Tephritid (Diptera: Insecta) fruit flies of economic importance under terai and hilly agro-ecological region of West Bengal

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By

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Dedicated

To

My Beloved Parents,

My Respected Chairman

and

My Mama Masi

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Certificate

*This is to certify that the work recorded in the thesis entitled, **"Tephritid (Diptera : Insecta) fruit flies of economic importance under terai and hilly agro-ecological region of West Bengal"** submitted by **Shri Gobinda Roy** partial fulfillment of the requirements for the Doctoral Degree (Agriculture) in Agricultural Entomology of Uttar Banga Krishi Viswavidyalaya, is a faithful and bonafide research work carried out under my personal supervision and guidance. The results of the investigation reported in the thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have been duly acknowledged.*

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We, the undersigned, having been satisfied with the performance of **ShriGobinda Roy,** in the viva-voce examination of thesis, conducted today, the ………………………, 201...., recommend that the thesis be accepted for the award of the degree.

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ABBREBIATIONS & ACRONYMS

@	:	at the rate
cm	:	centimeter
°C	:	Degree celsius
et al.	:	and others (Authors)
gm	:	gram
CD	:	Critical difference
Max.	:	maximum
Min.	:	minimum
sq.	:	square
mt.	:	meter
spp	:	species
S. Em.	:	Standard error of mean
Viz.	:	*videlicet* (namely)
/	:	per
%	:	percent
Ha	:	hectare
HAT	:	hours after treatment
CL		cluster

List of Plates

Abstract

Fruit flies were collected by using different kinds of traps like methyl eugenol, cuelure and food bait traps and by rearing infested fruits from different corners of northern part of West Bengal. Observation with regard to species diversity of tephritid specimen revealed that maximum catch was obtained by using methyl eugenol and cuelure traps. In all the instances methyl eugenol collections were dominated by *B. dorsalis*. In case of food bait trap *B. dorsalis, B. correcta and B. nigrotibialis* were detected in the plains of northern West Bengal but *B. minax* was noted only in Darjeeling district. However, in case of species diversity recorded from infested fruits (mango, guava and mandarin orange) *B. dorsalis* was observed to be dominant species and *B. minax* was found to be dominant species recovered from mandarin orange. *B. cucurbitae* dominated in the recovered specimens from infested cucurbitaceous vegetables viz., pumpkin, bitter gourd and bottle gourd.

Studies on intensity of infestation by fruit fly (*Bactrocera* spp.) on different fruit and vegetables varied from location to location at various corners of the region under consideration.

Observation on frequency and duration of visit by adult female *B. cucurbitae* revealed that maximum number of visits per hour (8.53±0.60) was recorded in mid-morning i.e., 09:00 to 10:00 hours, which indicates that the fly remain more active during morning, mid -morning as well as afternoon hours. However, during the observation period visitation number and duration of visits were always high on fully ripe fruit (M_1) was found to be most preferred stage by the fly followed by ripening stage(M_2) and Unripe stage (M_1) stage. Duration of visit by the fly on different coloured artificial mango fruit model varied significantly ($F_{0.05,\ 2,270}$= 60.471). More time per hour was spent on yellow coloured artificial fruit model (9.66±0.39 minutes per hour) and least time was spent on green coloured artificial fruit (5.65±0.25 minutes per hour) indicating preference of yellow colour as compared to green.

Analysis on impact of entomopathogenic nematode, *H. indica* at different concentrations on *B. cucurbitae* revealed that maximum percentage of mortality on third instar maggots at 24, 48 and 72 hours after treatments. The treatments were conducted at various moisture level of soil. Two way factorial RBD design was followed for this purpose. The results with regard to percent mortality of maggots,

deformed pupa and adult emergence were recorded, analysed and presented in tabular as well as graphical forms.

It appeared from the investigation that, a diverse number of tephritid fruit fly species are present in the terai and hilly agro-ecological region of West Bengal. The flies cause various yield reduction in several vegetable and fruit crops grown commercially under the region. It has also been reconfirmed that certain visual cues as well as stages of fruit maturity affect fruit preference by the fly. Again, for sustainable management of this dreaded insect pest, entomoparasitic nematode, *Heterohabditis indica* have been found to produce good mortality and hence appeared promising as a good biological tool for present day IPM.

CHAPTER-1

Introduction

Chapter-1

INTRODUCTION

The Hexapod family Tephritidae (=Trypetidae) comes under the order Diptera (true flies). Tephritids (true fruit flies) includes about 4000 species arranged in 500 genera (White and Elson-Harris, 1994). It is one of the largest as well as economically important Dipteran family. Immature stages of the flies are apodous (legless), acephalous and known as maggots. Maggots of most of the species develop in the seed bearing organs of plants and about 35% of species attack soft fruits, including commercially important fruits. Hence, the name is fruit flies.

Besides infesting soft fruits the maggots of about 40% of species develop in the flowers of Astaraceae (=Compositae) and most of the remaining species are associated with the flowers of other families, or their miners in leaf, stem or root tissues. About 250 species of the family Tephritidae have been known to attack fruits that are either grown commercially or harvested from wild. However, many of these species are very rare (White and Elson-Harris, 1994). Almost all the species are phytophagous except a very few of known biology that are non-phytophagous.

About 1500 (38%) of the described species of Tephritidae are probably fruit associated including the fruit vegetables like cucurbits and solanaceous. The tephritid fruit flies are the pests of global importance and traditionally occupy important position (Aluja and Liedo, 1993). The larvae of almost all Dacinae (e.g. *Bactrocera*, *Ceratitis* and *Dacus* spp.) and most Trypetinae (e.g. *Anastrepha* and *Rhagoletis* spp.) develop within fruit (White and Elson-Harris, 1994). The sexually mature adult females lay eggs just beneath the skin of tender fruits. Oozing out of resinous fluid from fruits results. The eggs hatch into white maggots which reach about 9 mm long. These maggots live and feed in the fruit for 7-12 days and then drop down to the ground and pupate by making hole or along with the infested dropped fruits. The pupae are about 5 mm long, elongated and brown in colour. The adult fruit fly emerges from the pupae after 10-14 days depending upon environmental condition. The whole life cycle takes 4 weeks under ideal environmental conditions. Cool and dry conditions can cause the life cycle to be extended past 4 weeks. The infested fruits become distorted and malformed. Infestation also causes premature dropping of fruits which are usually unfit for human consumption.

The fruit pest tephritids are distributed all over the fruit as well as fruit vegetable growing areas of the world. The flies are widespread from sea level to mountainous areas (over 7,000 ft. from MSL). Fruit vegetables include cucurbits, solanaceous vegetables etc. The pest is purely internal feeder and vulnerable stage of the same remains exposed in the environment only for a couple of minutes or so in its whole life cycle before entering in soil for pupation. Fruit flies create havoc in horticulture industries because they cause tremendous loss to fruit and vegetable growers all over the world. The hosts that are preferable to fruit flies larva include a broad array of fruits, vegetables, flower heads and seeds (Kapoor, 1993a). Thus, they attack the ultimate commercially important portion of the plant, i.e. fruits and thus cause economic loss to the producer. Monetary estimates of fruit production and fruit fly damage are not available for most of the countries. However, it has been estimated in Australia that with an annual production of over 850 billion australian dollar and potential losses if fruit flies were not controlled are believed to exceed 100 billion australian dollar (Anon., 1986). In India Dhillon *et al.* (2005) estimated that the tephritid fly *B. cucurbitae* alone can cause 30-100% crop loss in different cucurbits depending upon crop and growing season.

