesh with CV and LSD (0.05%) value

Fruit fly name	Zone	Abdomen(mm^2)	Thorax(mm^2)	Head(mm^2)	Wing(mm^2)
Melon fly	**Middle**	14.671 a	13.695 a	4.993 ab	29.089 b
Melon fly	**North**	15.170 a	14.498 a	4.013 b	35.076 a
Melon fly	**Hill tract**	12.422 a	13.390 a	5.556 a	38.203 a
CV		8.23	17.43	9.29	5.41
LSD (0.05%)		0.96	1.97	0.36	1.50

Pumpkin fly adults have orange-brown scutum marked with black stripes contains lateral two and median and yellow stripes. Females have pointed abdomen and male have round and male was smaller than female insect (Fig. 4.3.5). According to White and Hancock (1997) pumpkin fly head has pedicel+1st flagellomere and no longer than ptilinal suture. Face with a large dark spot in each antennal furrow. Frons - 2-3 pairs frontal setae, 1 pair

orbital setae (Fig. 4.3.5. A). Pumpkin fly thorax has predominant colour of scutum fuscous. Postpronotal (humeral) lobe entirely pale (yellow or orange). Notopleuron yellow. Scutum with lateral postsutural vittae (yellow/orange stripes), which are not tapered, and which extend beyond the intra-alar setae. With a medial vitta. Scutellum not partly dark marked. Anepisternal stripe as narrow as notopleural spot. Yellow marking on both anatergite and katatergite. Postpronotal lobe (humerus) without a seta. Notopleuron with anterior seta. Scutum with anterior supra-alar setae; with prescutellar acrostichal setae. Scutellum with basal as well as apical setae (Fig. 4.3.5. B). Abdomen predominant colour orange, brown. Tergites not fused. Abdomen not wasp aisted. Pattern distinct. Tergite 3 with a transverse band. Tergite 4 either with antero-lateral recatngular marks or dark laterally. Medial longitudinal stripe on T3-5. Sternites dark, not yellow (Fig. 4.3.5. C). Wing length 6.1-8.8 mm. Wing with a complete costal band, which may extend below R2+3, but not to R4+5; expanded into a spot at apex which reaches about halfway to M. Wing with an anal streak. Cells bc and c not coloured. No transverse markings. Cell bc and c without extensive covering of microtrichia. Cell br (narrowed part) with extensive covering of microtrichia (Fig. 4.3.5. D). Legs fore femur yellow / pale, sometimes with a dark preapical spot. Mid and hind femora pale (Fig. 4.3.5. E).

At hill tract zone, no pumpkin fruit flies were found. 15.40 mm^2 areas were measured at abdominal part of the pumpkin fly which was higher at middle zone compared to hill tract zone and the lowest value was 8.09 mm^2. According to thorax, 15.78 mm^2 areas were measured of the pumpkin fly which was higher at middle compared to hill tract zone and the value was 11.11 $mm^{2.}$ Moreover, same trend was observed in case of head and wing of the pumpkin fly of Bangladesh (Table 4.3.4).

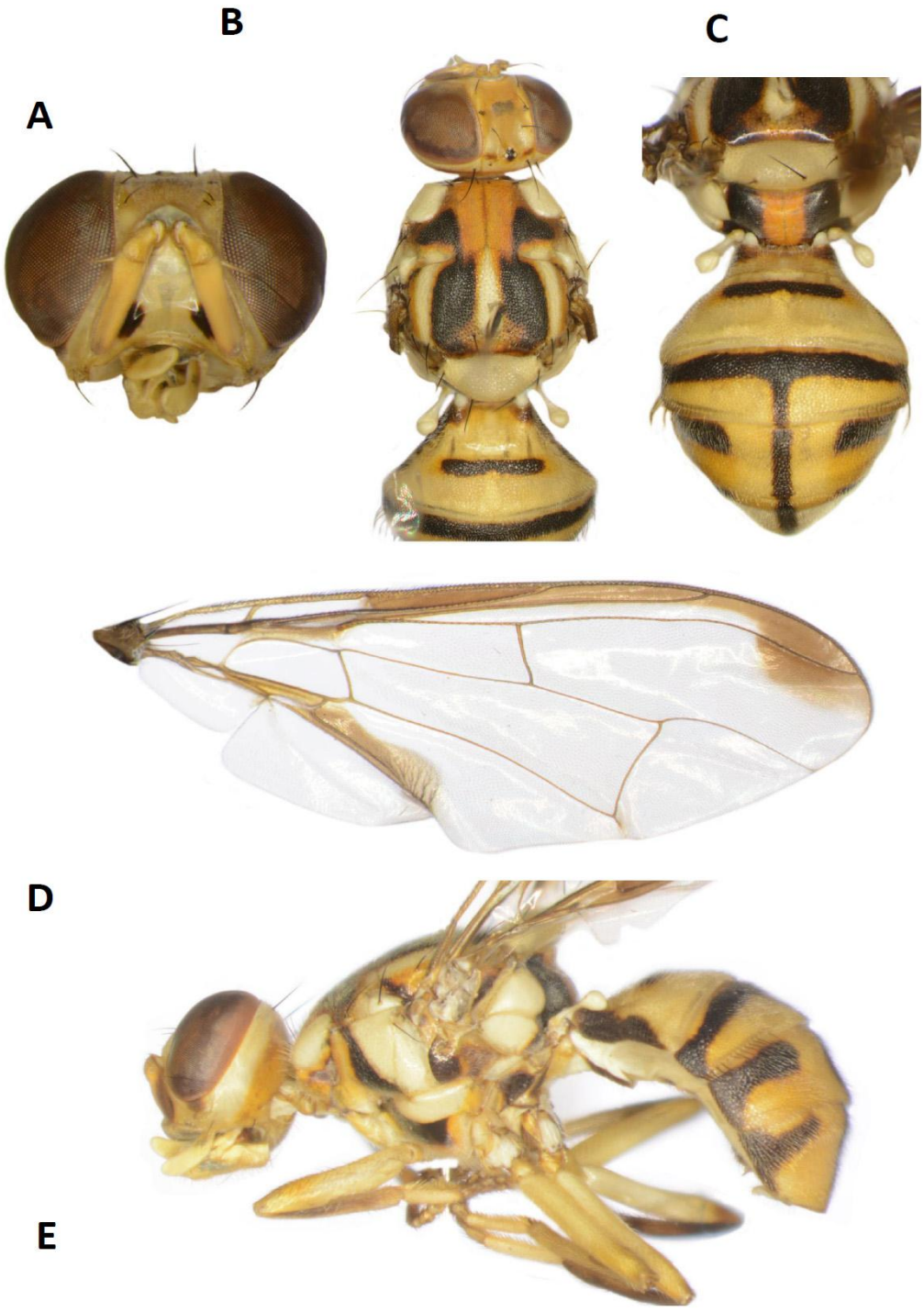

Figure 4.3.5. *Zeugodacus tau*, habitus and body details. (A) Head with compound eyes (B) scutum with a medial postsuturalvittae (C) abdomen, (D) wing and (E) legs.

Table 4.3.4. Size (length x width) of pumpkin fly (*Zeugodacus tau*) collected from pheromone trap at different zones of Bangladesh with CV and LSD (0.05%) value

Fruit fly name	Zone	Abdomen(mm^2)	Thorax(mm^2)	Head(mm^2)	Wing(mm^2)
Pumpkin fly	**Middle**	15.396 a	15.781 a	4.780 a	32.501 a
Pumpkin fly	**North**	8.089 b	11.110 a	3.086 a	23.646 a
	CV	21.14	16.59	19.18	20.74
	LSD (0.05%)	2.02	1.82	0.61	4.75

Significant differences were observed at different zone of Bangladesh in respect of pumpkin fly at abdominal body part. Therefore, middle zone pumpkin fly was prominent compared to other zone and pumpkin fly were almost same size and shape with melon fly. No pumpkin fly was found at hill tract zone of Bangladesh at guava orchard.

Bactrocera zonata adult was about 6 mm long and reddish brown with yellowish thoracic markings. Head higher than long and chaetotaxy reduced. Dark round spots in each antennal furrow (Fig. 4.3.6. A). In thorax, anterior supra-alar bristles present. Scutum orange, brown, or red brown. Scutum has two pale whitish to yellow lateral postsutural stripes (vittae), they extending to intra-alar bristles or beyond. Scutum without blackish dorsoventral stripe (Fig. 4.3.6. B). Abdomen ovate or parallel sided and yellow to orange, brown color. Abdominal tergites with medial dark stripe usually on T5; not brown with medial T-shaped yellow mark (Fig. 4.3.6. C).

Wings with sub-costal vein (Sc), which bends abruptly to the wing edge, combined with the presence of setulae along the dorsal side of vein R_1 and yellowish and brownish in color (Fig. 4.3.6. D). Femora legs of *Bactrocera zonata* are slender. Fore femora are with regular bristles and mid femur and hind femur are without spine bristles. Middle legs of ale is without feathering. All femora are entirely yellow without dark mark (Fig. 4.3.6. E).

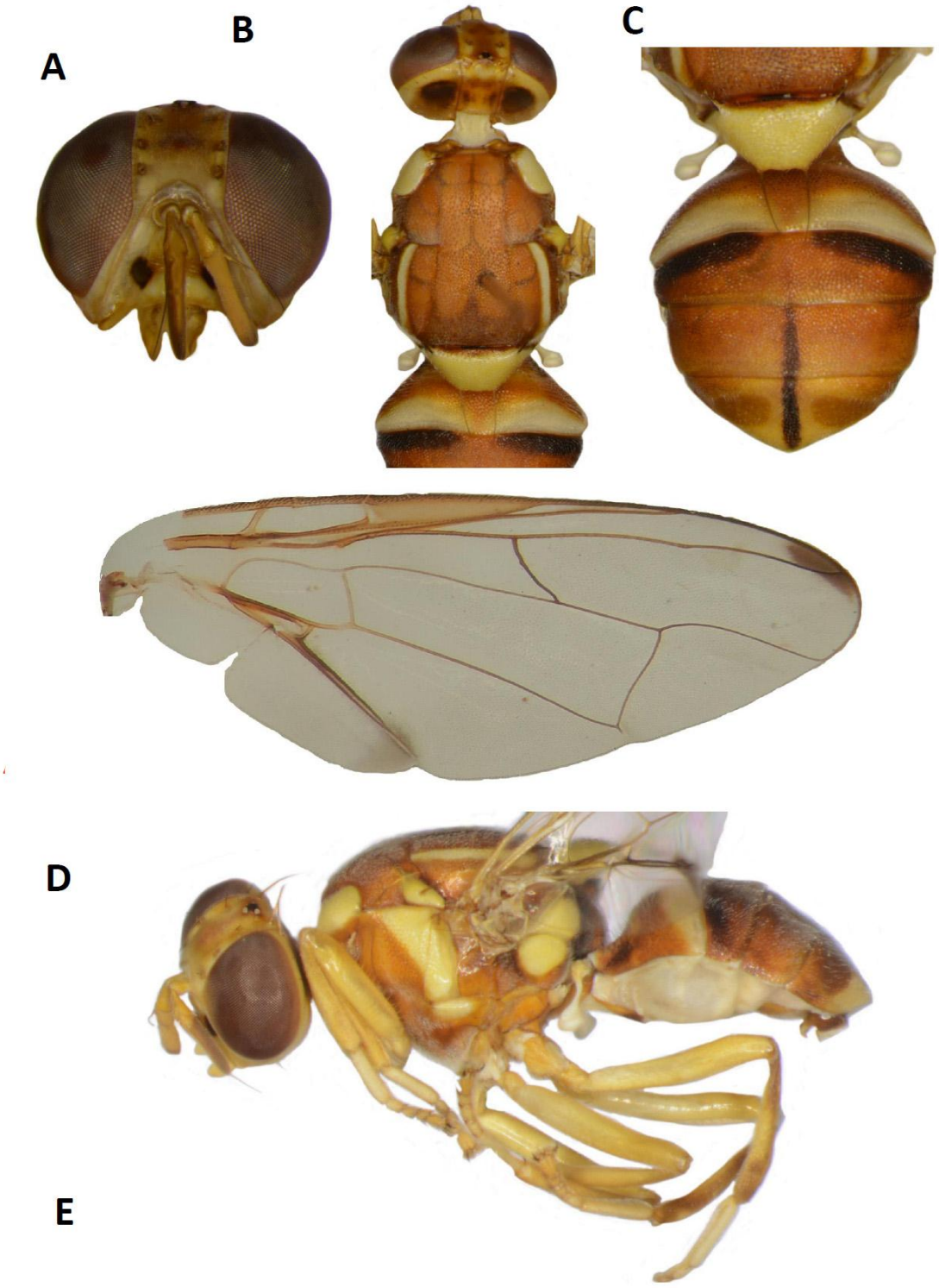

Figure 4.3.6. *Bactrocera zonata*, habitus and body details. (A) Head with compound eyes (B) scutum with a medial post sutural vittae (C) abdomen (D) wing, (E) Legs.

At hill tract zone, no peach fruit fly was found. 13.87 mm^2 area were measured at abdominal part of the peach fruit fly which was higher at middle zone compared to hill tract zone and the lowest value was 9.27 mm^2. In the thorax, 13.79 mm^2 area were measured of the peach fruit fly which was higher at middle as compared to hill tract zone and the value was 10.33 mm^2. Similar trend was observed for head and wing of the peach fruit fly of Bangladesh (Table 4.3.5). Significant difference was observed at different zones of Bangladesh regarding peach fruit fly *(Bactrocera zonata)* at abdominal body part. Therefore, middle zone peach fruit fly was prominent compared to other zones and peach fruit fly were almost same sized and shaped with melon fly. No peach fruit fly was found at hill tract zone of Bangladesh at guava orchard (Table. 4.3.5; 4.3.6).

Table 4.3.5. Size (length x width) of peach fruit fly (*Bactrocera zonata*) collected from pheromone trap at different zones of Bangladesh

Fruit fly name	Zone	Abdomen (mm^2)	Thorax (mm^2)	Head (mm^2)	Wing (mm^2)
Peach fruit fly	Middle	13.872 a	13.786 a	4.128 a	28.893 a
Peach fruit fly	North	9.271 b	10.332 a	4.098 a	25.224 a
CV (%)		5.62	14.01	23.07	9.32
LSD (0.05%)		0.53	1.37	0.77	2.05

Dacus longnicornis head (Fig 4.3.7.A) were fulvous with a pair of small irregularly oval black spots and scutum dark red-brown without distinct dark patterns (Fig.4.3.7.B), lateral and medial postsutural yellow vittae absent and scutellum yellow except for broad red-brown basal band. *D. longnicornis* legs with fore femora dark red-brown to fuscous, mid femora dark red-brown to fuscous except fulvous on basal 1/4, hind femora dark fuscous and wing with cells bc and c fuscous, dense microtrichia over all of cell c and

most of cell bc and abdomen strongly petiolate, abdominal terga III-V generally dark fuscous to black with a paler band often across posterior margin of tergum III, large orange-brown spots posterocentrally on terga IV and V with the spot on tergum V often expanded anteriorly into a medial longitudinal orange-brown band (Fig.4.3.7)

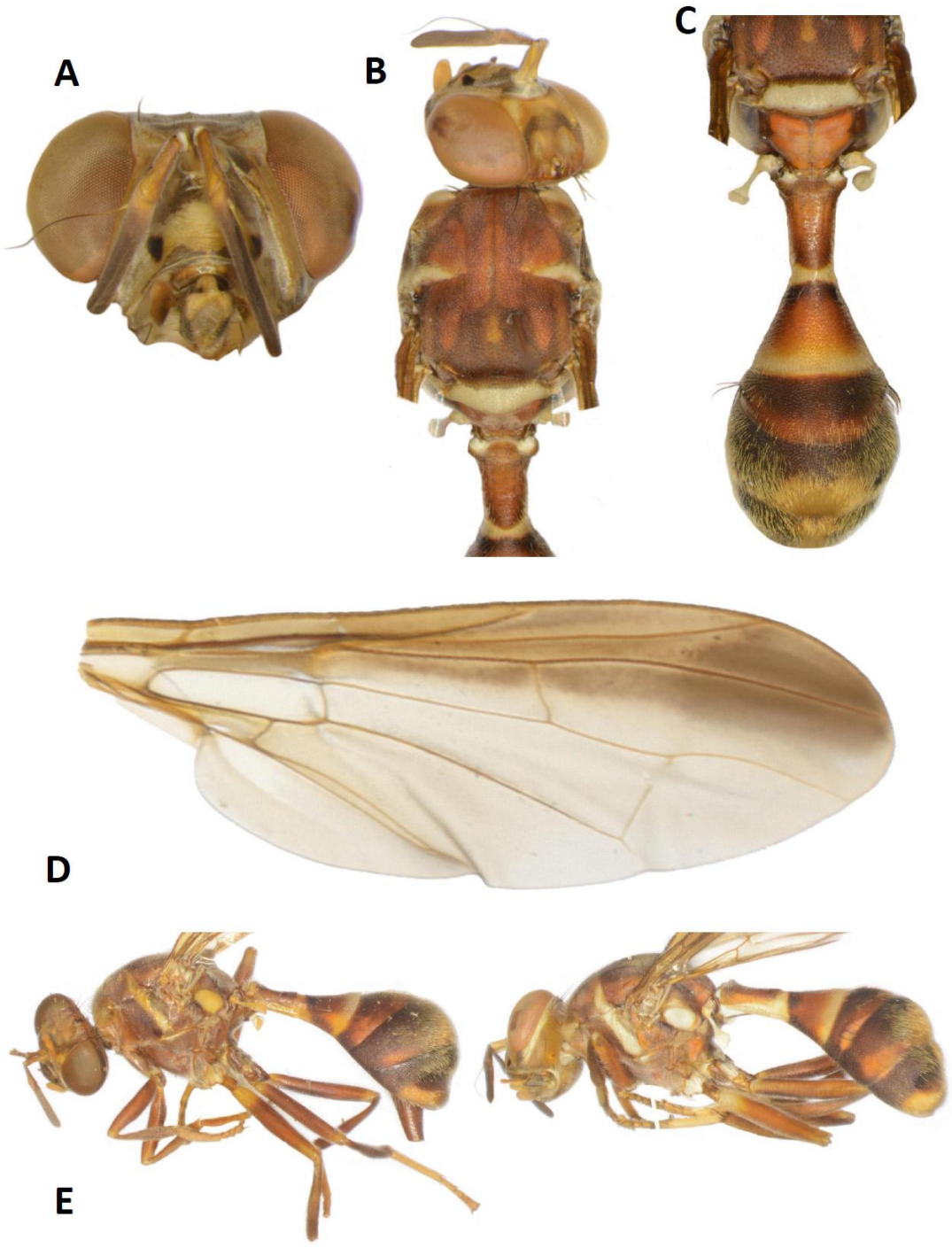

Figure 4.3.7. *Dacus longnicornis* habitus and body details. (A) Head with compound eyes (B) a scutum with a medial postsuturalvittae (C) Abdomen and hind legs (D) abdomen, (E) Legs.

Table 4.3.6. Body part size (Length and width) of four identified fruit fly species collected from pheromone trap at different zones of Bangladesh with (±SE) value

Species	Abdomen (±SE)	Scutum (±SE)	Head (±SE)	Wing (±SE)
Oriental fruit fly ***(Bactrocera dorsalis)***	L=3.97±0.46	L=4.51±0.08	L=1.70±0.23	L=9.33±0.37
	W=3.77±0.29	W=3.23±0.15	W=2.94±0.16	W=3.57±0.22
Melon fruit fly ***(Zeugodacus cucurbitae)***	L=4.18±0.05	L=4.46±0.09	L=1.56±0.05	L=9.6±0.1
	W=3.63±0.19	W=3.25±0.05	W=2.57±0.04	W=3.65±0.06
Pumkin fruit fly ***(Zeugodacus tau)***	L=2.97±0.17	L=3.95±0.26	L=1.26±0.05	L=7.77±0.42
	W=2.72±0.23	W=2.81±0.06	W=2.46±0.03	W=3.05±0.07
Peach fruit fly ***(Bactrocera zonata)***	L=3.72±0.04	L=4.23±0.15	L=1.60±0.3	L=8.96±0.06
	W=3.54±0.03	W=3.11±0.15	W=2.69±0.17	W=3.47±0.03

Figure 4.3.8. Morphological structure (Abdomen, scutum, head and wing) of identified five fruit fly species captured in pheromone trap at different zones of Bangladesh

Species	Abdomen	Scutum	Head	Wing
Oriental fruit fly ***(Bactrocera dorsalis)***				
Melon fruit fly ***(Zeugodacus cucurbitae)***				
Pumkin fruit fly ***(Zeugodacus tau)***				
Peach fruit fly ***(Bactrocera zonata)***				
Dacus Longnicornis				

Five different species were identified using stereomicroscope. The name of the fruit flies were Oriental fruit fly (*Bactrocera dorsalis*), melon fruit fly (*Zeugodacus cucurbitae*), pumpkin fruit fly (*Zeugodacus tau*), peach fruit fly (*Bactrocera zonata*) and. *Dacus longnicornis.* The numbers of oriental fruit fly were highest compared to other fruit fly. The highest infestations were observed at hill tract zone compared to other two zones in guava orchard. Middle zone oriental fruit fly was larger compared to other two zones and hill tract zones oriental fruit flies were small. Therefore, middle zone melon fruit fly was prominent compared to other two zones and melon fruit fly were larger compared to oriental fruit fly. No pumpkin fruit fly and peach fruit fly were found in hill tract zone of Bangladesh at guava orchard.

Experiment 4. Molecular Detection of guava infesting Fruit Fly Species in Bangladesh

Molecular genetics is a sub-field of genetics that applies an "investigative approach" to determine the structure and/or function of genes in an organism's genome using genetic screens. Researchers search for mutations in a gene or induce mutations in a gene to link a gene sequence to a specific phenotype. The molecular analysis help to identify the biofilm composition to the genus level and to determine shifts in the community due to environmental changes (Herbertet *et al.* 2003). The main objective of this work is the Standardization of the molecular detection protocols of different species and race(s) of fruit fly infesting guavas collected from different regions of Bangladesh and the work done at the Molecular Entomology lab at the Central Laboratory, Sher-e-Bangla Agricultural University, Dhaka.

Molecular detection augmented with morphometric was an efficient technique of insect detection which was not limited by sex and stage of development of the target species. Many kinds of molecular detection including microsatellites, internal transcribed spacer 1 (ITS1), amplified fragment length polymorphism, 16S rRNA, 12S rRNA, mitochondrial cytochrome oxidase I (mt COI), etc. had been employed as molecular detection approach and the mt COI used to standardize the detection of the fruit fly species infesting Bangladeshi guava. Different types of fruit flies were identified using conventional taxonomy on most morphological characters in the egg & adult stage which is the most important stage for detection of any pest. Fruit fly samples were collected at different locations of Bangladesh using trap (methyl-eugenol) (Simon *et al.* 1994).

The trap was set up in the guava field for 48 hours. After 48 hours the fruit fly samples were collected from the trap then washed and preserved with ethanol. The samples were analyzed with COI gene and then sequenced the fruit fly species and comparing the sequenced samples with a different source of fruit fly sequence to identify the species and races of fruit fly in Bangladesh.

Results and discussion

Among the collected fruit fly species in this study, five were morphologically identified to species level and three were identified upto genera (*Bactrocera* sp., *Zeugodacus* sp. and *Dacus* sp.). Only *Bactrocera dorsalis* were reared from guava host plant, but the rest of the species were not found from guava. It is probable that, the *Zeugodacus cucurbitae*, *Zeugodacus tau*, *Bactrocera zonata*, and *Dacus longnicornis* were come from other host plants especially from vegetabes and infested diverse host plant species.

Table 4.4.1. List of all fruit fly species with **Accession** number reported and found in samples in this study.

SL NO.	Scientific name	Geographical Location	Percent Identification (%)	Accession Length	Accession Number
1	*Bactrocera dorsalis*	South	99.33	605	OK083710.1
2	*Bactrocera dorsalis*	North	99.33	606	OK175561.1
3	*Bactrocera dorsalis*	South	100	607	OK083609.1
4	*Bactrocera dorsalis*	North	100	555	MZ960188.1
5	*Bactrocera dorsalis*	North	100	603	OK175615.1
6	*Bactrocera dorsalis*	North	99.83	603	OK175616.1
7	*Bactrocera dorsalis*	Middle	100	609	OK083601.1
8	*Bactrocera dorsalis*	Middle	100	582	OK083602.1
9	*Bactrocera dorsalis*	Middle	100	599	OK083603.1
10	*Bactrocera dorsalis*	South	100	569	OK087308.1
11	*Bactrocera dorsalis*	South	100	609	OK087309.1
12	*Bactrocera dorsalis*	South	100	599	OK087311.1
13	*Bactrocera dorsalis*	South	100	592	OK087313.1
14	*Bactrocera dorsalis*	North	100	605	OK175604.1
15	*Bactrocera dorsalis*	North	100	607	OK175605.1
16	*Bactrocera dorsalis*	South	100	599	OK087315.1
1	*Zeugodacus cucurbitae*	Middle	100	560	MZ960189.1
2	*Zeugodacus cucurbitae*	Middle	100	599	OK083606.1
3	*Zeugodacus cucurbitae*	Middle	100	599	OK083608.1
4	*Zeugodacus cucurbitae*	South	100	586	OK087317.1
5	*Zeugodacus cucurbitae*	South	100	589	OK087318.1
6	*Zeugodacus cucurbitae*	North	100	594	OK175606.1
7	*Zeugodacus cucurbitae*	North	100	579	OK175608.1
8	*Zeugodacus cucurbitae*	North	100	554	OK175609.1
1	*Zeugodacus tau*	Middle	100	558	MZ960191.1
2	*Zeugodacus tau*	Middle	100	604	OK083607.1
3	*Zeugodacus tau*	Middle	100	599	OK083608.1
4	*Zeugodacus tau*	North	100	589	OK175607.1
5	*Zeugodacus tau*	North	100	594	OK175610.1
6	*Zeugodacus tau*	North	100	602	OK175611.1
1	*Bactrocera zonata*	Middle	100	585	MZ960190.1
2	*Bactrocera zonata*	North	98.80	591	OK175612.1
3	*Bactrocera zonata*	North	98.63	592	OK175613.1
4	*Bactrocera zonata*	North	98.97	593	OK175614.1
1	*Dacus longicornis*	Middle	100	542	MZ960192.1

Evaluation of sequences from different regions and identification of haplotype and their distribution. To determine the genetic diversity and distribution, (609-542) bp trimmed nucleotide sequences of 35 mt-COI region represented by South (9), middle (11) & North (15) zones of three guava growing zones of Bangladesh were used for analysis. The homology search of sequences using NCBI BLAST matched with the mt-COI sequences of fruit fly in database, and the sequence similarity varied from 98–100% to that of available sequences of fruit fly populations. The nucleotide sequences were aligned using MEGA ver. X and used for further analysis. The trimmed sequences were deposited in NCBI Gene Bank and accession numbers (Table: 4.4.1.) were obtained.

Table 4.4.2. Results from Tajima's Neutrality Test of four different species of fruit fly where, m = number of sequences, n = total number of sites, S = Number of segregating sites, Ps = S/n, Θ = ps/a1, π = nucleotide diversity, and D is the Tajima test statistic.

	m	***S***	***Ps***	***Θ***	***π***	***D***
Bactrocera dorsalis	13	22	0.03	0.01	0.007	-1.64
Zeugodacus cucurbitae	6	2	0.003	0.001	0.001	-1.13
Zeugodacus tau	5	10	0.01	0.007	0.006	-0.89
Bactrocera zonata	4	13	0.02	0.01	0.01	0.10

The study identified a total of 24 (68.57%) unique haplotypes in fruit fly populations of the three guava growing zones of Bangladesh. Among the nucleotide mutations (42) found in different haplotypes (Table 4.4.2). Tajima's D of neutrality tests were executed for analysis of demographic history in Bangladeshi fruit fly populations.

Neutrality tests were rejected for all populations with significant negative values except *Bactrocera zonata* (table 4.4.2). The four neutrality tests were performed, and three values were negative for populations of fruit fly indicating there was an excess of rare mutations which favor population expansion or growth.

4.4.1. BLAST analysis of *Bactrocera dorsalis*

ACAAATGTTCAGCTACATAATCATTGCGGTACCCACAGGTATTAAAATTTTTAGTTGACTAGC
TACATTACACGGTACACAATTAAACTATTCACCAGCCATATTATGAGCCCTAGGGTTTGTATTC
CTATTTACAGTAGGAGGATTAACAGGAGTAGTTCTTGCTAATTCATCTGTAGATATTATTCTTC
ATGATACATATTACGTAGTAGCTCATTTCCACTATGTATTATCAATAGGAGCAGTCTTTGCTAT
TATAGCAGGATTCGTTCACTGATACCCCCTATTTACAGGGCTAGTATTAAATCCTAAATGATTA
AAAAGTCAATTTATTATCATGTTTATCGGAGTAAATTTAACCTTCTTCCCACAACACTTTTTAG
GATTAGCTGGTATACCTCGACGATATTCAGATTATCCAGATGCTTACACAACATGAAATGTAG
TTTCTACTATTGGTTCATCTATTTCTTTACTAGGAATTTTATTCTTCCTATTCATCATTTGAGAA
AGTTTAGTAACACAACGACAAGTAATTTACCCTATACAGCTTAGTTCTTCAATTGAATGACTTC
AAAATACCCCTCCAGCTGAACACAGTTATTCAGAACTACCTCTTTTAACTAATTATCTAATATG
GCAGATTAGTGGCAATGGAA

BLAST analysis revealed that the observed species shows 98-100% homology with the sequence of *Bactrocera dorsalis* submitted in NCBI GenBank with E value 0.0 and query cover 98%-100% (Table 4.4.1). It indicates that the observed sample was *Bactrocera dorsalis*. Chromatogram of *Bactrocera dorsalis* (forward primer) of FASTA sequence (642bp) (Fig. 4.4.1).

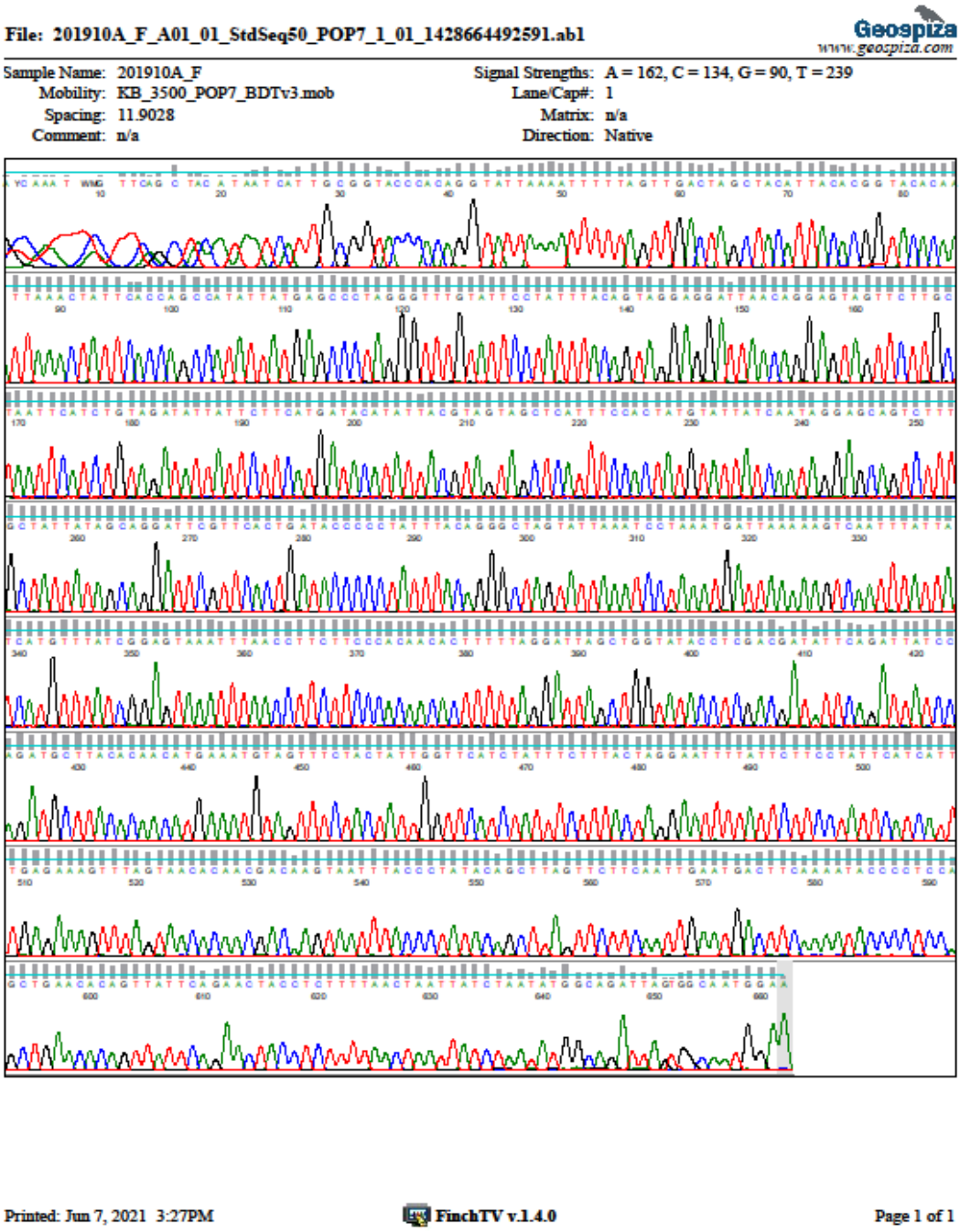

Figure 4.4.1. Chromatogram of *Bactrocera dorsalis* (using forward primer) of FASTA sequence (derived from forward primer, 642bp).

Table 4.4.3. Haplotype and nucleotide diversity of *Bactrocera dorsalis* in Bangladesh. N: Number of samples; H: Number of haplotypes; Number of segregating (S); Hd: Haplotypes diversity; K: Variance of haplotype diversity; pi: Nucleotide diversity.