Vegetables and fruits play an important role in nutrition of human beings as these are the highly valuable sources of carbohydrate, proteins, minerals, vitamins as well as different kinds of antioxidants. Majority of Indian populations are vegetarian and they depend mainly upon vegetables and fruits to fulfill their dietary requirement. In addition to that, production of vegetables and fruits are also directly related with employment generation as well as livelihood support of lions' share of rural population engaged in agriculture in India. Thus, cultivation of fruits and vegetables play a pivotal role in strengthening the agrarian Indian economy by way creating greater employment opportunity vis-à-vis providing raw materials to the agro-industries.

India is a large country endowed with diverse agro-climatic situation. Environmental variation exists from tropical to sub-tropical and even upto temperate. This typical agro-climatic situation favours for the cultivation of a number of fruits as well as vegetable crops in different corners of the country. In India major fruit growing states are Tamil Nadu, Maharashtra, Karnataka, Bihar, Orissa, and West Bengal etc. Different kinds of fruits are grown across the country including these major fruit growing states. Among fruits mango, guava, banana, apples, ber, peach, pear, plum and citrus are the most important which are being cultivated commercially. According to National Horticulture Board (NHB) during 2013-2014, India has produced 1,62,897 thousands MT of vegetables from 9,396 thousands ha of land. Diverse agro-climatic situation, wide variety of soils supplemented with substantial rainfall has made the state a forerunner in this sector.

In this aspect, tephritid fruit flies are one of the most high profile insect pests of commercial fruit and vegetable growers, marketing exporters, government regulatory agencies, and the scientific community. Locally, producers face huge losses without some management scheme manage fruit fly populations. At the national and international level, plant protection agencies strictly regulate the movement of potentially infested products. Consumers throughout the world also demand high quality, blemish-free produce. Partly to satisfy these demands, the costs to local, state and national governments are quite high and increasing as world trade, and thus risk increases.

The Dacine (Tephritidae: Diptera) fruit flies form the most important group of insect causing considerable yield losses to various fruits and vegetables in the world. India has nearly 200 species of fruit flies, which is about 5% of the known tephritid fauna of the world. The tephritids are one of the most diversified groups of insects and the sub- family Dacinae has the maximum number of the economically important species (Verghese *et al.*, 2002b). The major fruit fly pests in India belong to the genus *Bactrocera* are *B. cucurbitae* (Coquillett), *B. dorsalis* (Hendel) and *B. zonata* (Saunders). Other species of *Bactrocera*, such as *B. correcta* (Bezzi), *B. diversa* (Coquillett) and *B. latifrons* (Hendel), although moderate pests, are localized in their distribution. Five or six species of the *B. dorsalis* complex have been recorded in India, and at least 10 species may occur there, as well as three or four species of the *B. zonata* complex. *B. tau* (Walker) and *B. scutellaris* (Bezzi) have not been recognized even as moderate pests, whereas *B. caudata* (Walker) is still not fully confirmed in India. The pest status of *B. oleae* Gmelin has not yet been determined by the olive growers. *Dacus ciliatus* Loew sometimes becomes a serious pest of squash melons, dominating *B. cucurbitae* (Kapoor, 2006).

To date, with increasing globalization, it has become a challenge for our country not only to feed its own population but also to export fruits and vegetables to various developed countries so as to earn foreign currency. This requires strict quality control and restrictive quarantine measures. For example, the export of mango is increasing despite concern that fruit fly pests might be shipped along with these fruits. Being unable to use ethylene dibromide (EDB) fumigation, because of residue problems, the alternative non-toxic, ecofriendly innovative technologies to be exploited. Use of pesticides in managing the fruit fly menace also increase cost of production in addition to environmental degradation and ecological destabilisation. Another fact is that, it is very much difficult to manage the pest due to its concealed feeding habit and peculiar life history. Again, due to its concealed feeding habit it may sometimes transported from one country to another along with the consignment of exporting fruits and vegetables. It is, therefore, very important that quarantine entomologists can make rapid identifications of fruit flies intercepted with imported fruit produce, so that measures can be taken quickly to prevent the establishment of new fruit fly pests.

Some of the recent fruit fly problems all over the world have been observed in Australia and South pacific (Anon., 1986), Central and South America (Enkarlin *et al.*, 1989), Europe and temperate Asia (Fimiani, 1989), Hawaian islands and North America (Harris, 1989), Tropical Asia (Kapoor, 1993b) etc. The role of taxonomic services in helping to combat the fruit fly problem can not be ignored in this regard because details of import restrictions are too numerous and rapidly changing and as such knowledge base to be kept updated accordingly. Private travellers usually fail to understand the need to abide by regulations banning the import or export of fruits. The incidence of infested fruits in aircraft baggage can not be ignored as suggested by Satoh *et al.* (1985). Another little studied aspect of fruit fly problem is the effect of ingested larvae on human health. The only available information refers to *Anastrepha* spp. larvae causing abdominal pain and diarrhoea, particularly in children (Jiron and Zeledon, 1979).

The fruit flies cause varying degrees of damage to fruits. The loss due to infestation of fruit flies varies according to species, location and hosts. Ekesi *et al.* (2006) observed that the level of infestation in mango ranged from 3.0 to 97.02% flies per fruit in Kenya and 39.4% in Iran (Karim, 2011). Verghese *et al*. (2002a) reported 2.5% to 59.0% crop loss in mango due to *B. dorsalis* depending on variety in Bangalore, India. The level of infestation due to *B. dorsalis* in mango was as high as 26.66% in Gujarat (Sushil and Bhatt, 2002), and 30% in Karnataka (Babu and Virakthmath, 2003). The infestation by fruits flies to guava ranged from 5-7% (Verghese *et al*., 2002a) and 20-46 % (Hasseb, 2007). The oriental fruit fly *B. dorsalis* damaged the litchi fruits to the tune of 2.6% to 12.1% (Hung *et al.*, 2008).

Dhillon *et al.* (2005) reported 30-100% fruit infestation by *Bactrocera cucurbitae* on different cucurbits in India. Singh and Naik (2006) reported that *Bactrocera cucurbitae* infestation varied from 2 to 11% in different months on bitter gourd. According to Mote (1975), losses in India due to *B. cucurbitae* may be as high as 40-80%. Nearly 50% of cucurbits are reported partially or fully damaged by the pest every year in India (Agarwal *et al.*, 1987), while in bitter gourd, the infestation varied from 2-11% on different months (Singh and Naik, 2006). Cost of making a fruit fly free area are much more. In California, Dowel and Wange (1986) listed eight species of fruit flies which are major threat to the fruit production in California. They also estimated that the state wide establishment of those species would cause crop losses of US dollar 910 million and cost US dollar 290 million to control. The cost of eradicating a fruit fly from even a small island is very high using sterile insect technique (SIT).