Location	Number of sequence (N)	Number of Haplotypes (h)	Number of segregating (S)	Haplotype diversity (Hd)	Nucleotide diversity (Pi)	Variance of haplotype diversity (K)
Middle	3	3	8	1.000	0.0101	5.333
South	7	6	8	0.952	0.0057	3.047
North	6	5	8	0.933	0.0058	3.066
India	3	3	2	1.000	0.0025	1.333
China	4	4	4	1.000	0.0037	2.000
Total	23	18	20	0.968	0.0059	3.154

A total of 16 *Bactrocera dorsalis* was sequenced those collected from different regions of Bangladesh and 7 retrieved sequences samples (4 samples from China and 3 samples from India) previously reported from China and India population of *Bactrocera dorsalis.* In phylogenetic analysis of *Bactrocera dorsalis,* the descriptive statistics, Number of sequence (N), Number of Haplotypes (h), Number of segregating (S), Haplotype diversity (Hd), Number of difference (K) and Nucleotide diversity (Pi) were calculated with DnaSP ver. 6.10.01 software. For the descriptive statistics analysis the three guava growing zones (south, middle and north zones) of Bangladesh and China and India samples were used and 23 (N), 18 (h), 20 (S), 0.968 (Hd), 0.0059 (Pi) and 3.154 (K) for *Bactrocera dorsalis* which suggest that entire population exhibited low level of genetic diversity. In this study zone-wise clustering revealed that north zone recorded low level of Hd (0.93) as compared to middle (1.00) and China and India (1.00) (Table 4.4.3).

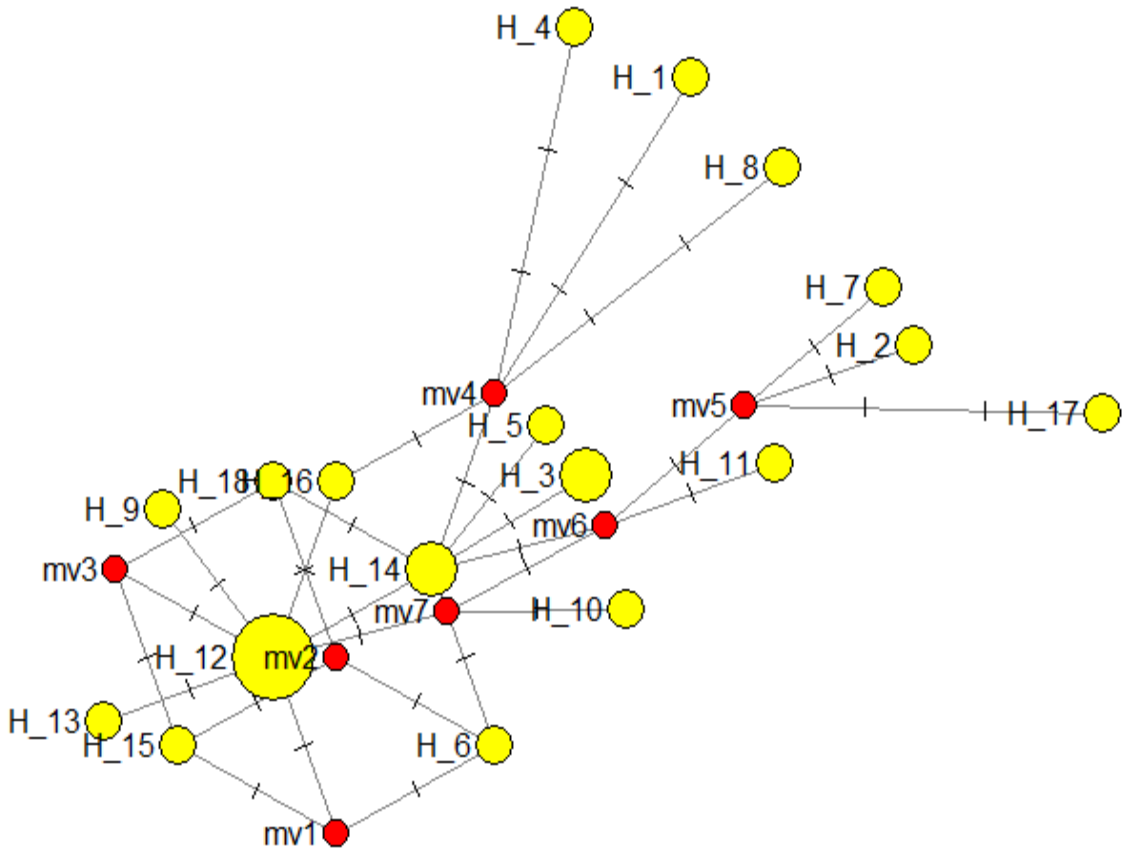

Figure 4.4.2. The haplotype network tree for the mitochondrial COI region of *Bactrocera dorsalis*, circles represent the haplotypes identified and the size of each circle are proportional to the frequency of the haplotypes. The lines between each haplotypes represents the mutations; each line represents single mutation. Where, Hap_1 [BDM2], Hap_2 [BDM1], Hap_3 [BDN5 BDN6], Hap_4 [BDN4], Hap_5 [BDN3], Hap_6 [BDN2], Hap_7 [NDN1], Hap_8 [BDM3], Hap_9 [BDS1], Hap_10 [BDS5], Hap_11 [BDS4], Hap_12 [BDS3 BDS2 India1 China1 Thai1], Hap_13 [India3], Hap_14 [India2 China2], Hap_15 [China4], Hap_16 [China3], Hap_17 [BDS7], Hap_18 [BDS6]

The pairwise population diversity values ranged from 0.01 to 990.00 for *Bactrocera dorsalis*. Total of comparisons, all showed positive and low genetic differentiation for *Bactrocera dorsalis*. (Table 4.4.4). The haplotype network tree for the mitochondrial COI region of *Bactrocera dorsalis* (Figure: 4.4.2). A total of 16 *Bactrocera dorsalis* sequenced those collected from different regions of Bangladesh and 7 retrieved sequences samples (4 samples from China and 3 samples from India) previously reported from China and India population of *Bactrocera dorsalis.* We were found 18 haplotypes. The yellow circles represent the haplotypes identified and the size of each circle are

proportional to the frequency of the haplotypes. The lines between each haplotypes represents the mutations, and each line represents single mutation.

Table 4.4.4. Estimates of **Evolutionary Divergence** between sequences of *Bactrocera dorsalis* species of the fruit fly. The method of calculation was p-distance with the software of MEGA-X.

	Hap_3	**Hap_1**	**Hap_2**	**Hap_5**	**Hap_4**	**Hap_6**	**Hap_8**	**Hap_7**	**Hap_10**	**Hap_9**	**Hap_13**	**Hap_12**	**Hap_11**	**Hap_15**	**Hap_14**	**Hap_16**	**Hap_18**	**Hap_17**
Hap_3																		
Hap_1	760.43																	
Hap_2	0.01	648.45																
Hap_5	506.93	586.00	513.54															
Hap_4	639.52	887.95	641.25	883.02														
Hap_6	570.39	483.16	597.38	610.94	609.90													
Hap_8	0.01	656.93	0.01	508.74	639.49	598.14												
Hap_7	546.36	779.57	547.49	585.68	606.15	539.94	546.36											
Hap_10	513.47	605.37	519.45	0.01	857.67	604.49	514.33	617.08										
Hap_9	761.66	0.01	649.38	587.56	886.51	485.10	657.83	787.32	6072.02									
Hap_13	904.81	627.05	922.76	823.22	916.71	941.98	906.83	559.49	8126.31	626.60								
Hap_12	509.25	586.41	513.54	0.01	883.02	610.86	508.74	585.68	0.00	587.96	8219.94							
Hap_11	761.83	0.01	649.39	587.15	886.51	485.21	657.83	787.32	6060.60	0.01	6253.38	5875.56						
Hap_15	926.80	918.08	914.96	783.16	636.84	982.36	935.84	839.85	8041.41	922.74	6438.87	7831.16	9162.29					
Hap_14	906.77	625.92	924.64	824.50	916.71	941.04	906.83	559.49	8136.63	626.60	0.00	8233.22	6253.38	6447.70				
Hap_16	971.56	605.74	959.00	906.31	627.51	449.21	975.49	609.52	8992.29	605.24	4679.96	9035.55	6069.96	5838.88	4679.96			
Hap_18	495.64	885.44	523.24	989.14	836.47	648.61	495.64	954.74	9852.39	887.72	8908.82	9890.14	8875.57	6495.52	8908.82	6435.45		
Hap_17	0.01	655.11	0.01	507.91	638.53	599.35	0.01	549.77	5134.47	656.03	9048.68	5079.91	6560.02	9360.10	9065.65	9790.54	4960.73	

Genetic distance is a measure of the genetic divergence between species or between populations within a species, whether the distance measures the time from a common ancestor or degree of differentiation (Nei 1987).

Populations with many similar alleles have small genetic distances. This indicates that they are closely related and have a recent common ancestor. The pairwise distance among the species to species and genera to genera was different. The close differences were similar genetically and suppose to come from a common ancestor. Genetic distance is useful for reconstructing the history of populations. For example, evidence from a genetic distance suggests that Sub-Saharan African and Eurasian people diverged about 100,000 years ago (Nei and Roychoudhury 1974). Genetic distance is also used for understanding the origin of biodiversity. For example, the genetic distances between different breeds of domesticated animals are often investigated to determine which breeds should be protected to maintain genetic diversity (Nei and Roychoudhury 1974).

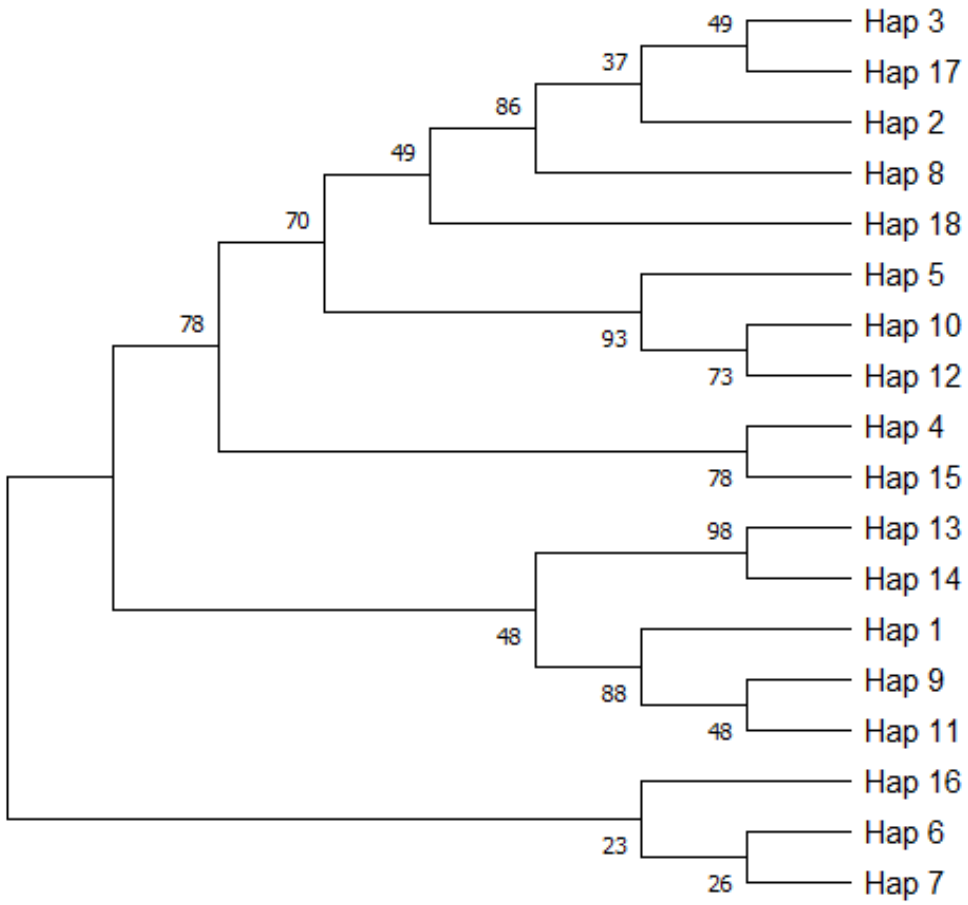

Figure 4.4.3. Phylogenetic tree of the 17 Haplotype of mtCOI DNA sample of *Bactrocera dorsalis*. Where, Hap_1 [BDM2], Hap_2 [BDM1], Hap_3 [BDN5 BDN6], Hap_4 [BDN4], Hap_5 [BDN3], Hap_6 [BDN2], Hap_7 [NDN1], Hap_8 [BDM3], Hap_9 [BDS1], Hap_10 [BDS5], Hap_11 [BDS4], Hap_12 [BDS3 BDS2 India1 China1 Thai1], Hap_13 [India3], Hap_14 [India2 China2], Hap_15 [China4], Hap_16 [China3], Hap_17 [BDS7], Hap_18 [BDS6].

Molecular diagnostics have also been used by insect researchers to infer phylogenetic relationships and for identification to species level (Chua *et al*. 2009, Jamnongluk *et al*. 2003). The evolutionary history was inferred using the Neighbor-Joining method (N. Saitouet *et al.* 1987). The optimal tree of *Bactrocera dorsalis* was shown (Fig. 4.4.3).

The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.* 2004) and were in the units of the number of base substitutions per site. This analysis involved 16 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Evolutionary analyses were conducted in MEGA X (Kumar *et al*. 2018).

In the phylogenetic tree, the horizontal dimension gave the amount of genetic change. The horizontal lines were branches and represent evolutionary lineage changing over time. The longer the branch in the horizontal dimension, the larger the amount of change. The tree is broken down into nodes and branches. There were two types of nodes, externals nodes, and internals nodes. The external nodes or tip represent the actual insect and sequenced. The internal node was represented the putative ancestor for the sample insect. The numbers next to each node represented a measure of support for the node. These were generally numbers between 0 to1. Where 1 represented maximal support. The branch length was usually nucleotide substitution per site-that is the number of changes or substitutions divided by the length of the sequence.

The measure of time, the greater the length of the branches the likelihood greater the distance between the species. In the study, the species were not closely related and have not a common ancestor. They have diverged from each other might be latitude and altitude difference and as well as the ancestor of two region species come from two different locations of the world. (Fig. 4.4.3)

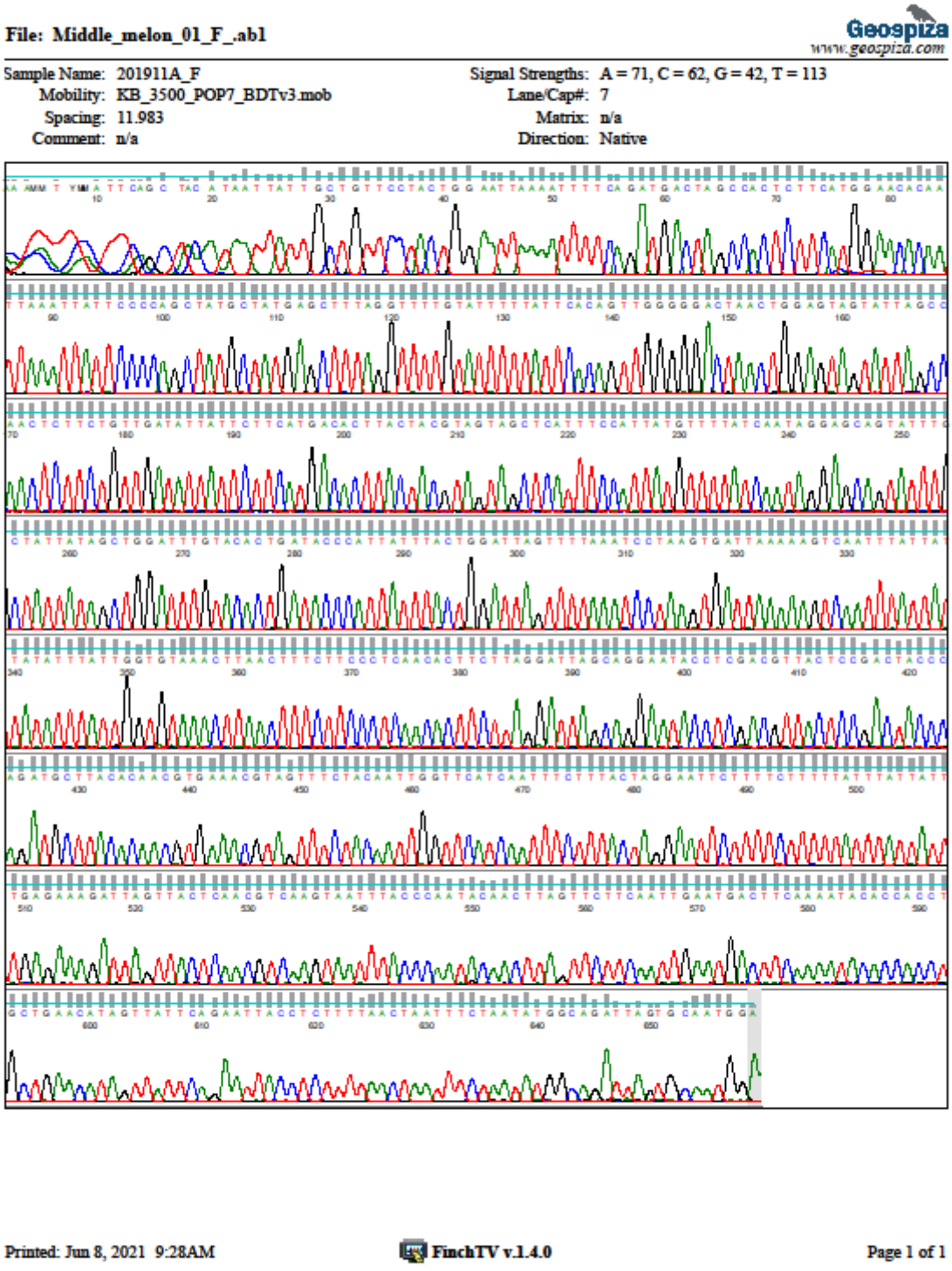

Figure 4.4.4. Chromatogram of *Zeugodacus cucurbitae* (using forward primer) of FASTA sequence (derived from forward primer, 633bp).

4.4.2. BLAST analysis *Zeugodacus cucurbitae*

TTGCTGTTCCTACTGGAATTAAAATTTTCAGATGACTAGCCACTCTTCATGGAACACAATTAAA
TTATTCCCCAGCTATGCTATGAGCTTTAGGTTTTGTATTTTTATTCACAGTTGGGGGACTAACT
GGAGTAGTATTAGCCAACTCTTCTGTTGATATTATTCTTCATGACACTTACTACGTAGTAGCTC
ATTTCCATTATGTTTTATCAATAGGAGCAGTATTTGCTATTATAGCTGGATTTGTACACTGATA
CCCATTATTTACTGGATTAGTTTTAAATCCTAAGTGATTAAAAAGTCAATTTATTATTATATTT
ATTGGTGTAAACTTAACTTTCTTCCCTCAACACTTCTTAGGATTAGCAGGAATACCTCGACGTT
ACTCCGACTACCCAGATGCTTACACAACGTGAAACGTAGTTTCTACAATTGGTTCATCAATTTC
TTTACTAGGAATTCTTTTCTTTTTATTTATTATTTGAGAAAGATTAGTTACTCAACGTCAAGTA
ATTTACCCAATACAACTTAGTTCTTCAATTGAATGACTTCAAAATACACCACCTGCTGAACAT
AGTTATTCAGAATTACCTCTTTTAACTAATTTCTAATATGGCAGATTAGTGCAATGGA

BLAST analysis revealed that the observed species shows 98-100% homology with the sequence of *Zeugodacus cucurbitae* submitted in NCBI GenBank with E value 0.0 and query cover 98%-100% (Table 4.4.1). It indicated that the observed sample was *Zeugodacus cucurbitae*. Chromatogram of *Zeugodacus cucurbitae* (forward primer) of FASTA sequence (633bp) (Fig. 4.4.4).The results of fruit fly sequencing based on the COI genes were searched for homology in GenBank using BLAST software. Some fruit fly species nucleic acid sequence data were found in GenBank data that had similarities to fruit flyfound in the north, middle south, and hilly areas of Bangladesh. The BLAST result obtained 200 data from GenBank which had identical levels from 100% to 98% as shown in (Table 4.4.1).

Table 4.4.5. Haplotype and nucleotide diversity of *Zeugodacus cucurbitae* in Bangladesh. N: Number of samples; H: Number of haplotypes; Number of segregating (S)Hd: Haplotypes diversity; K: Variance of haplotype diversity; pi: Nucleotide diversity.

Location	Number of sequence (N)	Number of Haplotypes (h)	Number of segregating (S)	Haplotype diversity (Hd)	Number of difference (K)	Nucleotide diversity (Pi)
Middle	2	2	1	1.000	1.000	0.001
South	2	2	1	1.000	1.000	0.001
North	3	2	1	0.666	0.666	0.001
USA	4	1	0	0.000	0.000	0.000
India	4	1	0	0.000	0.000	0.000
Total	15	4	3	0.371	0.400	0.0007

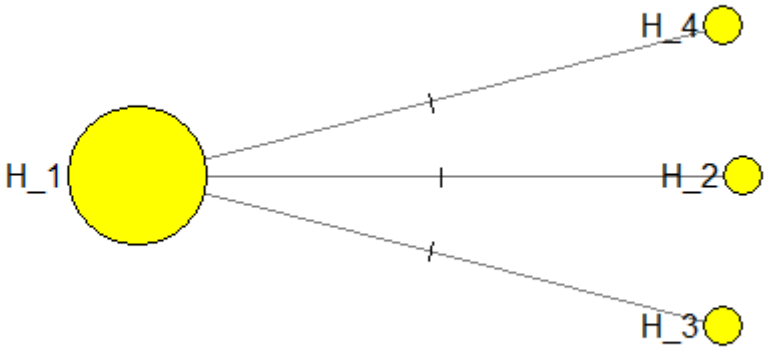

Figure 4.4.5. The haplotype network tree for the mitochondrial COI region of *Zeugodacus cucurbitae*, circles represent the haplotypes identified and the size of each circle are proportional to the frequency of the haplotypes. The lines between each haplotypes represents the mutations; each line represents single mutation. Where, Hap_1: 12 [USA_4 USA_3 USA_2 USA_1 India_4 India_3 India_2 India_1 BDN1 BDS1 BDN2 BDM1], Hap_2: 1 [BDN3], Hap_3: 1 [BDS2], Hap_4: 1 [BDM2].

Table 4.4.6. Estimates of Evolutionary Divergence between of *Zeugodacus cucurbitae* species of the fruit fly. The method of calculation was p-distance with the software of MEGA-X.

	Hap_4	Hap_3	Hap_2	Hap_1
Hap_4				
Hap_3	659.43			
Hap_2	523.26	590.09		
Hap_1	533.82	597.81	0.00	

A total of 7 *Zeugodacus cucurbitae* was sequenced that was collected from different region of Bangladesh and 8 retrieved sequences samples (4 samples from USA and 4 samples from India) previously reported from USA and India population of *Zeugodacus cucurbitae.*

In phylogenetic analysis of *Zeugodacus cucurbitae* the total number of haplotypes was 4 and the number of mutation was 3 (Table 4.4.5; Fig. 4.4.5) and the descriptive statistics Number of sequence (N), Number of Haplotypes (h), Number of segregating (S), Haplotype diversity (Hd), Number of difference (K) and Nucleotide diversity (Pi) were calculated with DnaSP ver. 6.10.01 software. For the descriptive statistics analysis the three guava growing zones (south, middle and north zones) of Bangladesh were used and 15(N), 4(H), 3(S), 0.371(Hd), 0.400(Pi) and 0.0007(K) for *Zeugodacus cucurbitae* which suggest that entire population exhibited low level of genetic diversity (Table 4.4.5). In this study zone-wise clustering revealed that north zone recorded low level of Hd (0.6666) as compared to south (1.00) (Table 4.4.5). Demographic history analysis. Tajima's D of neutrality tests were executed for analysis of demographic history in Bangladeshi fruit fly populations. Neutrality tests were rejected for all populations with significant negative values except *Bactrocera zonata*.

The four neutrality tests were performed, and three values were negative for populations of fruit fly indicating there was an excess of rare mutations which favor population expansion or growth. The pairwise population diversity values ranged from 660.00 to 0.00 *Zeugodacus cucurbitae*. For total of comparisons, all showed positive and low genetic differentiation for *Zeugodacus cucurbitae*. (Table 4.4.6).

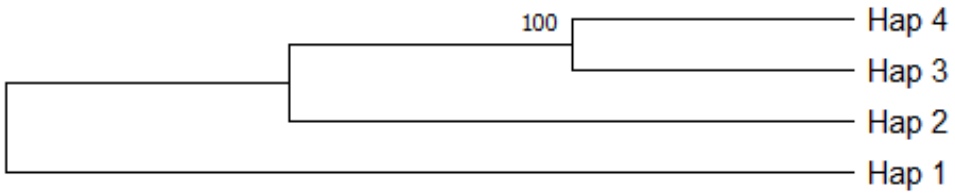

Figure 4.4.6. Phylogenetic tree of the 4 haplotypes of mtCOI DNA sample of *Zeugodacus cucurbitae*. Where, Hap_1: 12 [USA_4 USA_3 USA_2 USA_1 India_4 India_3 India_2 India_1 BDN1 BDS1 BDN2 BDM1], Hap_2: 1 [BDN3], Hap_3: 1 [BDS2], Hap_4: 1 [BDM2].

Molecular diagnostics have also been used by insect researchers to infer phylogenetic relationships and for identification to species level (Chua *et al*. 2009, Jamnongluk el al. 2003, Smith and Bush 1997). The pairwise distance among the species to species and genera to genera was the same. They closely similar genetically and suppose to come from a common ancestor. The measure of time, the greater the length of the branches the likelihood greater the distance between the species. They came from the same ancestor might be latitude and altitude same or have no relation with that and as well as the ancestor of two region species come from the same location of the world. They have a common ancestor and are closely related to each other and in the case of measurement of time, they came from each other (Fig. 4.4.6).

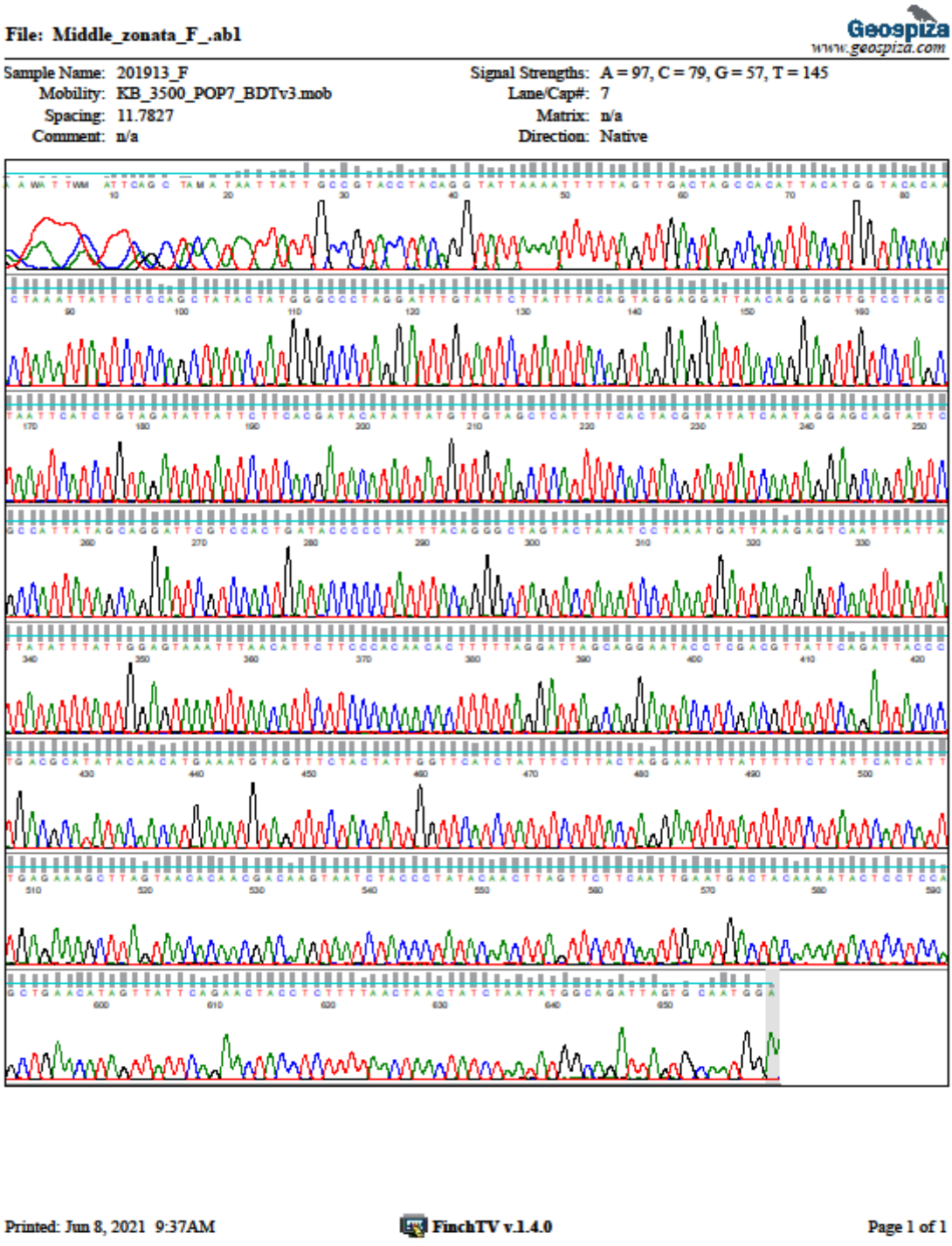

Figure 4.4.7. Chromatogram of *Bactrocera zonata* (using forward primer) of FASTA sequence (derived from forward primer, 659bp).

4.4.3. BLAST analysis *Bactrocera zonata*

ATTCAGCTAMATAATTATTGCCGTACCTACAGGTATTAAAATTTTTAGTTGACTAGCCACATT
ACATGGTACACAACTAAATTATTCTCCAGCTATACTATGGGCCCTAGGATTTGTATTCTTATTT
ACAGTAGGAGGATTAACAGGAGTTGTCCTAGCTAATTCATCTGTAGATATTATTCTTCACGAT
ACATATTATGTTGTAGCTCATTTTCACTACGTATTATCAATAGGAGCAGTATTCGCCATTATAG
CAGGATTCGTCCACTGATACCCCCTATTTACAGGGCTAGTACTAAATCCTAAATGATTAAAGA
GTCAATTTATTATTATATTTATTGGAGTAAATTTAACATTCTTCCCACAACACTTTTTAGGATT
AGCAGGAATACCTCGACGTTATTCAGATTACCCTGACGCATATACAACATGAAATGTAGTTTC
TACTATTGGTTCATCTATTTCTTTACTAGGAATTTTATTTTTCTTATTCATCATTTGAGAAAGCT
TAGTAACACAACGACAAGTAATCTACCCTATACAACTTAGTTCTTCAATTGAATGACTACAAA
ATACTCCTCCAGCTGAACATAGTTATTCAGAACTACCTCTTTTAACTAACTATCTAATATGGCA
GATTAGTGCAATGGA

Moreover, when compared with *Bactrocera zonata* with an identical rate of 100% and 98.0% respectively (Table 4.4.1). The results of sequence alignment indicate that the *Bactrocera zonata* COI nucleic acid from the middle sites showed the highest homology with identical levels of 100% but *Bactrocera zonata* COI nucleic acid from the middle sites showed the homology with *Bactrocera correcta* (MT257793.1) identical levels of 98.93%. Chromatogram of *Bactrocera zonata* (forward primer) of FASTA sequence (659bp) (Fig. 4.4.7).

Table 4.4.7. Haplotype and nucleotide diversity of *Bactrocera zonata* in Bangladesh. N: Number of samples; H: Number of haplotypes; Number of segregating (S); Hd: Haplotypes diversity; K: Variance of haplotype diversity; pi: Nucleotide diversity.

Location	Number of sequence (N)	Number of Haplotypes (h)	Number of segregating (S)	Haplotype diversity (Hd)	Number of difference (K)	Nucleotide diversity (Pi)
BD	4	4	13	1.000	7.166	0.012
USA	4	4	4	1.000	2.000	0.003
Total	8	8	16	1.000	5.178	0.008

Table 4.4.8. The pairwise distance calculated of *Bactrocera zonata* species of the fruit fly. The method of calculation was p-distance with the software of MEGA-X.

	Hap4	Hap5	Hap3	Hap1	Hap2	Hap7	Hap8	Hap6
Hap4								
Hap5	0.00							
Hap3	0.01	0.01						
Hap1	0.01	0.01	0.01					
Hap2	0.01	0.01	0.02	0.02				
Hap7	0.00	0.00	0.01	0.01	0.01			
Hap8	0.00	0.00	0.01	0.01	0.01	0.00		
Hap6	0.00	0.00	0.01	0.01	0.01	0.00	0.00	

A total of 4 *Bactrocera zonata* sequenced that was collected from different region of Bangladesh and 4 retrieved sequences samples (4 samples from USA) previously reported from USA population of *Bactrocera zonata.* In phylogenetic analysis of *Bactrocera zonata* the total number of haplotypes were 8 and the number of mutation was 16 (Table 4.4.7) and the descriptive statistics Number of sequence (N), Number of Haplotypes (h), Number of segregating (S), Haplotype diversity (Hd), Number of difference (K) and Nucleotide diversity (Pi) were calculated with DnaSP ver. 6.10.01 software. For the descriptive statistics analysis, the three guava growing zones of Bangladesh were used and 8(N), 8(H), 16(S), 1.000(Hd), 0.008(Pi) and 5.178(K) for *Bactrocera zonata* which suggest that entire population exhibited low level of genetic diversity (Table 4.4.7). In this study zone-wise clustering revealed that Bangladesh recorded of Hd (1.000) as compared to USA (1.00) (Table 4.4.7).

Demographic history analysis, Tajima's D of neutrality tests were executed for analysis of demographic history in Bangladeshi fruit fly populations. Neutrality tests were rejected for all populations with significant negative values except *Bactrocera zonata*. The four neutrality tests were performed, and three values were negative for populations of fruit fly indicating there was an excess of rare mutations which favour population expansion

or growth. The pairwise population diversity values ranged from 0.02 to 0.00 for *Bactrocera zonata*. Total of comparisons, all showed positive and low genetic differentiation for *Bactrocera zonata* (Table 4.4.8). The pairwise distance among the species to species and genera to genera was different. They are closely similar genetically and suppose to come from a common ancestor. In the study, *Bactrocera zonata* collected from the middle region and north region and differences were 0.02.