The overall scenario of fruit fly infestation on commercially cultivated crops in West Bengal is more or less similar with that of other parts of our country. In northern part of West Bengal also is not an exception, rather more serious. Because a number of export oriented fruit crops like mango, mandarine orange, litchi, pineapple etc are being cultivated here commercially. In addition to that northern part of West Bengal substantially contribute in different kinds of vegetable production. In both fruits and vegetable production fruit flies appears regularly as one of the major hindrances. Fruit fly infestation being the major obstacle in the production and marketing of the mango and guava, successful cultivation and export are highly dependent on sound management of fruit fly.

As they remain within the fruit, the conventional contact and stomach insecticides do not come in contact with the pest. Again systemic insecticides have the disadvantage of leaving residual toxicity in fruits which is very dangerous to the consumers. Among IPM strategies, the only viable and effective method to manage them is to use pheromone traps. The pheromones are also species specific and one pheromone is not enough for all the species infesting all the fruits and vegetables. Trapping fruit flies is one of the most important alternatives to manage pest in an ecofriendly way. Use of chemical attractants like methyl eugenol and cue lure along with appropriate trapping technique has been reported effective in monitoring, suppressing and even complete eradication of various fruit flies (Grewal and Kapoor, 1987). Correct identification of species has immense value to promote sound biological evidence for the specific status of sibling species which ultimately helps in developing and adopting effective management strategies. Most of the fruit flies are having limited distribution perhaps due to physical, climatic and vegetative factors and host specificity (Ukey *et al.*, 2013).

In this regard for successful suppression of fruit fly population with a view to reap optimum yield potentiality of several commercially grown fruits and vegetable crops all possible bio-ecological, behavioural aspects need to be explored in addition to the correct identification of species and intensity of infestation. The introduction of affordable integrated pest management (IPM) technologies based on classical biological control, baiting techniques, bio-pesticides, male annihilation technique and orchard sanitation to control the fruit fly *Bactrocera* spp. *h*ave been reported by many workers with varying degrees of success. These may be exploited in framing efficient strategies against this dreaded pest of fruits and vegetables particularly in Indian sub-continent where most of the rural population engaged in agriculture, more specifically horticulture for their livelihood. That will also help in maintaining quality of environment.

Colour of fruit play important role in attracting fruit fly for oviposition on the fruits. Prokopy and Owens (1983) indicated that the color yellow is a supernormal visual equivalent of plant foliage and is attractive to many phytophagous insects. Color preferences of fruit flies may be related to the color of their host fruits. *Bactrocera dorsalis* is attracted to a yellow color which occurs in almost all their host fruit species (Vargas *et al*., 1991; Cornelius *et al*., 1999a and 1999b; Alyokhin *et al*., 2000). *Bactrocera tryoni*, in contrast, prefers bluish fruit-mimicking spheres which have a slightly enhanced level of ultraviolet reflectance, similar to the reflectance of their native host fruits *Gmelina* spp. (Verbenaceae) and *Elaeocarpus grandis* (Elaeocarpaceae), while yellow or orange spheres, which resemble the color of near ripe or ripe wild tobacco host fruit, are most attractive to tobacco fruit fly, *B. cacuminata* (Drew *et al*., 2003). Raghuvanshi and Misra (2008) observed that BAT + MAT (bait application technique + male annihilation technique) was the most effective tactic for fruit fly management with less fruit damage (17.97 and 16.44%) than other tactics. Gill and Mann (2008) reported lowest the percent guava fruit infestation ranging from 0 to 16.67% in IPM treatment compared to 0-76.67% in the untreated control in Punjab. In studies with various colour traps, Ravikumar and Viraktamath (2007) reported from Dharwad that yellow colour traps attracted high number of *B. correcta* in guava (70.45 fruit flies/trap/week) while black colour traps in mango (8.68 fruit flies/trap/week). Methyl eugenol trap attracted much higher number of mango fruits fly *B. invadens* than home-made baits (Ndiaye *et al*., 2008).

Certain entomopathogenic nematodes have their good role in suppressing the population of several soil dwelling insect pests. Tephritid flies are not soil dwelling pests but mature maggots pupate in soil and thus there remain immense possibility of managing it using EPNs. Several authors also tried *H. indica*, *Steinernema* spp. etc for the same in different corners of the world. The impact of entomopathogenic nematode (EPN) species against *Bactrocera oleae* was very effective whenever infective juveniles (IJs) were sprayed over naturally infested fallen olives, many larvae died in the soil and *Steinernema feltiae* caused the highest overall mortality of 67.9% (Sirjani and Kaya, 2009). Nyasani *et al.* (2008) also reported that the nematode species, *Heterorhabditis indica*, *Steinernema karif, S. wesieri, Steinernema* spp. and *Heterorhabditis* spp. caused 96.0, 93.3, 92.0, 88.0 and 86.7% mortality respectively in the DBM larvae within 72 hours. Anbesse and Gebru (2008) reported that two entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora* and *Steinernema yirgalemense* caused 88.7% and 88.6% mortality to barley chafer grub *C. curtipennis*. With high concentrations of EPN wettable powders, Ebeling *et al.* (1953) obtained effective protection against *Dacus cucurbitae, D. dorsalis* and *Ceratitis capitata* in cucumbers, water melons and tomatoes respectively.

Keeping in view the aforementioned phenomenon with regard to the rich biodiversity, economic importance and seriousness of fruit fly problem and possible scope of its management in India as a whole and northern tract of West Bengal in particular the present investigation had been undertaken with the following objectives:

i) Determination of species diversity of *Bactrocera* spp. under terai and hilly agro-ecological region of West Bengal.
ii) Determination of intensity of infestation by this pest on different important fruits and vegetables.
iii) Identification of the preferred stage(s) of fruit(s) for oviposition by the adult female fruit flies.
iv) Detection of role of visual cues in host plant selection by the fruit flies.
v) Finding out the effect of Entomopathogenic nematode (EPN), *Heterorhabditis indica* on fruit fly maggot under laboratory condition.

CHAPTER-2

Review of Literature

Chapter-2

REVIEW AND LITERATURES

Apart from several other biotic and abiotic stresses, insects are often a major obstacle to the successful production of vegetables including cucurbits (York, 1992) as well as fruit crops. They can severely reduce yield, injure or kill plants, spread disease and adversely affect fruit quality. Scientists of the concerned discipline are doing their work round the world including India. Remarkable findings have also been come out on different aspects of pest management with special reference to management strategies.

2.1. Insect pests of vegetable and fruit crops:

A number of insect pests, mites and nematodes have been found to infest vegetables but fortunately, in India. Tephritid fruit flies are the most important pests of a number of vegetables particularly cucurbitaceous crops. In addition to that cucurbits are also infested by a few species of beetles, aphids etc. although these pests seldom cause severe damage. Other insect pests reported to infest the vegetables are pumpkin caterpillar, *Diaphania indica* (Sounders), snake gourd semilooper, *Plusia peponis* (Fab.), white fly, *Bemisia tabaci* (Genn.), stink bugs, *Apspongopus* spp. (Thunberg.) and flea beetle, *Phyllotreta* spp. (Goeze), fruit and shoot borer, *Leucinodes orbonalis* (Guen), diamond back moth, *Plutella xylostella* (Linn), leaf hopper, *Amrasca biguttula biguttula* (Ishida), thrips, *Thrips tabaci* (Linn.). Among non-cucurbitaceous, fruit flies sometimes cause damage to solanaceous vegetables like tomato, brinjal, chilli etc. but not as dreaded as cucurbits.