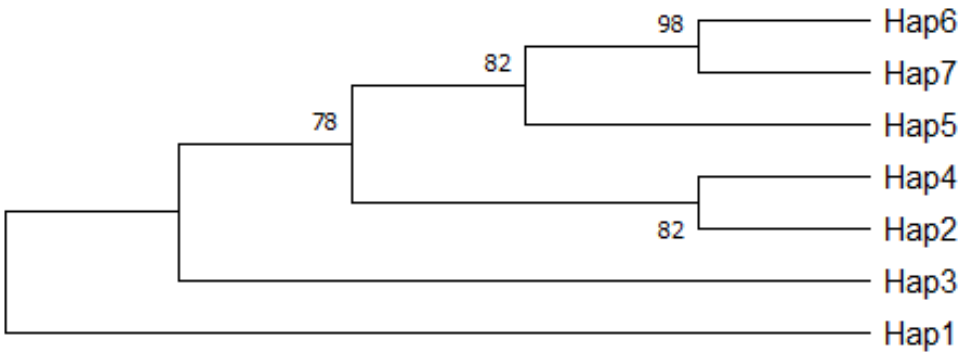

Figure 4.4.8. Phylogenetic tree of COI *Bactrocera zonata* gene at Bangladesh location compared with data from NCBI with the closest resemblance and comparison. Where, Hap_1: 1 [BDN2], Hap_2: 1 [BDN1], Hap_3: 1 [BDN3], Hap_4: 1 [USA2], Hap_5: 1 [USA1], Hap_6: 1 [BDM1], Hap_7: 1 [USA4], Hap_8: 1 [USA3].

The measure of time, the greater the length of the branches the likelihood greater the distance between the species. In the study, the middle region and north region were not closely related and have a different ancestor. They came from different ancestor might be latitude and altitude were different and as well as the ancestor of two region species come from different location of the world (Fig. 4.4.8). In phylogenetic analysis of *Bactrocera zonata* the total number of haplotypes was 8 and the number of mutation was 16 (Fig. 4.4.9)

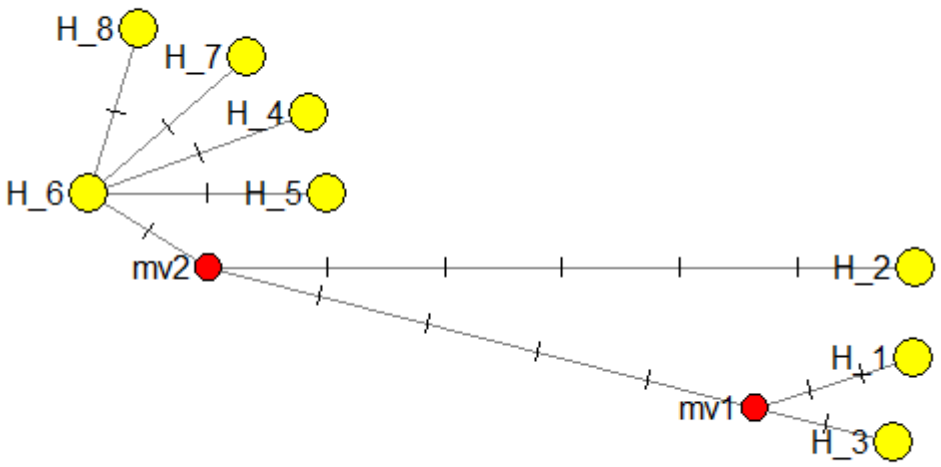

Figure 4.4.9. The haplotype network tree for the mitochondrial COI region of *Bactrocera zonata*, circles represent the haplotypes identified and the size of each circle are proportional to the frequency of the haplotypes. The lines between each haplotypes represents the mutations; each line represents single mutation. Where, Hap_1: 1 [BDN2], Hap_2: 1 [BDN1], Hap_3: 1 [BDN3], Hap_4: 1 [USA2], Hap_5: 1 [USA1], Hap_6: 1 [BDM1], Hap_7: 1 [USA4], Hap_8: 1 [USA3].

4.4.4. BLAST analysis *Zeugodacus tau*

CAGCTACATAATTATTGCTGTTCCTACTGGAATTAAAATTTTCAGTTGATTAGCCACTCTTCAT
GGGACACAATTAAATTATTCACCAGCTATATTATGAGCTTTAGGATTTGTGTTTTTATTCACAG
TTGGAGGACTAACTGGAGTAGTATTAGCAAACTCTTCTGTTGATATTATTCTTCATGACACTTA
CTACGTAGTAGCTCATTTCCATTATGTTTTATCAATAGGAGCAGTATTTGCTATTATAGCCGGA
TTTGTACATTGATACCCACTATTTACTGGATTAGTTTTAAACCCTAAGTGATTAAAGAGCCAAT
TTATTATTATATTTATTGGTGTAAACTTAACTTTCTTCCCTCAACACTTCTTAGGATTAGCGGG
AATGCCTCGACGATATTCTGACTACCCAGATGCTTACACAACATGAAATGTAGTTTCTACAAT
TGGTTCATCAATTTCTTTATTAGGAATTCTTTTCTTTTTATTTATTATTTGAGAAAGATTAGTTA
CTCAACGTCAAGTAATTTATCCAATACAACTTAGTTCTTCAATTGAATGACTTCAAAATACGCC
GCCTGCTGAACACAGTTATTCAGAACTACCTCTTTTAACTAATTTCTAATATGGCAGATTAGTG
CAATGGAA

Whereas when compared with *Zeugodacus tau* from the middle, *Zeugodacus tau* from the north with an identical rate of 100-98 *Zeugodacus tau* COI nucleic acid from the middle sites showed the highest homology with identical levels of 100%.Chromatogram of *Zeugodacus tau* (forward primer) of FASTA sequence (659bp) (Fig. 4.4.10).

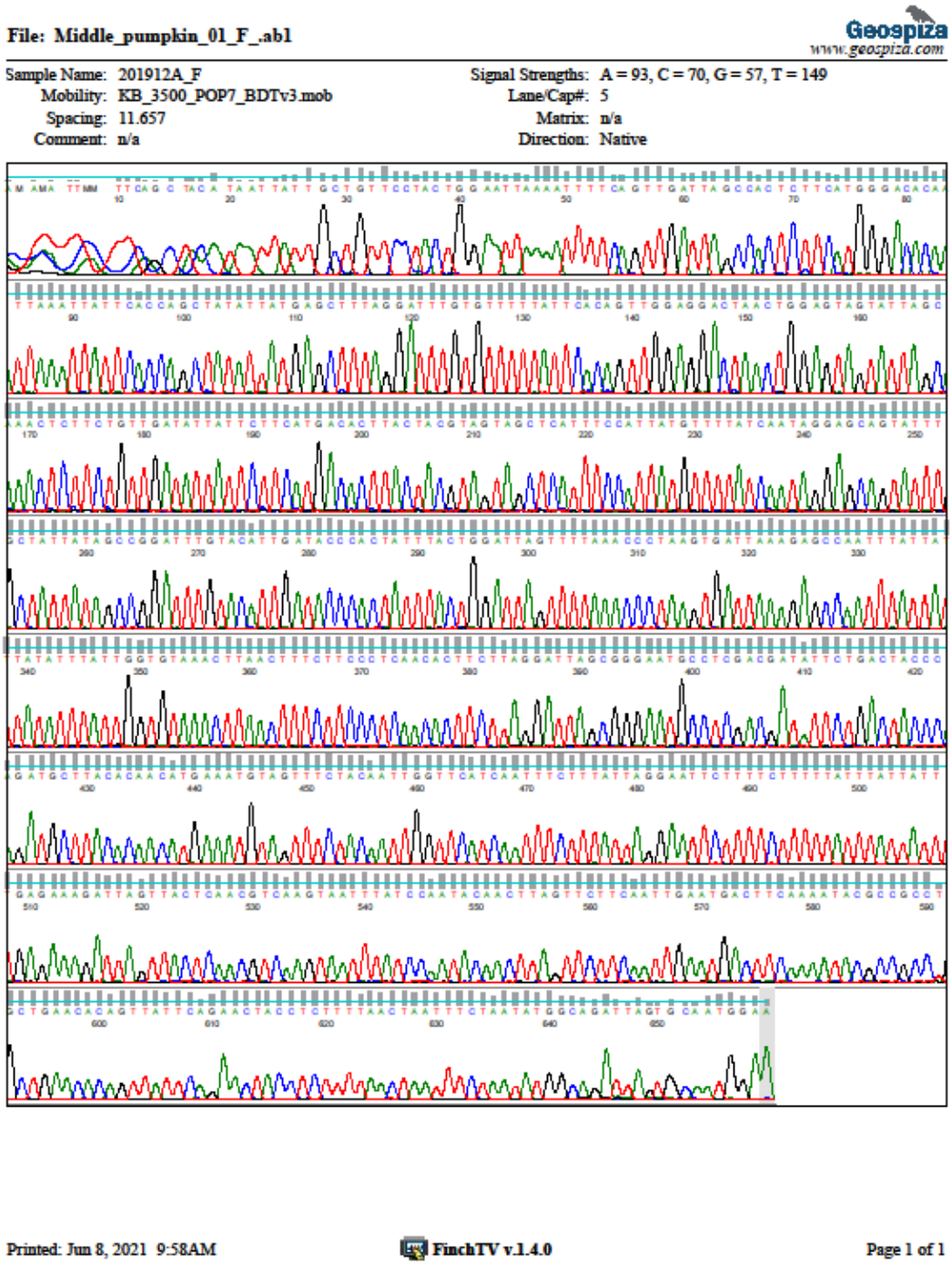

Figure 4.4.10. Chromatogram of *Zeugodacus tau* (using forward primer) of FASTA sequence (derived from forward primer, 659bp).

Table 4.4.9. The pairwise distance calculated of *Zeugodacus tau* species of the fruit fly. The method of calculation was p-distance with the software of MEGA-X.

	Hap6	Hap5	Hap4	Hap3	Hap2	Hap7	Hap1
Hap6							
Hap5	6.85						
Hap4	12.83	13.89					
Hap3	9.25	7.72	8.41				
Hap2	14.48	12.43	6.75	8.90			
Hap7	0.00	6.86	12.82	9.24	14.48		
Hap1	9.12	7.60	8.28	0.00	8.77	9.11	

The pairwise distance among the species to species and genera to genera was different. They closely similar genetically and suppose to come from a common ancestor. In the study, *Zeugodacus tau* collected from the middle region and north region, and the difference were 14.5-0.00. There was a difference between them (Table 4.4.9).

Table 4.4.10. Haplotype and nucleotide diversity of *Zeugodacus tau* in Bangladesh. N: Number of samples; H: Number of haplotypes; Number of segregating (S);Hd: Haplotypes diversity; K: Variance of haplotype diversity; pi: Nucleotide diversity.

Location	**Number of sequence (N)**	**Number of Haplotypes (h)**	**Number of segregating (S)**	**Haplotype diversity (Hd)**	**Number of difference (K)**	**Nucleotide diversity (Pi)**
Middle	2	2	6	1.000	6.000	0.011
South	-	-	-	-	-	-
North	3	3	3	1.000	2.000	0.003
India	4	1	0	0.000	0.000	0.000
USA	4	3	3	0.833	1.500	0.002
Total	13	7	10	0.794	2.153	0.004

A total of 5 *Zeugodacus tau* sequenced that collected from different region of Bangladesh and 8 retrieved sequences samples (4 samples from USA and 4 samples from India) previously reported from USA and India population of *Zeugodacus tau.* In phylogenetic analysis of *Zeugodacus tau* the descriptive statistics Number of sequence (N), Number of

Haplotypes (h), Number of segregating (S), Haplotype diversity (Hd), Number of difference (K) and Nucleotide diversity (Pi) were calculated with DnaSP ver. 5.10.01 software. For the descriptive statistics analysis, the three guava growing zones (south, middle and north zones) of Bangladesh were used and 13(N), 7(H), 10(S), 0.794(Hd), 0.004(Pi) and 2.153(K) for *Zeugodacus tau* which suggest that entire population exhibited low level of genetic diversity. In this study zone-wise clustering revealed that north zone recorded same level of Hd (1.00) as compared to south (1.00) (Table 4.4.10). Demographic history analysis, Tajima's D of neutrality tests were executed for analysis of demographic history in Bangladeshi fruit fly populations. Neutrality tests were rejected for all populations with significant negative values except *Bactrocera zonata*. The four neutrality tests were performed, and three values were negative for populations of fruit fly indicating there was an excess of rare mutations which favor population expansion or growth.

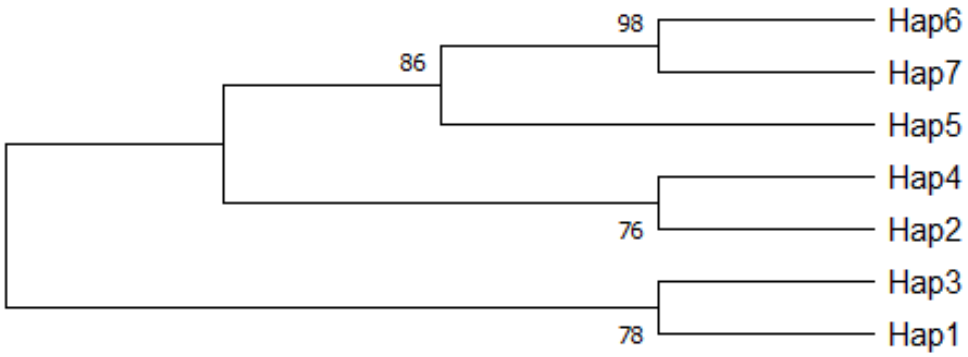

Figure 4.4.11. Phylogenetic tree of COI *Zeugodacus tau* gene at Bangladesh location compared with data from NCBI with the closest resemblance and comparison. Where, Hap_1: 6 [India_4 India_3 India_2 India_1 BDN3 USA_4], Hap_2: 1 [BDM1], Hap_3: 1 [BDM2], Hap_4: 1 [BDN1], Hap_5: 1 [BDN2], Hap_6: 1 [USA1], Hap_7: 2 [USA3 USA2].

The measure of time, the greater the length of the branches the likelihood greater the distance between the species. In the study, the middle region and north region were not closely related and have a different ancestor (Fig.4.4.11). They came from different ancestor might be latitude and altitude were different and as well as the ancestor of two region species come from different location of the world. In phylogenetic analysis of *Zeugodacus tau* the total number of haplotypes was 5 and the number of mutation was 10 (Fig. 4.4.12)

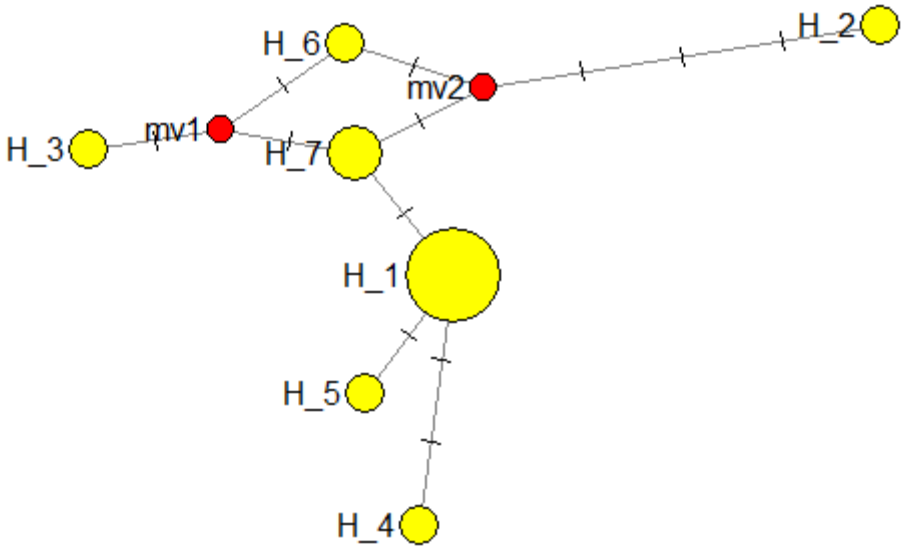

Figure 4.4.12. The haplotype network tree for the mitochondrial COI region of *Zeugodacus tau*, circles represent the haplotypes identified and the size of each circle are proportional to the frequency of the haplotypes. The lines between each haplotypes represents the mutations; each line represents single mutation. Where, Hap_1: 6 [India_4 India_3 India_2 India_1 BDN3 USA_4], Hap_2: 1 [BDM1], Hap_3: 1 [BDM2], Hap_4: 1 [BDN1], Hap_5: 1 [BDN2], Hap_6: 1 [USA1], Hap_7: 2 [USA3 USA2].

DNA barcodes have been used successfully for the identification of fruit flies of the family Tephritidae in many geographic regions (Blacket *et al.* 2012, Virgilio *et al.* 2012).

DNA barcode sequences were effective for species identification with >94% of the specimens being correctly identified (Kunprom and Pramual 2016).

B. dorsalis and *B. carambolae* were treated as valid species (Schutze *et al.* 2015). Other members of this important species complex require further taxonomic investigations. For *B. carambolae,* although multidisciplinary data (molecular genetics, cytogenetics, host use, sexual compatibility, and chemoecology), indicated that it is a valid species separated from other members of the complex, the COI sequences failed to discriminate it from the others (Jiang *et al.* 2014, Kunprom and Pramual 2016). *B. carambolae* identity could not be confirmed molecularly (COI) because of degradation. Possible explanations for unsuccessful identification based on COI gene sequences for this species could be either the COI sequences have inadequate variation (thus molecular markers such as other gene sequences that are more variable are required (Boykin *et al.* 2014) or that some specimens are misidentified (Jiang *et al*. 2014) which is possible as this species is morphologically similar to other members of *B. dorsalis* complex. Identifications of other closely related species were also ambiguous. A similar situation was also observed for *B. zonata* and *Z. tau,* they were morphologically very similar. These species share several morphological characteristics, but certain characters of the wings and legs can readily differentiate them (Drew and Romig 2016, 2013).

The objective of this study was to identify firstly the different fruit fly species and barcoding the specific species and haplotypes of fruit fly, major insect of guava and secondly to see its distribution in the different agro-ecological zones and in the sampled localities of Bangladesh on the basis of its occurrence and resistance development to guava growing region. After a careful alignment of the 35 COI mitochondrial gene sequences, we have investigated the genetic diversity and structure of 35 individuals of 4

populations sampled throughout their main area of distribution in Bangladesh. The mitochondrial COI DNA sequence region was used in this study because it was more prone to genetic drift than nuclear markers and because of the smaller effective population size and maternal gene flow.

The studies revealed that total of 24 unique haplotypes were identified in 35 individuals from three guava growing region of Bangladesh, with low values of nucleotide and haplotype diversity. In this study zone-wise clustering revealed that the middle zone recorded low level of Hd as compared to south and north zones. Low mitochondrial DNA variations were reported in taxa that might have undergone severe bottlenecks or founder effects.

Experiment 5. Development of management approaches of guava attacking fruit fly species in Bangladesh

Several fruit fly species are invasive pests that damage quality fruits in horticulture crops and cause significant value losses. The management of fruit flies is challenging due to their biology, adaptation to various regions and wide range of hosts. Introduction to managing fruit flies points out simple but essential management tools that each grower should consider whenever planting crops that are hosts to fruit flies. However, it does not provide a single, "one-answer" solution to the fruit fly problem, nor does it cover postharvest treatments for export. The main goal of this experiment is to find out the effective and sustainable management technique to improve the guava production by reducing the fruit fly infestation.

To fulfill the specific objective an experiment was conducted at on-station and Farmers' field of Savar of Dhaka district, Salna of Gazipur district and Horticulture Farm, Sher-e-Bangla Agricultural University, Dhaka for testing and rearing the collected sample. For immediate sustainable management purpose, efficacy of different selected pesticides (organic & chemical) was evaluated in the laboratory for suggesting in the field trials as sole &/ (or) IPM (Integrated Pest Management) component.

4.5.1. Results and discussion

The number of the healthy fruits and infested fruits harvested at late fruiting stages of the season. The mean number of healthy fruits at late stages from T_4 (Wrapping of twig and fruits with micro nets) treatment showed the higher level of mean number (12.67) compared to T_5 (Setting of Pheromone trap at plant canopy) treatment (82.00) which was the lowest number of healthy fruit and T_8 untreated (control) (85.66) (Table 4.5.1). However, significantly the best mean number of infested fruits were harvested at late stages from T_4 (Wrapping of twig and fruits with micro nets) treatment compared to treatment package of T_2, T_3,T_5,T_6 and untreated control (Table 4.5.1). Wrapping of twig and fruits with micro nets (Kumar *et al*. 2011) showed the better fruit fly control than other management technique and might be full cover this reduced infestation and the fruit surface with micro nets. The number of total fruits is depending on varietal genetic composition (Aktaruzzamn *et al.* 1999). The mean number of total fruits of late stages harvested from T_2 (Spraying of Neem oil 5 m1/L of water trix 5g at 7 days' interval) (161.00) showed the highest level compared to T_5 (Setting of Pheromone trap at plant canopy) (124.00) which was the lowest number of total fruits harvested (Table 4.5.1).

Table 4.5.1. Effect of cultural and chemical practice on number of healthy fruits, number of infested fruit and total number of guava fruits growing in different regions of Bangladesh

Treatment	Number of healthy fruits 3 plants plot^{-1}	Number of infested fruits 3 plants plot^{-1}	Total number of fruit
T_1	109.33 b	24.33 cd	133.67 cd
T_2	104.00 bc	57.00 ab	161.00 a
T_3	103.33 bc	38.66 bc	142.00 abcd
T_4	129.67 a	9.00 d	138.67 bcd
T_5	82.00 d	42.00 bc	124.00 d
T_6	93.33 cd	36.66 bc	130.00 cd
T_7	118.67 ab	27.33 cd	146.00 abc
T_8	85.66 d	68.33 a	154.00 ab
CV	5.30	21.36	5.08
LSD (0.05%)	4.47	6.61	5.85

[T_1: Setting of Pheromone trap at plant canopy + spraying of Neem oil 5 m1L^{-1}of water + trix 5g at 7 days interval; T_2: Spraying of Neem oil 5 m1L^{-1} of water trix 5g at 7 days interval; T_3: Spraying of carbosulfan 20 EC @ 2.0mlL^{-1} of water at 7 days interval + Setting of Pheromone trap at plant canopy; T_4: Wrapping of twig and fruits with micro nets; T_5: Setting of Pheromone trap at plant canopy; T_6: Bait Application Technique (BAT), in which food baits are mixed with a small amount of insecticide to attract and kill adults; T_7: Male Annihilation Technique (MAT), in which synthetic pheromones are mixed with insecticide, applied to a suitable substrate to allow slow release, and will be used selectively to attract and kill male flies and T_8: Untreated control.]

The weight of the healthy fruits and infested fruits harvested at late fruiting stages of the season. The mean weight of healthy fruits of late stages harvested from T_4 (Wrapping of twig and fruits with micro nets) (42.79 kg) treatment which showed the high level of mean weight but statistically comparable to treatment T_1 (37.29 kg) & T_7 (40.78 kg) (Table 4.5.2) with T_8 (control) (26.55 kg) which was the lowest number of healthy fruit weight (Table 4.5.2). This was significantly different from T_2 (33.64 kg), T_3 (34.10 kg), T_5 (28.88 kg), T_6 (31.73 kg) including untreated control (26.55 kg) (Table 4.5.2).

The mean weight of infested fruits of late stages from T_4 (Wrapping of twig and fruits with micro nets) (2.79 kg) showed the lowest level of mean weight compared to

treatment T_1(7.54 kg) and T_7 (8.47 kg), but statistically different from all other treatment and untreated control.

The mean weight of total fruits of late stages harvested from T_2 (Spraying of Neem oil 5 $m1L^{-1}$ of water trix 5g at 7 days' interval) (50.17 kg) showed the highest level but not significantly different from those of T_1 (44.83 kg), T_3 (45.70 kg), T_4 (45.58 kg),T_7 (49.26 kg) and untreated control T_8 (46.37kg) (Table 4.5.2). However, it was significant different from T_5 (42.32kg) and T_6 (42.73 kg) but they were not significantly different from each other.

Table 4.5.2. Effect of cultural and chemical practice on weight of healthy fruit, weight of infested fruit and total weight of fruit in guava growing different regions in Bangladesh.

Treatment	Weight of healthy Fruit (kg.)	Weight of infested fruit k(g.)	Total weight of fruit (kg)
T_1	37.29 ab	7.54 cd	44.83 ab
T_2	33.64 bc	16.53 ab	50.17 a
T_3	34.10 bc	11.60 bc	45.70 ab
T_4	42.79 a	2.79 d	45.58 ab
T_5	28.88 cd	13.44 abc	42.32 b
T_6	31.73 bcd	11.00 bc	42.73 b
T_7	40.78 a	8.47 cd	49.26 a
T_8	26.55 d	19.81 a	46.37 ab
CV (%)	5.95	21.47	4.79
LSD (0.05%)	1.67	1.99	1.79

[T_1: Setting of Pheromone trap at plant canopy + spraying of Neem oil 5 $m1L^{-1}$ of water + trix 5 g at 7 days interval; T_2: Spraying of Neem oil 5 $m1L^{-1}$ of water and trix 5g at 7 days interval; T_3: Spraying of carbosulfan 20 EC @ 2.0mlL^{-1} of water at 7 days interval + Setting of Pheromone trap at plant canopy; T_4: Wrapping of twig and fruits with micro nets; T_5: Setting of Pheromone trap at plant canopy; T_6: Bait Application Technique (BAT), in which food baits are mixed with a small amount of insecticide to attract and kill adults; T_7: Male Annihilation Technique (MAT), in which synthetic pheromones are mixed with insecticide, applied to a suitable substrate to allow slow release, and will be used selectively to attract and kill male flies and T_8: Untreated control.]

In experiment field the effects of different treatment on fruit fly infestation by number on guava field are presented in (Fig. 4.5.1). Statistically higher number of infestation (%)

was observed in untreated control plant. The lowest fruit infestation by number was obtained from the wrapping of twig and fruits with micro nets treated plant. Percent number reduction per fruit due to fruit fly infestation calculated for the entire reproductive stages and its reduction over control are presented in (Fig. 4.5.1). The overall number of reductions per fruit ranged from 0.00 % to 84.70%. The lowest number of reductions per fruit was obtained from T_4 (Wrapping of twig and fruits with micro nets) treated plot. The highest weight reduction occurred in fruits of untreated T_8 (control) plot. Number reduction over control was higher in T_4 (Wrapping of twig and fruits with micro nets) treated plot and lower in T_2 (Spraying of Neem oil 5 m1L^{-1} of water trix 5g at 7 days' interval) treated plot (Fig. 4.5.1).

According to Sultana *et al.* (2010) on effectiveness of some mechanical and cultural methods for suppressing fruit fly in cucumber. There was significant difference among mechanical control, field sanitation and untreated control at the different fruiting stages of cucumber by percent fruit fly infestation by number. The mean percent of infested fruits of all stages from mechanical control (25.36%) showed the lower level of infestation compared with field sanitation (29.68%) and control (30.12%).

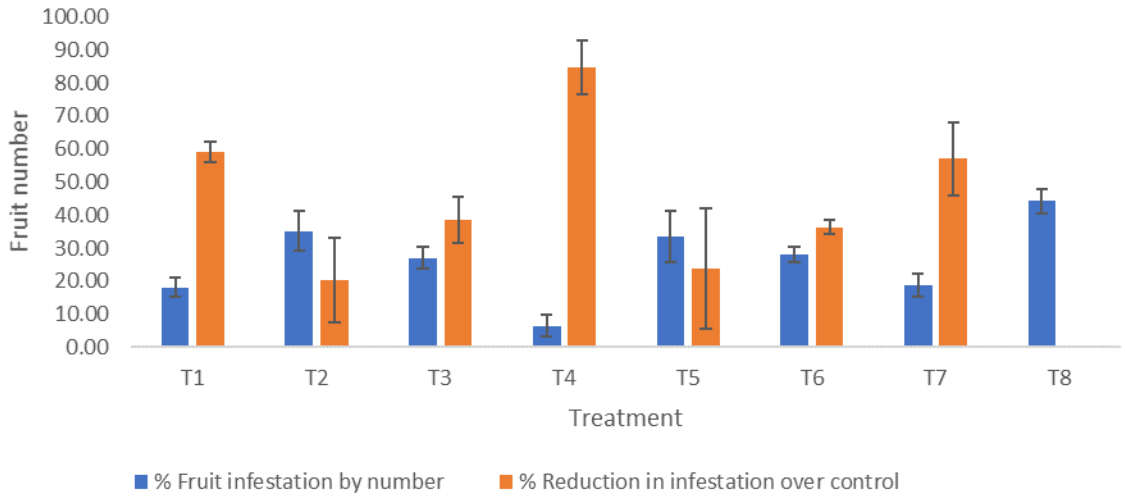

Figure 4.5.1. Effect of different treatments on percent fruit infestation by number and percent of reduction in infestation by number over control of different fruit fly infestation at guava orchards

[T_1: Setting of Pheromone trap at plant canopy + spraying of Neem oil 5 m1L^{-1} of water + trix 5g at 7 days interval; T_2: Spraying of Neem oil 5 m1L^{-1} of water and trix 5g at 7 days interval; T_3: Spraying of carbosulfan 20 EC @ 2.0 mlL^{-1} of water at 7 days interval + Setting of Pheromone trap at plant canopy; T_4: Wrapping of twig and fruits with micro nets; T_5: Setting of Pheromone trap at plant canopy; T_6: Bait Application Technique (BAT), in which food baits are mixed with a small amount of insecticide to attract and kill adults; T_7: Male Annihilation Technique (MAT), in which synthetic pheromones are mixed with insecticide, applied to a suitable substrate to allow slow release, and will be used selectively to attract and kill male flies and T_8: Untreated control.]

The percent weight of fruit infestation was significantly lower (6.19%) in T_4 (Wrapping of twig and fruits with micro nets) treatment. Fruit infestation in T_1 (Setting of Pheromone trap at plant canopy + spraying of Neem oil 5 m1L^{-1} of water + trix 5 g at 7 days interval) treated and T_7 (Male Annihilation Technique (MAT) treated plot had comparable level of infestation in T_4 (Wrapping of twig and fruits with micro nets) treated one and were statistically similar (Fig.4.5.2). According to Akhtaruzzaman *et al.* (1999), the best performance in suppressing fruit fly was obtained from treatment involving bagging of fruits at 3 days after anthesis and retaining the bag for 5 days. The treatment of bagging at 3 days after anthesis (DAA) left for 5 days resulted significant suppression of fruit fly at initial, early, mid and late fruiting stages. The percent reduction

in weight of fruit infestation was significantly higher (84.99%) in T_4 (Wrapping of twig and fruits with micro nets) treatment (Fig 4.5.2).

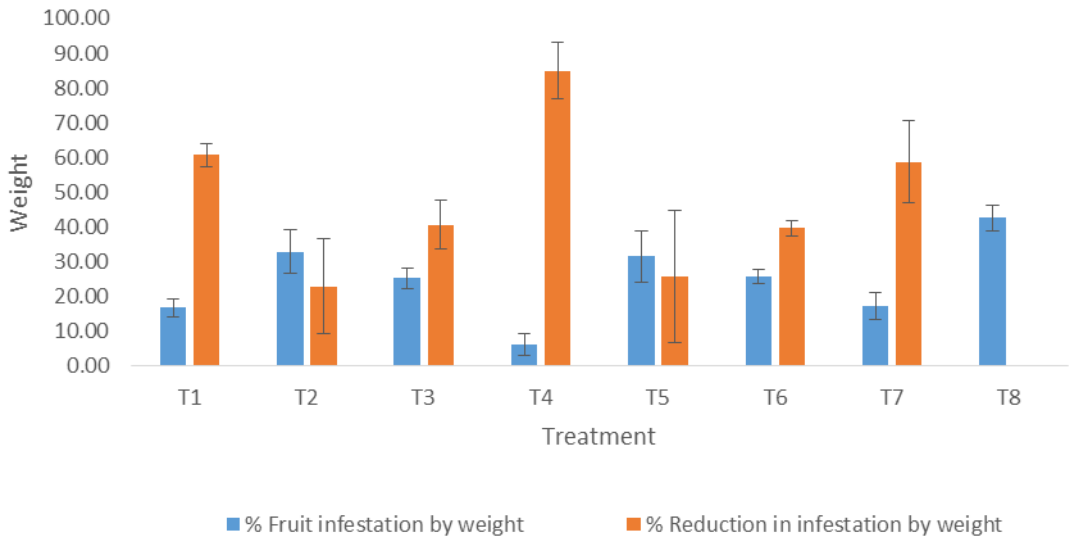

Figure 4.5.2. Effect of different treatments on fruit infestation by weight and percent reduction of infestation

[T_1: Setting of Pheromone trap at plant canopy + spraying of Neem oil 5 m1L^{-1} of water + trix 5.0 g at 7 days interval; T_2: Spraying of Neem oil 5 m1L^{-1} of water trix 5g at 7 days interval; T_3: Spraying of carbosulfan 20 EC @ 2.0 mL^{-1} of water at 7 days interval + Setting of Pheromone trap at plant canopy; T_4: Wrapping of twig and fruits with micro nets; T_5: Setting of Pheromone trap at plant canopy; T_6: Bait Application Technique (BAT), in which food baits are mixed with a small amount of insecticide to attract and kill adults; T_7: Male Annihilation Technique (MAT), in which synthetic pheromones are mixed with insecticide, applied to a suitable substrate to allow slow release, and will be used selectively to attract and kill male flies and T_8: Untreated control.]

Percent reduction of fruit infestation under T_2 (Spraying of Neem oil 5 m1L^{-1} of water trix 5 g at 7 days' interval) treatment and T_5 (Setting of Pheromone trap at plant canopy) treatment and T_4 (Wrapping of twig and fruits with micro nets) treatments were statistically similar (Fig.4.5.2). Significantly the lowest percent of fruit infestation by weight was obtained in fruits harvested from bagging at 3 DAA for 5 days' treatment according to Akhtaruzzaman *et al.* (1999) found that the mean value of weight reduction per fruit over control ranged from 34.36 to 76.87%. Significantly the lowest weight

reduction per fruit over control was obtained in cucumber harvested from mechanically controlled plots which was statistically similar to that of Malathion treated plots. The highest weight reduction was observed in untreated plots which was statistically similar to those from cultural control plots.