Like vegetables, the fruit crops are also get infested by several number of insect pests viz., fruit fly, *Bactrocera* spp., mango mealy bugs, *Drosicha mangiferae* Stebbins, mango stone weevil, *Sternochetus mangiferae* (Fab), lemon butter fly, *Papilio demoleus* (Linnaeus), citrus leaf minor, *Phyllocnistis citrella* (Stainton) *Diaphorina citri* (Kuwayana), bark eating caterpillar, *Inderbela quadrinotata* (Walker), mealy bug, *Ferrisia virgata* (Cockerell), banana weevil, *Cosmopolites sordidus* (Germar) etc. These biotic pressures on the crops are increasing day by day due to round-the-year intensive cultivation of photo and thermo insensitive cultivars as well as indiscriminate use of toxic agro-chemicals in the present era of chemicalized agriculture.

Thus, in both vegetables and fruit crops, the most common and devastating insect pest is fruit fly, *Bactrocera* spp.. Nowadays, its a part of national and international importance that has the potentiality to damage crops upto the extend of 100% depending upon crops and growing season. The pest is a fruit infester and has peculiar mode of infestation and very difficult to keep under control. It has immense quarantine importance also due to its internal feeding habit and typical life history.

2.2. Tephritids of economic importance:

Tephritids, the true fruit flies come under the order Diptera (true flies) that includes about 4000 species arranged in 500 genera (White and Elson-Harris, 1994). It is one of the largest as well as important Dipteran family. True fruit flies are insects belonging to the dipteran family Tephritidae, which contains many of the most economically important fruit pests in the world (Fletcher, 1987). Within the Tephritidae, more than 1000 species are placed in the subfamily Dacinae and nearly all of these are fruit feeding species (Diaz-Fleischer *et al.*, 2001). Immature stages of the flies are legless, acephalous and known as maggots. Maggots of most of the species develop in the seed bearing organs of plants and about 35% of species attack soft fruits, including commercially important fruits. Hence the name is fruit flies. They are one of the most diversified groups of insects and ranked high among the world’s most serious pests of horticultural crops (Billah, 2007 and Billah *et al.*, 2006). Almost all the species are phytophagous except a very few are non-phytophagous.

In this aspect tephritid fruit flies are one of the most important biotic stresses in limiting optimum yield potentially of a crop and ultimately results in low productivity in addition to deteriorating quality of produce. The Dacine (Tephritidae: Diptera) fruit flies form the most important group of insect causing considerable yield losses to various fruits and vegetables in the world. India has nearly 200 species of fruit flies, which is about 5% of the known tephritid fauna of the world. The tephritids are one of the most diversified groups of insects and the sub-family Dacinae has the maximum number of the economically important species (Verghese *et al.*, 2002b). The pest is purely internal feeder and vulnerable stage of the same remains exposed in the environment only for a couple of minutes or so in its whole life cycle before entering in soil for pupation. They attack the ultimate commercially important portion of the plant, i.e. fruits and thus cause economic loss to the producer. Monetary estimates of fruit production and fruit fly damage are not available for most of the countries. However, it has been estimated in Australia that with an annual production at over 850 billion australian dollar and potential losses if fruit flies were not controlled are believed to exceed 100 billion australian dollar (Anon., 1986). In India Dhillon *et al*., (2005) estimated that on the tephritid fly *B. cucurbitae* alone can cause 30-100% crop loss in different cucurbits depending upon crop and growing season.

Several workers investigated various aspects of this tephritids of economic importance round the world as well as in India with a view to suppress their population effectively and sustainably. Numerous interesting informations have so far been come out and a number of technologies have also been formulated by different workers round the universe. Some of such frontal technologies and informations are delineated hereunder.

2.3. Biological features of 'true' fruit flies (Tephritidae: Diptera):

The tephritid fruit flies remain active throughout the year on one or the other host. During severe winter months, they hide and huddle together under dried leaves of bushes and trees and during the hot and dry season, the flies take shelter under humid and shady places and feed on honeydew of aphids infesting the fruit trees. For Dacine fruit flies, fruits are central to their life history as they represent an essential external resource required for the completion of the life cycle (Aluja and Mangan, 2008).

The female fruit flies puncture the soft and tender fruits with their stout and hard ovipositor and lay eggs just beneath the epidermis. They lay 4-10 eggs per fruit each time. Eggs are 1.0 to 1.5 mm long, whitish in colour, elongate cylindrical in shape, beautifully sculptured, slightly curved and tapering at both ends. A single female can lay about 200 eggs in her life span of 8 to 10 weeks. A puncture made by one female is often used by other flies also for ovipositing and a single fruit may have more than one puncture made by one or more females (Srivastava and Butani, 2009). The pre oviposition period of flies fed on cucumbers ranged between 11 to 12 days. Incubation period of melon fruit fly has been reported to be 1.1 to 1.8 days on bitter gourd, cucumber and sponge gourd (Gupta and Verma, 1995). On hatching the maggots feed from within the fruit and they undergo three larval instars before going to pupate. The larval period lasts for 3.0 to 21 days depending on temperature and host. After completion of larval period the mature third instar maggots come out from the fallen fruits and pupate in soil. The larvae pupate in the soil at a depth of 0.5 to 15 cm. The depth up to which the larvae move in the soil for pupation, and survival depend on soil texture and moisture (Pandey and Misra, 1999). Larvae are restricted to a single fruit piece and, because of this, oviposition by the parental fly is critical for the subsequent survival and fitness of her offspring (Fitt, 1986). The availability of fruiting host plants is not only essential to individual reproduction, but fruit also becomes a central driver of fruit fly population dynamics (Drew *et al.*, 1984, May, 1963, Muthuthantri, 2008 and Pritchard, 1969).

When fruit do not fall, the maggots either pupate inside the fruit (although it is not usual) or come out and drop down in soil for pupation. Pupae are 5-8 mm. long, barrel-shaped and brown to ochraceous in colour. Type of pupa is known as puparium. Pupal period is about 6-9 days in summer and for about four weeks during the months of December- January (Nair, 1995). In general, the pupal period lasts for about 6 to 9 days during the rainy season, and 15 days during the winter (Narayanan and Batra, 1960). On different hosts, the pupal period varies from 7.7 to 9.4 days on bitter gourd, cucumber, and sponge gourd and 6.5 to 21.8 days. Adult life lasts for up to 56 days in male and 66 days in female when fed. When starved the fly does not survive for more than two days.

2.4. Species diversity of fruit flies (Tephritidae: Diptera):

Accurate identification of pest species and information thereof are essential for any pest management programme and regulating the entry of pest species to a pest free zone particularly the pests of quarantine importance like tephritid fruit flies. Several workers documented varied number of fruit fly species from all over the world as well as Indian sub-continent.