Table 4.5.3. Effect of treatment on the yield over control by the infestation of fruit fly on guava growing orchard and Price of the harvested fruit in Bangladesh

Treatment	Number of fruits plant^{-1}	Number of healthy fruits 3 plants plot^{-1}	Weight of healthy fruits (Kg)	Yield (t ha^{-1})
T_1	43	109	37.29	29.83 ab
T_2	54	104	33.65	26.91 bc
T_3	47	103	34.10	27.28 bc
T_4	48	130	42.79	34.23 a
T_5	42	82	28.88	23.10 cd
T_6	43	93	31.79	25.38 bcd
T_7	46	118	40.79	32.62 a
T_8	51	85	26.56	21.24 d
CV				5.95
LSD (0.05%)				1.33

[T_1: Setting of Pheromone trap at plant canopy + spraying of Neem oil 5 m1 L^{-1} of water + trix 5g at 7 days interval; T_2: Spraying of Neem oil 5 m1L^{-1} of water trix 5g at 7 days interval; T_3: Spraying of carbosulfan 20 EC @ 2.0 mlL^{-1} of water at 7 days interval + Setting of Pheromone trap at plant canopy; T_4: Wrapping of twig and fruits with micro nets; T_5: Setting of Pheromone trap at plant canopy; T_6: Bait Application Technique (BAT), in which food baits are mixed with a small amount of insecticide to attract and kill adults; T_7: Male Annihilation Technique (MAT), in which synthetic pheromones are mixed with insecticide, applied to a suitable substrate to allow slow release, and will be used selectively to attract and kill male flies and T_8: Untreated control.]

Effect of different treatment on yield was evaluated in terms of total, healthy and infested fruit yield obtained during the entire reproductive period of the crop. The results thus obtained including the percent increase/ decrease of yield over control is presented in (Table 4.5.3). In this study, the total fruit yield was significantly higher 34.23 kg (three plant/plot) in the plots treated with the components of T_4 (wrapping of twig and fruits

with micro nets) which was statistically similar with T_7 (Male Annihilation Technique MAT, in which synthetic pheromones were mixed with insecticide, applied to a suitable substrate to allow slow release, and was used selectively to attract and kill male flies) and T_1 (29.83 t ha^{-1}) comprising setting of pheromone trap at plant canopy plus spraying of neem oil @ $5ml^{-1}$ of water plus trix @ 5 g at 7 days interval (Table 4.5.3).

However, the total fruit yield was significantly lower 21.24 kg (three plant $plot^{-1}$) in the plots of untreated control plant T_8 (Table 4.5.3) which was statistically comparable to T_5 (23.10) and T_6 (25.38) t ha^{-1}. According to Rahman, (2005) the total fruit yield was significantly higher (31.64 t ha^{-1}) in the plots treated with the components of IPM (Cypermethrin (@ 0.5 ml L^{-1} of water) applied at 10 days interval + bait spray with Malathion and molasses) which was statistically similar with of Cypermethrin (@ 0.5 ml L^{-1} of water applied at 10 days interval + bagging fruits at 3 DAA for 5 days).

The sorupkathi guava variety was cultivated at Pirojpur and Jhalokathi area and covered almost 80% area of total guava growing location (Table 4.5.4). This variety was locally improved and resistant to fruit flies. The south part of Bangladesh was close to sea water and tidal water come frequently. So, the pH was low and salinity was high with high humidity. However, humid and high temperature enhances the fruit fly growth, but reverse scenario was observed at south part of the Bangladesh. The numbers of healthy fruits were high compared with other grafted variety. Thai guava variety was most susceptible compared to grafted guava variety.

Table 4.5.4. Number of healthy, infested, and total fruits plant^{-1} in different varieties of guava cultivated at different locations of Bangladesh

Area	Name of the Variety	Total number of fruits 3 plants plot^{-1}	Percent of healthy fruits 3 plants plot^{-1}	Percent of infested fruits 3 plants plot^{-1}
North	Thai-3, thai-5	145	72.41	27.59
South	Kazipayara	129	69.77	30.23
	Kanchonnagar	155	77.42	22.58
	Sorupkathi	150	83.33	16.67
Middle	Thai-3, BAU-2	138	64.49	35.51
	Mukundupuri	132	76.52	23.48

Economic Analysis of treatments in fruit fly management

Economic analysis of various treatments in this study has been presented in Table 4.5.5 and Table 4.5.6. In this study the untreated control did not required any pest management cost, but rest of the seven treatments required various pest management costs. All these costs were calculated per hectare basis.

The Treatment T_1 comprising of setting pheromone trap at plant canopy + spraying of Neem oil @ 5 m1L^{-1} of water + trix @ 5g at 7 days' interval involved labor cost for spraying, trap preparation and setting, cost of pheromone trap, neem oil and trix. A total of Tk.117333 were spent for this treatment (Table 4.5.5).

In treatment T_2 comprised of spraying Neem oil @ 5 m1L^{-1} of water and trix @ 5g at 7 days' interval. These treatments also include the cost of labor, neem oil and trix. A total Tk.80000 were needed for T_2.

The treatment T_3, the cost of pheromone trap, carbosulfun 20 EC and labor cost for trap preparation, spraying and setting were involved. A total of Tk. 117333.00 were required in this purpose.

Treatment T_4 used in this study involved the cost of micronets and labor cost for operations and setting. A total Tk. 53332.00 were spent in this treatment.

The treatment T_5 consisting of setting pheromone trap at plant canopy. Here cost of labor and pheromone trap were involved which costs the total amount of Tk.66666.00.

In the treatment T_6 (Bait Application Technique), the cost for preparation of bait and labor cost were involved. A total cost of Tk. 80000.00 were needed for this treatment.

In addition, the treatment T_7, Male Annihilation Technique (MAT), in which synthetic pheromones are mixed with insecticide, applied to a suitable substrate to allow slow release, and was used selectively to attract and kill male flies. The cost of synthetic pheromone, insecticide and labor costs were involved here. A total cost of Tk. 93333.00 was needed in this treatment (Table 4.5.5).

Table 4.5.5 Costs incurred per hectare in different treatments applied against fruit fly infesting guava

Treatment T_1. Setting of Pheromone trap at plant canopy + spraying of Neem oil 5 $m1L^{-1}$ of water + trix 5g at 7 days' interval.

Treatment	Item	Cost TK. Three plant $plot^{-1}$	Cost TK. 800 plants ha^{-1}
T_1	Price of Pheromone trap with box	150.00	40000.00
	Price of the Neem oil	50.00	13333.00
	Price of Trix	40.00	10666.00
	Total labor cost (Spraying+ Trap preparation and setting)	200.00	53333.00
	Total cost	440.00	117333.00

Treatment T_2. Spraying of Neem oil 5 $m1L^{-1}$ of water trix 5g at 7 days interval.

Treatment	Item	Cost TK Three plants $plot^{-1}$	Cost TK 800 plants ha^{-1}
T_2	Price of the Neem oil	50.00	13333.00
	Price of Trix	50.00	13333.00
	Total number of labour (Spraying)	200.00	53333.00
	Total Cost	300.00	80000.00

Treatment T_3. Spraying of carbosulfan 20 EC @ 2.0 mlL^{-1} of water at 7 days interval + Setting of Pheromone trap at plant canopy

Treatment	Item	Cost TK. Three plants $plot^{-1}$	Cost TK. 800 plants ha^{-1}
T_3	Price of Pheromone trap	150.00	40000.00
	Price of the carbosulfan 20 EC	90.00	24000.00
	Total number of labour (Spraying+ Trap preparation and setting)	200.00	53333.00
	Total cost	440.00	117333.00

Treatment T_4. Wrapping of twig and fruits with micro nets.

Treatment	Item	Cost TK. Three plants $plot^{-1}$	Cost TK. 800 plants ha^{-1}
T_4	Price of micro net (100 pscX1.00)	100.00	26666.00
	Total number of labour (preparation and setting)	100.00	26666.00
	Total cost	200.00	53332.00

Treatment T_5. Setting of Pheromone trap at plant canopy.

Treatment	Item	Cost TK. Three plants $plot^{-1}$	Cost TK. 800 plants ha^{-1}
T_5	Price of Pheromone trap	150.00	40000.00
	Total number of labor (Trap preparation and setting)	100.00	26666.00
	Total cost	250.00	66666.00

Treatment T_6. Bait Application Technique (BAT), in which food baits are mixed with a small amount of insecticide to attract and kill adults.

Treatment	Item	Cost TK. Three plants $plot^{-1}$	Cost TK. 800 plants ha^{-1}
T_6	Price of Bait Application Technique (BAT) (Preparation)	200.00	53333.00
	Total number of labor (preparation and setting)	100.00	26666.00
	Total cost	300.00	80000.00

Treatment T_7. Male Annihilation Technique (MAT), in which synthetic pheromones are mixed with insecticide, applied to a suitable substrate to allow slow release, and will be used selectively to attract and kill male flies.

Treatment	Item	Cost TK. Three plants plot^{-1}	Cost TK. 800 plants ha^{-1}
T_7	Price of Male Annihilation Technique (MAT) (Preparation)	200.00	53333.00
	Total number of labour (preparation and setting)	150.00	40000.00
	Total cost	350.00	93333.00

T_8: Untreated control

No treatment was applied, so zero treatment cost.

Market price of guava @ Tk. 50 kg^{-1}

In this study, the BCR was significantly higher 10.69 (three plant/plot) in the plots treated with the components of T_4 (wrapping of twig and fruits with micro nets) and this BCR was numerically higher than all other treatment packages including untreated control plant (Table 4.5.6).

Table 4.5.6. Cost return analysis of different treatments for the management of fruit fly infesting guava grown in Savar, Gazipur and SAU campus, Dhaka, Bangladesh

Treatment	Yield t ha^{-1}	Costs in treatment (3 plants $plot^{-1)}$	Price of the harvested fruits (3 plants $plot^{-1)}$	Costs in treatment (800 plants $ha^{-1)}$	Gross return/Price of the harvested fruits (800 plants $ha^{-1)}$	Cost Benefit Ratio (BCR)
T_1	29.83	440	1864.7	117333	497244.4	4.23
T_2	26.91	300	1682.3	80000	448622.2	5.60
T_3	27.28	440	1705.0	117333	454666.7	3.87
T_4	34.23	200	21.39.5	53332	570533.3	10.69
T_5	23.10	250	1444.0	66666	385066.7	5.77
T_6	25.38	300	1586.7	80000	423111.1	5.28
T_7	32.62	350	2039.3	93333	543822.2	5.82
T_8	21.24	0	1327.8	0	354088.9	0.00

[T_1: Setting of Pheromone trap at plant canopy + spraying of Neem oil 5 $m1L^{-1}$ of water + trix 5g at 7 days interval; T_2: Spraying of Neem oil 5 $m1L^{-1}$ of water trix 5g at 7 days interval; T_3: Spraying of carbosulfan 20 EC @ 2.0mlL^{-1} of water at 7 days interval + Setting of Pheromone trap at plant canopy; T_4: Wrapping of twig and fruits with micro nets; T_5: Setting of Pheromone trap at plant canopy; T_6: Bait Application Technique (BAT), in which food baits are mixed with a small amount of insecticide to attract and kill adults; T_7: Male Annihilation Technique (MAT), in which synthetic pheromones are mixed with insecticide, applied to a suitable substrate to allow slow release, and will be used selectively to attract and kill male flies and T_8: Untreated control.]

The total fruit yield was significantly higher in the plots treated with the components of wrapping of twig and fruits with micro nets (T_4) which was statistically similar with Male Annihilation Technique (MAT) (T_7). The benefit/cost ratio was significantly higher in the plots treated with the components of wrapping of twig and fruits with micro nets (T_4) which was statistically different from the benefit/cost ratio in the plots treated with other treatment packages including untreated control.

Wrapping of twig and fruits with micro nets showed the higher level of mean number of healthy fruit and weight of healthy fruits and total number of fruits. The lowest weight reduction per fruit was obtained from wrapping of twig and fruits with micro nets treated plot. The highest weight reduction occurred in fruits of untreated control plot. So the percent weight of fruit infestation was significantly lower in rapping of twig and fruits with micro nets.

Since the health of human beings and the safety of the environments are of prime concern now a days an organic farming is being inspired and rewarded by enhanced price of the commodities that are grown without synthetic chemicals. Therefore, it would be wise to adopt the suitable integrated package for managing fruit fly that might require no or less chemicals. This approach might satisfy both the growers and consumers for the production and use of guava in Bangladesh.

Chapter-V

SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

Several fruit fly species are invasive pests that damage the quality of fruits and cause significant losses. The management of fruit flies is challenging due to their biology, adaptation to various regions and wide range of hosts. Introduction to managing fruit flies points out simple but essential management tools that each grower should consider whenever planting crops that are hosts to fruit flies. However, it does not provide a single, "one-answer" solution to the fruit fly problem, nor does it cover postharvest treatments for export.

The guava production was encountered mostly in north, middle and southern areas of Bangladesh. The guava growing farmer age were ranged from 15 to 65 years and categorized into five different age range. The highest numbers of guava growers were in 26-35 years age groups and lowest in 15-25 years age groups. Thirty four percent guava growers come from 26-35 years age groups and numbers of young farmer were high. However, only 7 percent farmer comes from 56–65-year age groups. Therefore, more than 90 percent farmers were young and most of them were male personnel. Farmers were categorized into four different groups on the basis of their farm size. Landless farmer has less than 0.02 ha of land, but 27 percent landless farmer were involved in guava farming because of guava production recently has become most profitable farming in Bangladesh and landless farmers want to change their economic condition through guava production. The highest numbers of guava growers were primary and junior school educated and the numbers of honors passed guava growers were low. Thirty four percent

guava growers were primary to junior school educated. Most popular guava cultivated variety was thai payara and 31 percent area were covered with that variety at surveyed location. Mainly thai payara were cultivated at northern area of Bangladesh but guava growers of other area like central, southern area cultivated that variety too. The second most popular variety was kazipayara which mostly cultivated at southern and hilly area and middle part of Bangladesh. Moreover, other two popular local varieties viz. Shorupkati and kanchannagar were also popular in southern part of Bangladesh and cultivated at Pirojpur and Patiya.

The availability of fruit fly and white fly at surveyed area were 97 percent and both fruit fly and white fly were destructively affected the production of guava. Hundred percent farmers were aquanted the fruit fly infesting their field. So, all the farmers at surveyed area knew about fruit fly. Almost twenty to twenty five percent yield loss due to fruit fly attack were recorded around 27 percent of total surveyed area. Fifteen to twenty percent yield loss were found around 35 percent areas due to attract of fruit fly and white fly.

Management was an important practice to reduce the yield loss. In Bangladesh most popular practice was mechanical control measure. Guava farmers basically used polybag to reduce the fruit fly infestation. Eighty five percent guava farmers usually practiced trap with pheromone and polybag simultaneously. Five different species were identified using stereomicroscope. The name of the fruit flies were Oriental fruit fly (*Bactrocera dorsalis*), melon fly (*Zeugodacus cucurbitae*), pumpkin fruit fly (*Zeugodacus tau*), peach fruit fly (*Bactrocera zonata*) and. *Dacus longnicornis.*

Total 78.54 percent of oriental fruit flies were found in total number of trap which was the highest number of fruit fly in guava field. However, 5.19 percent of peach fruit fly

were identified which was the lowest number among fruit flies trapped. Moreover, 9.16 and 7.10 percent of pumpkin fly and melon fly were collected from pheromone trap, respectively. Only 5 *Dacus longnicornis* samples were collected from pheromone trap. Therefore, numbers of oriental fruit fly was the highest compare to other fruit flies collected.

The highest infestations were observed at hill tract zone compared to other two zones of guava field. Oriental fruit fly of central zone was larger in size compared to other two zones and hill tract zone whose oriental fruit flies were small in size. Therefore, melon fly of central or middle zone was prominent compared to other two indicate zones. Melon fruit fly were larger compared to oriental fruit fly. No pumpkin fruit fly and peach fruit fly were found in guava field at hill tract zone of Bangladesh.

The dominant polyphagous fruit pest *B. dorsalis* (78.54 % of all trapped flies), followed by cucurbit fruit fly *Z. cucurbitae* (7.10 %), *Z. tau* (9.16 %), and *B. zonata* (5.19 %) in guava field when methyl eugenol trap was used. Cucurbit pest *D. longicornis* were collected in much smaller numbers. The very high number (almost 100%) of fruit flies (*B. dorsalis*) were collected using methyl eugenol traps at hilly tract and it maintained consistent peaks of abundance during the wet season or summer months. At northern part (Chapainawabgong, Natore, Rajshahi, Noagoan) of Bangladesh the number of oriental fruit fly reduced almost 50% compared to hilly tract but the total number of oriental fruit flies were almost samilar.

Seasonal abundance was positively correlated with rainfall, temperature, and host availability for most of the fruit infesting species, and especially for *B. dorsalis* in Bangladesh. The southern part of Bangladesh was close to sea water and tidal water come frequently. So, the pH was low and salinity was high with high humidity. However,

humid and high temperature enhances the fruit fly growth, but reverse scenario observed at southern part of the Bangladesh. The numbers of healthy fruits were high in local improved variety (eg., kanchannagar) compared with other grafted variety. Thai guava variety was most susceptible compared with grafted guava variety. Wrapping of twig and fruits with micro nets showed the higher level of mean number of healthy fruit and weight of healthy fruits and total number of fruits. The lowest reduction of infestation per fruit was obtained from wrapping of twig and fruits with micro nets treated plot. The highest weight reduction over control occurred in fruits of untreated control plot.

The percent weight of fruit infestation was significantly lower in rapping of twig and fruits with micro nets. The total fruit yield was significantly higher in the plots treated with the components of wrapping of twig and fruits with micro nets which was statistically similar to that of Male Annihilation Technique (MAT).

The benefit/cost analysis was significantly higher in the plots treated with the components of wrapping of twig and fruits with micro nets which was statistically different from the benefit/cost analysis when spraying of carbosulfan 20 EC @ 2.0ml/L of water at 7 days' interval + Setting of Pheromone trap at plant canopy in the plots.

The guava fruit fly (*Bactrocera correcta*) was not found during the study rather the present study got five different fruit fly species. These five species were morphologically identified to species level and three were identified into genera (*Bactrocera* sp., *Zeugodacus* sp. and *Dacus* sp.). Only *Bactrocera dorsalis* were reared from guava host plant species, but the rest of the species were not found from guavaProbably, the *Zeugodacus cucurbitae*, *Zeugodacus tau*, *Bactrocera zonata*, and *Dacus longnicornis* did come from other host plants especially from vegetables and infested diverse host plant species.

BLAST analysis revealed that the observed species shows 95-100% homology with the sequence of *Bactrocera dorsalis* submitted in NCBI GenBank with E value 0.0 and query cover 95%-100%. It indicates that the observed sample was *Bactrocera dorsalis*.

RECOMMENDATIONS

1. Awareness building and group mobilization program among farmers' level should be started immediately on most severe and vulnerable stages of infestation of this devastating insect. Government may take proper initiative through DAE (Department of Agricultural Extension).

2. The present study have surveyed the limited number of locations. It would be better to include the whole country.

3. In biological study, researcher found a single species *Bactrocera dorsalis* Hendel in four regions. Further study on the areas of whole country may be conducted for more confirmation.

4. In morphometric part of study, five distinguished species viz., *B. dorsalis, Zeugodacus cucurbitae, Zeugodacus tau, B. zonata and B. longicornis* were found. For more clarification, whole country should be covered for further research.

4. In molecular study used MEGA software; but there are other opportunities to follow different software for more imaging. Besides a present work was limited on haplotype, but for identifying races/species furthermore studies are required.

5.Present studies indicated highly sustainable and effective treatment was polybag rapping on guava which was normally practiced by the farmers and suggested to follow integrated approaches along with this common practice.

6. Find out alternative host plant and maintain isolation distance to reduce the infestation of fruit fly.

7. Introduction of the MAT, BAT technique to reduce the number of fruit flies.

8. Fruit fly resistant variety need to develop to reduce the rate of infestation. Local variety were resistance against fruit fly and locally improved high yielding variety need to be introduced as an alternative source.

Researcher's desire is to convey the finding messages among farmers' level through Department of Agriculture Extension.

Chapter-VI

LITERATURE CITED

CHAPTER VI
LITERATURE CITED

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LIST OF APPENDICES

Appendix I:

Plate showing the different experimental activities

Figure 7.1.1: Study on biology of different infested fruit fly collected from different location of Bangladesh.

Figure 7.1.2: Study on biology of different infested fruit fly collected from different location of Bangladesh.

Figure 7.1.3: Rearing sample of *Bactrocera dorsalis* fruit fly collected from different location of Bangladesh.

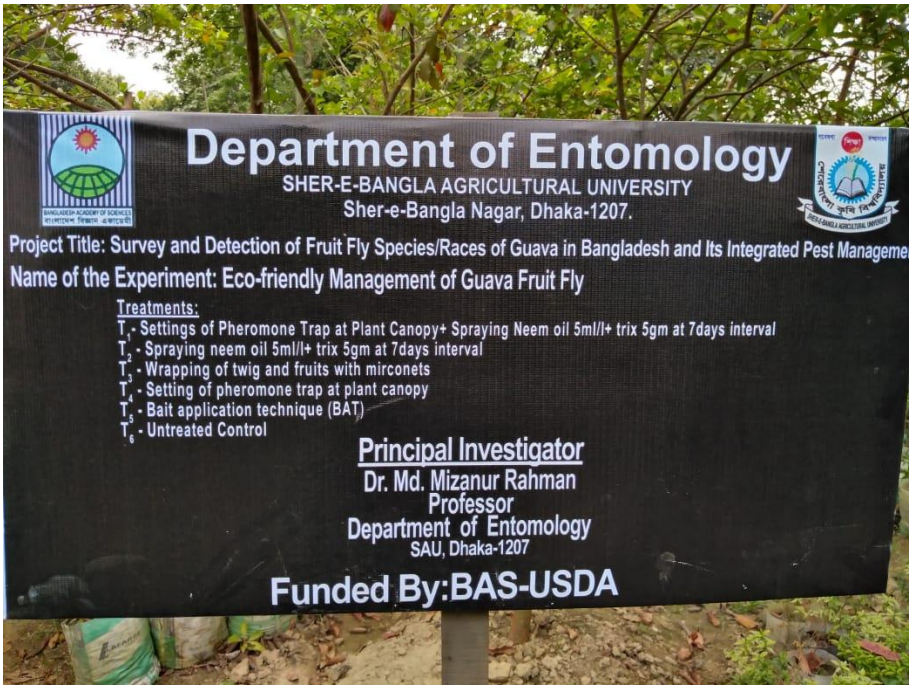

Figure 7.1.4 An experiment on fruit fly management approach at SAU campus

Figure 4.1.5 An experiment on fruit fly management approach at SAU campus

Figure 7.1.4: Weighing harvested fresh guava collected from different locations of Bangladesh.

Apendix II : Table showing the experimental sites average maximum and minimum temperature, Rainfall and Relative Humidity from November-17 to May-19 (2017-2019) under study area.

Month	Av. T°C max	Av. T°C min	Av. T°C	Total Rainfall	Rain	Av. RH	Av. T° C	Rain	Average monthly R.H	Number of fruit flies	Hill tract	North	south
Nov-17	29.6	20.22	24.9	25	0.83	73.43	24.9	0.83	2.45	1	2.00	4	1.00
Dec-17	27.75	17	22.4	0	0.00	72.83	22.4	0.00	2.43	3	2.00	3	1.00
Jan-18	26.36	14.86	20.6	0	0.00	61.56	20.6	0.00	2.05	2	1.00	3	2.00
Feb-18	29.56	17.58	23.6	2	0.07	52.66	23.6	0.07	1.76	3	4.00	2	2.00
Mar-18	30.48	20.38	25.4	100	3.33	62.33	25.4	3.33	2.08	10	18.00	16	7.00
Apr-18	32.78	23.94	28.4	236	7.87	70.45	28.4	7.87	2.35	28	30.00	28	20.00
May-18	34.5	25.92	30.2	202	6.73	68.22	30.2	6.73	2.27	25	31.00	28	22.00
Jun-18	33.04	26.51	29.8	403	13.43	76.76	29.8	13.43	2.56	40	60.00	49	33.00
Jul-18	32.05	26.48	29.3	585	19.50	80.35	29.3	19.50	2.68	60	70.00	64	45.00
Aug-18	32.58	26.74	29.7	608	20.27	81.19	29.7	20.27	2.71	65	78.00	71	51.00
Sep-18	33.07	26.88	30.0	383	12.77	79.95	30.0	12.77	2.67	35	40.00	38	30.00
Oct-18	31.99	24.8	28.4	381	12.70	76.33	28.4	12.70	2.54	40	42.00	34	31.00
Nov-18	30.18	20.56	25.4	6	0.20	64.4	25.4	0.20	2.15	2	3.00	2	1.00
Dec-18	26.71	17.05	21.9	33	1.10	75.06	21.9	1.10	2.50	5	4.00	4	1.00
Jan-19	23.56	12.75	18.2	0	0.00	69.45	18.2	0.00	2.32	1	2.00	4	2.00

Month	Av. T°C max	Av. T°C min	Av. T°C	Total Rainfall	Rain	Av. RH	Av. T° C	Rain	Average monthly R.H	Number of fruit flies	Hill tract	North	south
Feb-19	29.21	18.11	23.7	20	0.67	62.32	23.7	0.67	2.08	2	3.00	3	2.00
Mar-19	33.31	22.3	27.8	17	0.57	60.25	27.8	0.57	2.01	9	10.00	8	5.00
Apr-19	33.03	22.53	27.8	328	10.93	70.1	27.8	10.93	2.34	26	30.00	28	19.00
May-19	32.67	22.56	27.6	301	10.03	68.05	27.6	10.03	2.27	28	32.00	30	26.00

Appendix III: Line graph showing the experimental sites average maximum and minimum temperature, Rainfall and Relative Humidity from November-17 to May-19 (2017-2019) under study area.

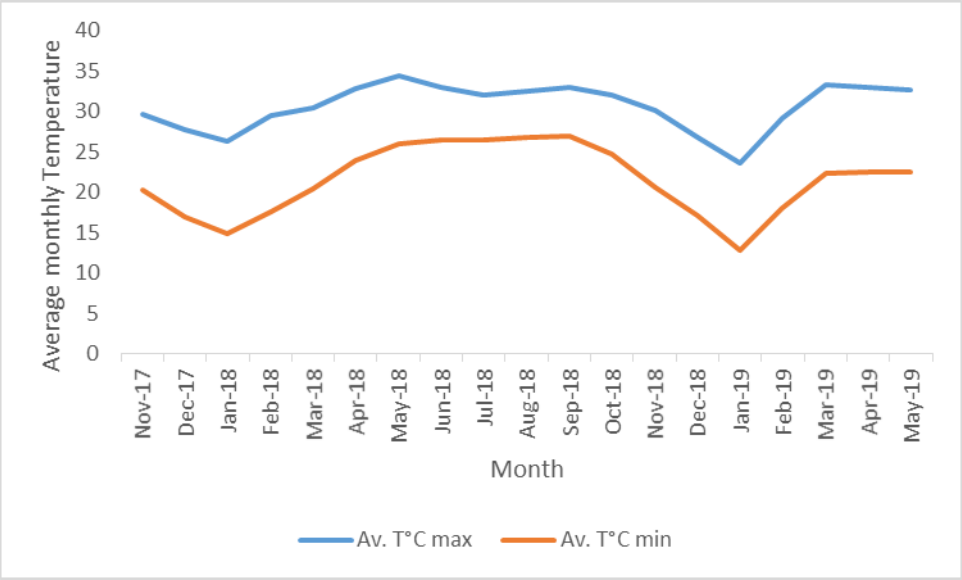

Figure. 7.2.1. Line graph showing the experimental sites average maximum and minimum temperature from November-17 to May-19 (2017-2019) under study area

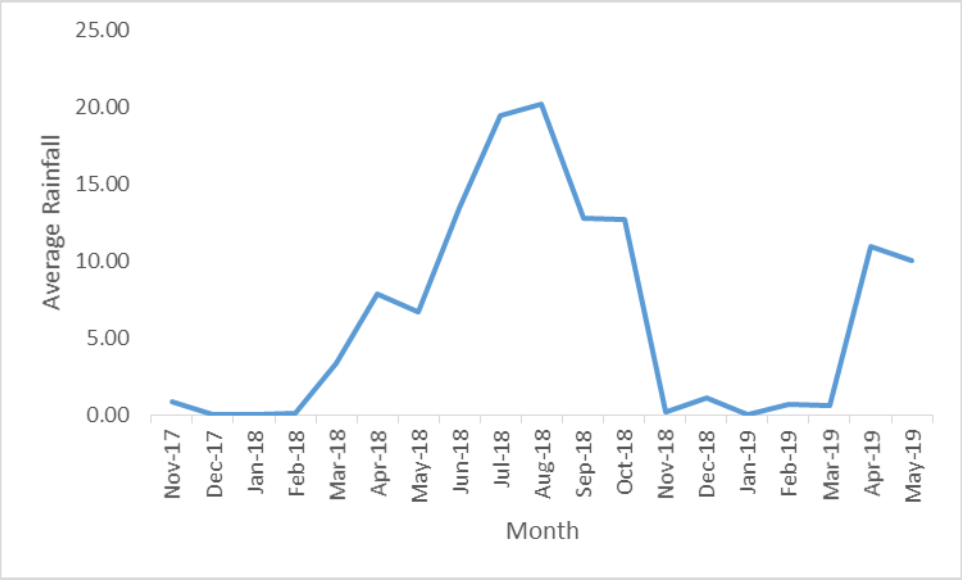

Figure. 7.2.2. Line graph showing the experimental sites average Rainfall from November-17 to May-19 (2017-2019) under study area

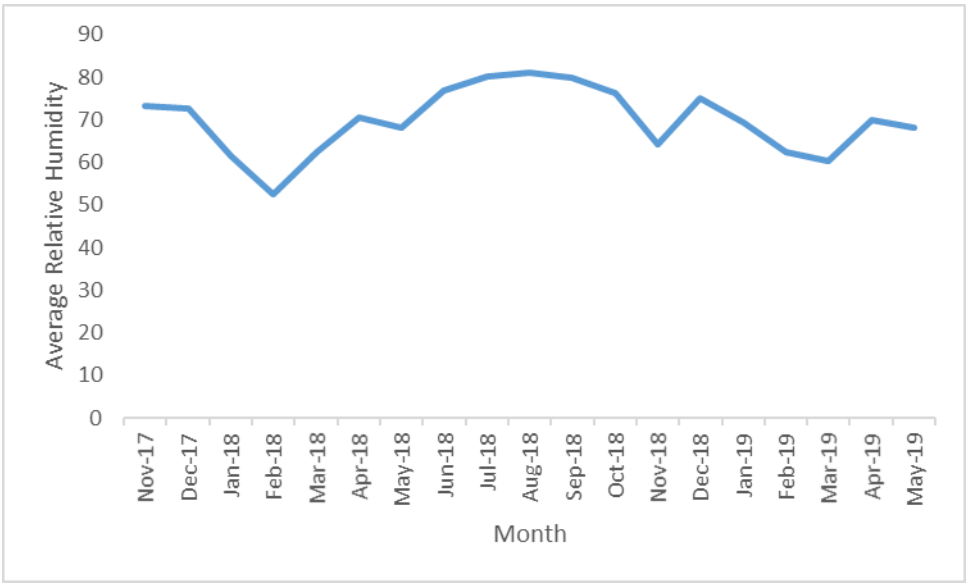

Figure. 7.2.3. Line graph showing the experimental sites average Relative Humidity (RH) from November-17 to May-19 under study area

Appendix IV: Measurement of fruit fly for morphometric analysis

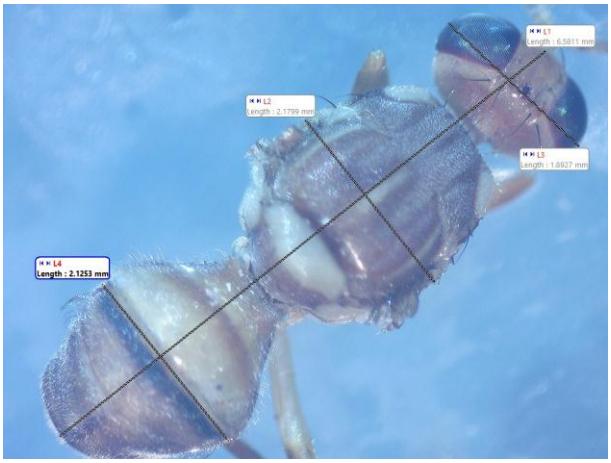

Figure 7.3.1: Measurement of fruit fly head, abdomen and thorax for morphometric analysis.

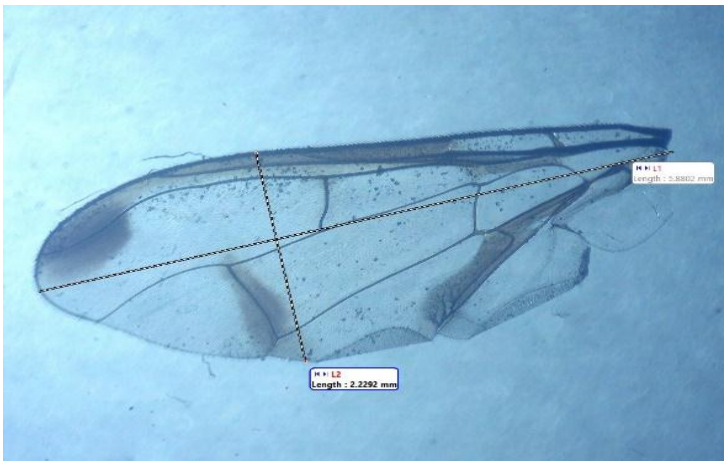

Figure 7.3.2: Measurement of fruit fly wing for morphometric analysis.

Appendix V: Variable costs per unit of item in different treatments applied against fruit fly on guava

Labor cost = 40 Tk./hour

Cost of single pheromone trap with lure = 50 Tk.

Cost of neem oil = 25 TK./ml.

Cost of single micronets = 1 Tk./piece

Cost of carbosulfan 20 EC = 15 Tk./ml.

Cost of trix = 5 Tk./g

Cost of single bait (BAT) = 65Tk./ bait

Cost of Male Annihilation technique (MAT) = 65 Tk./ MAT

Appendix VI: Questionnaire for Collecting Information from the Farmers

SL NO. Date:

1. **Farmers information**

    a. Name:
    b. Address:
    c. Age:
    d. Educational qualification:
    e. Sex:
    f. Marital status:

2. **Farm related information**

    a. Farm size:
    b. Name of cultivated guava variety:
    c. Age of seedlings:
    d. Time of harvesting:
    e. How many times you harvest:
    f. Yield:

3. **Pests and diseases related information**

    a. Usually what types of insects attack your plant?
    b. Usually what types of diseases affect your plant?
    c. How much yields are affected due to attack of pests?

4. Guava Fruit Fly related information:

    a. Do you know guava fruit Fly?
    b. Do you have any infestation of guava fruit fly?
    c. What type of control measure do you use for management of guava fruit fly?
    d. How much yields are affected due to guava fruit fly infestation?

THANKS