The Dipteran family, Tephritidae consists of over 4000 species, of which nearly 700 species belong to Dacine fruit flies (Fletcher, 1987). Nearly 250 species are of economic importance and are distributed widely in temperate, sub-tropical and tropical regions of the world (Christenson and Foote, 1960). Bezzi (1913) listed 39 species from India. Forty three species of fruit flies have been described under the genus *Bactrocera* from Asia, Africa, and Australia by several authors (Syed, 1969; Cavalloro, 1983; Drew and Hooper, 1983; Munro, 1984; Fletcher, 1987). *B. cucurbitae* (Coquillett) is a major threat to cucurbits (Shah *et al*., 1948). Endamically, 75 described species of tropical fruit flies were found in South East Asia including the small number of international significance polyphagous pests which are *B. carambolae, B. papayae*, *B. philippinensis* and *B. dorsalis* (Clarke *et al.*, 2005). According to Chua (1998), approximately 100 species of *B. dorsalis* complex were found in Malaysia and only half of them have been recorded. The species include the carambola fruit fly, *B. carambolae* which had been widely infesting more than 151 kinds of fruits and vegetables including starfruit, mango, cashew, lemon, grape fruit, guava, mandarin, tomato, jackfruit, orange, avocado, sugar palm, breadfruit and sapodilla (Vijaysegaran, 1984). Among the species reported worldwide, 325 species of fruit flies are known to occur in the Indian subcontinent, of which 243 in 79 genera are from India alone under four subfamilies, namely Dacinae, Phytalmiinae, Tephritinae and Trypetinae (Agarwal and Sueyoshi, 2005; David and Ramani, 2011).

Kapoor (2005) reported 325 species of fruit flies occurring in the Indian subcontinent, of which 205 are from India. The author noted major pest species belong to the genus *Bactrocera* as follows: *B. cucurbitae, B.dorsalis* and *B. zonata,* while other species, such as *B. correcta*, *B. diversa* and *B. latifrons*, are still localized in distribution. *B. versicolor* (Bezzi) (an earlier synonym of *B. dorsalis*), is very similar to *B. zonata*, but it differs from the latter in the presence of a black spot at the apex of the scutellum and in having a narrow (but complete) costal band. It is recorded from guava. *B. nigrofemoralis* White and Tsuruta (known as *B. nigrotibialis* in the Indian literature) was reported from south India on *Coffea canephoria* (Narayanan and Batra, 1960). It is known from South India and Sri Lanka and the only confirmed host is *Terminalia catappa* (Tsuruta and White, 2001). *B. (Tetradacus) minax* (Enderlein) is also known as *Callantra minax* and *Polistomimetes minax* (in the Indian literature) or Chinese citrus fly. It is a pest of citrus and has already been recorded from northern India as causing heavy damage to tangerine (*Citrus reticulata*). *Carpomya vesuviana* Costa has extended its intensity of infestation in various parts of India, causing concern to the growers of ber (*Zizyphus mauritiana*) both in northern and southern India. The infestation is becoming so serious that sometimes not a single fruit is spared.

Distribution of tephritid flies is cosmopolitan covering tropical, subtropical and temperate regions and they occupy habitats ranging from rainforests to open savannah except in Arctic and Antarctic regions (Norrbom *et al.*, 1998; Agarwal and Sueyoshi, 2005; De Meyer *et al.*, 2010). These flies are widespread over the entire world and richly redominant in the tropical and subtropical areas. Although the economically important species account for only about 5 per cent of all tephritid species, they are a driving force for various tephritid studies, including taxonomic ones. In Indian sub-continent, the knowledge of family Tephritidae has been based largely upon the monumental monograph of Bezzi published in 1913 (Kapoor *et al.*, 1980). Agarwal and Sueyoshi (2005) noted 243 known species out of which 79 genera were from India, 41 species of 27 genera have been reported from Himachal Pradesh. Bhatia and Gupta (2003) conducted a survey during 1991-99 in six districts of Himachal Pradesh, India and reported that fruit fly (*Bactrocera cucurbitae*) on cucurbits was the most serious pests in different parts of the state. Kapoor (2004) stated that two hundred species of fruit flies were known from India. Only 35 to 40 species were so far associated directly or indirectly with their host plants.

Recently, six fruit fly species were reported for the first time from Himachal Pradesh by Prabhakar *et al.* (2012). Out of 47 species of fruit flies reported from Himachal Pradesh, 13 species belong to genus *Bactrocera* and *Dacus* and majority of them are economically important pests of agricultural and horticultural crops of several countries including India.

A pictorial key for 13 species of fruit flies under 2 (two) genera viz., *Bactrocera* and *Dacus* of sub-family Dacinae (Diptera: Tephritidae) have been documented based on actual photographs of fruit flies collected from north western Himalaya of India during 2009-2010. Among them, *Bactrocera diversa* (Coquillett), *Bactrocera scutellaris* (Bezzi), *Bactrocera tau* (Walker), *Bactrocera cucurbitae* (Coquillett), *Bactrocera zonata* Saunders), *Bactrocera correcta* (Bezzi), *Bactrocera dorsalis* (Hendel), *Bactrocera latifrons* (Hendel) and *Dacus ciliatus* Loew are the pests of agricultural and horticultural ecosystems. *Bactrocera latifrons*, *Bactrocera nigrofemoralis* White & Tsuruta, *Dacus longicornis* Wiedemann and *Dacus sphaeroidalis* (Bezzi) are the new records from the region of which host range has yet to be investigated (Prabhakar *et al.*, 2012). Ukey *et al.* (2013) found different species of fruit flies such as, *B. zonata*, *B. dorsalis*, *B. correcta* and *B. verbascifoliae* infest guava in Ahmednagar District, Maharastra, India.

Bhagat (2014) found a total of 48 species of fruit fly, belonging to 21genera from Jammu and Kashmir, India. These species belonging to two separate families, viz. Drosophilidae (Sub-family Drosophilinae), Tephritidae (Sub-family Dacinae, Tephritinae and Trypetinae). However, from the nearby Ladakh region, so far only two species of Tephritid are known (Chandra and Kaur, 2009). Ukey *et al.* (2013) revealed that different species of fruit flies viz., *B. zonata, B. dorsalis*, *B. correcta* and *B. verbascifoliae* found to infest guava in Ahmednagar District of Maharastra, India. Among the four species, *B. dorsalis* was observed to be dominant species with highest mean number of flies 50.25 (49.95%) emerged out per cage. *B. zonata* was found next dominant species recorded 38.5 flies (31.36%) emerged out per cage. *B. correcta* recorded 24.5 flies (19.95%)

emerged out per cage. Very low infestation of *B. verbascifoliae* found during that investigation which was recorded 9.5 flies (7.73%) per cage.

2.5. Lures and traps in fruit fly collection:

Male Dacine fruit flies are highly attracted to various chemical lures often referred to as parapheromones, although this phenomena remains a complex mystery of tephritid biology (Cunningham, 1989). Male lures are often used with suitable traps for fruit fly detection, population assessment in ecological studies, quarantine surveys, suppression and eradication programs (Drew and Hooper, 1981). It was first discovered to attract male oriental fruit flies, *B. dorsalis* and many other species of fruit flies. Cuelure attracts both male melon fly and the Queensland fruit fly and tri-medlure attracts the males of the Mediterranean fruit fly (Chambers, 1977). Methyl eugenol and cue-lure traps have been reported to attract *B. cucurbitae* males from mid July to mid November (Ramsamy *et al*., 1987, Zaman, 1995 and Liu and Lin, 1993).

Sounder Rajan *et al*. (1996) reported that moist fishmeal was more attractive to the tephritid than fermented palm juice, methyl eugenol, fermented mollasses, jack fruit and rice gruel. Banana was found to be as attractive as soybean hydrolysate probably because of its higher sugar content (Bose and Mitra, 1990) since fermenting sugars attracts fruit flies (McPhail, 1937). Lall and Singh (1969) used palm juice and dried mango juice along with citronella oil to trap adult *B. cucurbitae*. The flies respond positively to the sources of ethyl alcohol and acetic acid (Barrows, 1907). Usually the baits in traps dry up in 2-3 days due to weather factors (Taneja *et al*., 1986) and once the moisture content decreases the fermentation process considerably reduced with little release of volatile compounds from the baits. Cue-lure traps have been used for monitoring and mass trapping of the melon fruit flies in bitter gourd (Permalloo *et al.*, 1998; Seewooruthun *et al.*, 1998). Chowdhury *et al.* (1993) captured 2.36 to 4.57 flies/ trap/ day in poison bait traps containing trichlorfon in bitter gourd.

A new protein bait GF-120 Fruit Fly Bait containing spinosad as a toxicant have been found to be effective in the area wide management of melon fruit fly in Hawaii

(Prokopy *et al.*, 2003). The GF-120 Fruit Fly Bait would be highly effective, when applied to sorghum plants surrounding cucumbers against protein-hungry melon flies but would be less effective in preventing protein-satiated females from arriving on cucumbers. Among the various protein baits tested, yeast, soybean, fruit fly diet, protone and casein were more female selective. When total fruit flies were considered, soybean + sugar + banana was the most superior protein bait with a fruit fly capture of 4.5/trap/week in guava, while casein + sugar + papaya attracted more female fruit flies with a mean capture of 4.33 in mango (Rajitha, 2004).

In guava orchard, yeast + sugar + guava pulp (1: 1: 1) + 5% ammonium acetate, fish meal + 5% ammonium acetate, yeast + sugar + guava pulp (1: 1: 1) + 5% acetic acid and fish meal + 5% acetic acid attracted 1.5, 0.59, 0.33 and 0.25 female fruit flies/trap/week respectively. Yeast + sugar + mango pulp (1: 1: 1) + 5% ammonium acetate attracted 0.63 female fruit flies/trap/week, but there was no fruit flies found in fish meal + 5% ammonium acetate, yeast + sugar + mango pulp (1: 1: 1) + 5% acetic acid and fish meal + 5% acetic acid baited traps in mango orchard during peak fruiting season of guava (1^{st} week of October to 4^{th} week of November, 2005) and mango (six weeks during April-May, 2006) at Dharwad, Karnataka (Ravikumar and Viraktamath, 2007). In sponge gourd plantations, 10 per cent mixture of methyl eugenol and cue-lure was the most effective concentration to melon fly. Madhura and Viraktamath (2003) recorded five species of fruit flies *viz., B. dorsalis, B. correcta, B. erbascifoliae*, *B. affinis* and *B. zonata* which were attracted to methyl eugenol traps located at Bangalore. Morde (2003) observed *B. caryeae*, *B. dorsalis* and *B. zonata* in the trap installed in guava orchard in Konkan area. Kawashita *et al.* (2004) reported catches of *B. correcta, B. dorsalis* and *B. zonata* in methyl eugenol while *B. cucurbitae* in cue lure. Satarkar *et al*. (2009) reported four fruit flies species i.e. *B. caryeae*, *B. zonata*, *B. affinis* and *B. correcta* attracted to the methyl eugenol trap in coastal region of Goa. The highest population of *B. dorsalis* was trapped to methyl eugenol traps installed in guava orchard and lowest population of fruit fly *B. verbascifoliae* was trapped due to the least prevalence of this species in the guava orchards of Ahmednagar district of Maharastra, India while in cuelure traps, only one species *B. cucurbitae* was noticed throughout the year. The fruit fly species, *B. zonata* and *B. correcta* were also observed in the fruit fly methyl eugenol trap as noted by Ukey *et al.* (2013).

2.6. Intensity of infestation by fruit flies:

Intensity of fruit fly infestation varies from crop to crop, season to season and even from cultivar to cultivar of a single crop. In various agro-ecological regions also the population intensity varies. In different commercially cultivated crops like vegetables and fruits the intensity of fruit fly infestation differ. Some of the informations with regard to intensity of infestation of fruit flies are cited here.

2.6.1. Intensity of infestation on vegetables:

The losses due to insect pests infestation constitute major one among different complex production constraints (Dent, 2000). Information on yield losses caused by insect pests is of great interest and value in decision making. Farmers require yield loss information for adopting management practices. Among the vegetables the cucurbits badly suffer from melon fly, *Bactrocera cucurbitae* (Coq.) infestation. It has been estimated that infestation of cucurbits by *D. cucurbitae* ranged from 40-80% between July to October (Pruthi, 1941) while the damage was estimated to the tune of 3-100% on little gourd, *Coccinia grandis* (Linn.) (Patel, 1976). Several authors also observed fruit infestation in bitter gourd (*Momordica charantia* Linn.) by the melon fly that varied from 41-89% (Narayanan and Batra, 1960, Kushwaha *et al*., 1973, Rabindranath and Pillai, 1986 and Gupta and Verma, 1978). Singh *et al*. (2000) reported 31.27% damage on bitter gourd (*Momordica charantia* Linn.) and 28.55% on watermelon (*Citrullus lanatus* Thunb.) in India.

Outside India, the fruit fly has also reported to infest 95% of bitter gourd in New Guinea and 90% snake gourd, 60-87% pumpkin fruits in Soloman Island (Hollingsworth *et al.,* 1997) and in Pakistan. Anwar (1956) reported that up to 60% loss occurred to melon due to fruit fly. In Nepal, fruit fly preferred young and immature summer squash fruits and caused a loss of 9.7% female flowers. Of the total fruits set, more than onefourth (26%) fruits dropped or were damaged just after set, and 14.04% fruits were damaged during the harvesting stage due to fruit fly infestation, yielding only 38.8% fruits of marketable quality in summer squash (Sapkota *et al.*, 2010).

The field experiments on assessment of losses caused by cucurbit fruit fly in different cucurbits been reported 28.7-59.2, 24.7-40.0, 27.3-49.3, 19.4-22.1 and 0-26.2% yield losses in pumpkin, bitter gourd, bottle gourd, cucumber and sponge gourd respectively in Nepal (Pradhan, 1976). It is also the most common and destructive pest of cucurbits throughout Indo-Pakistan subcontinent (Jain *et al.*, 2008) and damage to about 20 cucurbitaceous and solanaceous host all over the Pakistan (Syed, 1971). This fruit fly causes 50% or more damage in Indo-Pakistan sub-continent, Japan, Hawaii and USA (Armostrong, 1991). The losses caused by this species amount to 60% in Pakistan (Alam, 1969).

The losses caused by this highly destructive *Bactrocera* genus of fruit flies (Diptera: Tephritidae) to tree fruit and cucurbit vegetables falling particularly heavily on poor farmers without recourse to cover sprays of insecticides. Losses without control have been estimated as 21% of fruit and 24% of cucurbits in Pakistan (Stonehouse *et al*., 1998) and 12% of fruit and 21% of cucurbits in India (Mumford *et al*., 2005). Fruit infestation by fruit fly in bitter gourd has been reported to vary from 41 to 89% (Lall and Sinha, 1959; Kushwaha *et al*., 1973). The fruit fly has been reported to infest 95% of bitter gourd fruits in Papua (New Guinea) and 90% snake gourd and 60 to 87% pumpkin fruits in Solomon Islands (Hollingsworth *et al*., 1997).

In south Gujarat, the damage in bitter gourd fruits (29.18 %) and little gourd (1-48 %) as recorded at Navsari, India by Patel, (1989) and Patel, (1994) respectively. In north Gujarat, 33.75 percent fruit damage on pumpkin was observed at Sardar krushinagar (Anonymous, 2005). Fruit fly, *Carpomyia vesuviana* is one of the major insect pests contributing towards low yield and poor quality of fruits. It can generally reduce yield/plant from 13 to 20 %. (Bagle, 1992). *Bactrocera cucurbitae* is dominant in all the locations of Bangladesh followed by *Bactrocera tau* and *Dacus ciliatus* (Akhtaruzzaman *et al.*, 1999). Among 15 cucurbits, fruit fly prefer bitter gourd, the extent of losses varies between 30 to 100%, depending on the cucurbit species and the season (Anonymous, 2004). Crop losses in mango (12-60%), guava (40-90%) and papaya (12-60%) have also been recorded by Allwood and LebLanc (1997).

Singh *et al*. (2000) reported 31.27% fruit fly damage on bitter gourd and 28.55% on watermelon in India. Damage due to *B. cucurbitae* in Goa during *kharif* ranged from about 5 to 20 percent (Anonymous, 2005). The melon fly cause cucurbitaceous crops losses were estimated from 10 to 30% of annual agricultural produces in Bangladesh (Naqvi, 2005). Singh and Naik (2006) reported that *Bactrocera cucurbitae* infestation varied from 2 to 11% in different months on bitter gourd. For cucurbits, especially bitter gourd, *Momordica charantia* Linn., the melon fruit fly damage is the major limiting factor in obtaining good quality fruits and high yield (Srinivasan, 1959; Lall and Singh, 1969; Mote, 1975; Rabindranath and Pillai, 1986). Due to melon fruit fly infestation, 75.65% damage was reported on ridge gourd (Kumar *et al.*, 2006, Chaudhury and Patel, 2012).

2.6.2. Intensity of infestation on fruits:

Fruit flies (Diptera: Tephritidae) are also among the most economically important pests attacking fruits worldwide and usually attack commercial fruits (White and Elson-Harris, 1994). They have great economic importance in Pakistan due to their heavy losses to fruits at the farm level with estimated loss of 200 million US dollar annually and the small farmers suffer in particular, being the main growers of highly susceptible guava, mango, peach and cucuribits are being unable to afford existing protection measures (Stonehouse *et al*., 2002). The attack of fruit flies reduces fruit yield and quality. Among 400 species of fruit flies distributed all over the world, *Bactrocera dorsalis* (Hendel) is the most destructive pest occurring in homesteads of Kerala (Verghese *et al.*, 2002b) causing 25-50% fruit loss in mango when harvested at the mature ripe stage. The extent of damage may go up to 80% when the pest incidence occurs in an epidemic form

(Abdullah, 2002 and Latif, 2004). In addition to mango this species also attacks guava, sapota and papaya as reported by Jiji *et al.* (2009).

Oriental fruit fly, *Bactrocera dorsalis* (Hendel) is the most serious pest species that cause about 5-100% loss to various fruits. Highest loss of 80 percent in guava fruit was reported by Kafi (1986) and Ishtiaque *et al.* (1999) in Pakistan. Similarly, Jalaluddin *et al*. (1999) reported 60-80% loss in guava fruit by *Bactrocera correcta* (Bezzi). The peach fruit fly, *Bactrocera zonata* (Saunders) is another devastating pest species found most abundantly in most of the ecological regions of Pakistan and causing losses from 3-100% in different fruits. The Ber fruit fly (*Carpomya vesuviana*) may cause 90-100% damage to Ber fruit (Kapoor, 1993).

Verghese *et al*. (2002a) reported 2.5% to 59.0% crop loss in mango due to *B. dorsalis* infestation depending on variety in Bangalore, India. The level of infestation due to *B. dorsalis* in mango was as high as 26.66% in Gujarat (Sushil and Bhatt, 2002) and 30% in Karnataka (Babu and Virakthmath, 2003). The infestation by fruit flies on guava ranged from 5-46% as reported by Verghese *et al*. (2002a) and Hasseb (2007). The oriental fruit fly, *B. dorsalis* damages litchi fruits to the tune of 2.6 to 12.1% (Hung and Chien, 2008).

2.7. Host selection by the tephritid fruit flies:

Interaction of tephritid fruit flies with their host plants is very much complex. Host plants and parts thereof which the flies prefer to oviposit or settle play an important role in all stages of the life cycle of fruit flies. It is also established that the evolution of fruit flies is considered to be linked with their host plants. Host fruit and foliage attract female flies as they serve as oviposition sites and food substrates.

Under natural conditions, female flies rely on several factors to find suitable host fruit for oviposition. The factors may vary depending on the existing environment and the physiological status of the female fly. Impact of such visual cues as well as different maturity level of fruits have been investigated in the present study. Earlier workers revealed various kinds of findings from all over the world and some of the findings are discussed hereunder.

2.7.1. Role of visual cues on host fruit selection:

Host plant searching is an active process by which phytophagous insects recognise and select suitable substrates for food, mates, oviposition and refuge by using physical cues (color, size, sense of touch) and chemical cues (volatile, nutrition) of host plant (Jolivet, 1992; Panda and Khush, 1995). The fruit flies also use both visual and olfactory cues when searching for their host plants (Dalby-Ball and Meats, 2000, Pinero *et al*., 2006). Adult feeding, mating, and oviposition of tephritid fruit flies are considered to have close evolutionary and ecological associations with their larval host plants (Metcalf, 1990; Drew and Hancock, 2000). The hosts are recognised and then accepted by the fly for oviposition after a series of activities of searching.

It has been documented that the fruit flies are influenced by visual cues in search of their host fruits or plants in addition to their attraction to semiochemicals and other chemical cues from the hosts. Fletcher and Prokopy (1991) noted that host preference of tephritids for oviposition can be driven by the colour of the fruit. Owens and Prokopy (1996) reported that *R. pomonella* was attracted to a colour reflectance range between 350-580 nm and with decreasing reflectance intensity the attractiveness of fruit increased. That is, the fly species is attracted to darker coloured fruit over light coloured fruit, with a fruit colour preference going from black, to orange, to red (Messina, 1990). Similarly, *Rhagoletis cerasi* was attracted to dark colours (red, black and dark orange as observed by Prokopy and Boller (1971). Owens and Prokopy (1996) found that *R*. *pomonella* was not sensitive to the colour hue, but the intensity of reflectance was critically important.

It is now an established fact that the color yellow is a supernormal visual equivalent of plant foliage and is attractive to many phytophagous insects. Color preferences of fruit flies may be related to the color of their host fruits. *Bactrocera dorsalis* is attracted to a yellow color which occurs in almost all their host fruit species (Vargas *et al*., 1991 and Alyokhin *et al*., 2000). On the other hand, *Bactrocera tryoni*, prefers bluish fruit-mimicking spheres that have a slightly enhanced level of ultraviolet reflectance, similar to the reflectance of their native host fruits, while yellow or orange spheres, which resemble the color of near ripe or ripe wild tobacco host fruit, are most attractive to tobacco fruit fly, *B. cacuminata* (Drew *et al*., 2003). *Rhagoletis pomonella*, which attacks red fruits (apple and hawthorn berries), were attracted to red color spheres more than other colors (Prokopy and Hauschild, 1979 and Reynolds *et al*., 1996). *Anastrepha suspensa* were most attracted to an orange colour with a peak reflectance wave length range of only 580-590 nm (Greany and Szentesi, 1979). On the other hand, *B. tryoni*, *B. neohumeralis* (Hardy) (lesser Queensland fruit fly), *B. cacuminatus* (Hering) (solanum fruit fly) and some other tephritids found in south eastern Queensland showed significant preference to colours with a peak wavelength between 520-540 nm (Hill and Hooper, 1984).

Henneman and Papaj (1999) found the role of cues, colour is often the most important. The authors noted that *Rhagoletis juglandis* females showed significantly greater attraction to green coloured real fruit and green coloured fruit models (i.e. no flavour) over yellow coloured real fruits and fruit models, indicating that colour itself is the major cue for that species in choosing oviposition sites. Colour preference by *B. tryoni* to blue or white fruit mimics did not differ with size of the fruit models (Drew *et al.*, 2003).

Stark and Vargas (1992) indicated that visual cues are involved in the search for methyl eugenol by *Bactrocera dorsalis* (Hendel), although probably only in a secondary manner but also found high capture rate in white and yellow traps. Studies were made on attraction of different species of fruit flies to different coloured traps in guava and mango orchards during 2005-06 near Dharwad. Yellow and transparent traps attracted significantly high number of *B. correcta* in guava (70.45 fruit flies / trap / week) and mango (5.13 fruit flies / trap / week) orchard respectively. Green and orange coloured traps in guava (3.79 and 3.75 fruit flies / trap / week respectively) black coloured traps in mango (3.88 fruit flies / trap / week) were attractive to *B. dorsalis. B. zonata* was attracted to red coloured traps (3.75 fruit flies / trap / week) in mango ecosystem. Rajitha and Virkthamath (2005) also observed similar trends and noted that orange and green colour traps were more attractive to *Bactrocera dorsalis*.

Reflectance of yellow and orange colour might also be a factor in the attractiveness of fruit flies to these colours (Robacker *et al*., 1990 and Stark and Vargas, 1992). Stark and Vargas (1992) used coloured bucket traps baited with methyl eugenol for monitoring males of *D. dorsalis* in guava orchard. White and yellow traps caught the largest number of flies, whereas green, red and black caught the fewest. They further found that when traps were placed in guava trees, no significant difference in fly capture occurred. The responses of fruit flies to visual stimuli are dependent on colour, shape and size of the stimulus as noted by Katsoyannos (1989).

Recent reports from China have revealed that for *B. dorsalis*, UV and green stimuli would enhance the attractiveness of a coloured paper, while blue stimuli would diminish the attractiveness (Wu *et al*., 2007). However, after studying the response of *B. cucurbitae* female to host associated visual and olfactory stimuli, Pinero *et al*. (2006) suggested that a combination of both of these stimuli needed to elicit high levels of response compared to each stimulus offered alone. Earlier, Prokopy and Owens (1983) and Fletcher and Prokopy (1991) also opined that adult flies use visual and olfactory stimuli to locate hosts and the traps that combine visual and olfactory cues proved to be the most efficient for capturing fruit flies.

The advantage of using coloured traps is that both male and female are attracted. Guava fruit fly, *B. correcta* (Bezzi) was attracted to yellow followed by orange coloured traps coated with methyl eugenol (Jalaluddin *et al.*, 1999). *B. tau* was attracted to yellow traps (Sood and Nath, 1998). A deep yellow colour (585 nm) was found to attract more fruit flies than fluorescent yellow, brown and orange. In addition, an increase in trap area was directly correlated to the number of fruit flies trapped (Madhura, 2001).

Experiment was conducted at farmer’s field in Nadia and North 24 Parganas districts of West Bengal during 2012 to study the population variation of melon fruit fly on four cucurbitaceous vegetable crops (pointed gourd, ridge gourd, bitter gourd and cucumber) with four different coloured traps (yellow, transparent, red and blue) along with cue-lure blocks as attractants. Four holes (0.5 cm in radius) were done on four sides of the coloured traps along with 250 ml water at the bottom of the traps. Yellow traps attracted significantly high number of *B. cucurbitae* in pointed gourd and transparent traps attracted significantly high number of *B. cucurbitae* (Coq.) in ridge gourd, bitter gourd and cucumber. Blue coloured traps were least attractive in all four cases (Bhowmik *et al*., 2013).

The preference of female flies to host fruit for oviposition may depend on factors other than fruit size. Visual response of fruit flies has also been found to be related with the size of colored spheres (i.e., fruit mimics) as revealed by several authors (Cornelius *et al.*, 1999a and Brevault and Quilici, 2007a). Moericke *et al*., (1975) found that females of apple maggot fly, *R. pomonella,* preferred smaller red models, while a red sphere of about 10 cm diameter appears optimal for attracting Western cherry fruit fly, *Rhagoletis indifferens* Curran (Mayer *et al*., 2000). Nakagawa *et al*. (1978) reported that the attraction of *C. capitata* increased as the size of fruit models increased from 1.5 to 18 cm.

In addition to visual cues, the habitat pattern also plays an important role in the behavior of tephritid fruit flies. Fruit maturity, abundance of host fruit within the canopy and species of host fruit all affect host fruit orientation by female flies (Katsoyannos *et al.*, 1998, Dalby-Ball and Meats, 2000). The efficiency of visual traps tend to decrease when competing with host fruit, but trap efficiency rises when placed within the canopy of host plants without host fruit (Rull and Prokopy, 2003 and Brevault and Quilici, 2007b).

2.7.2. Stages of fruits preferred by the adult fruit fly:

Tropical fruit flies (Diptera: Tephritidae: Dacinae) are important agricultural pests, depositing their eggs into fruits and vegetables, the flesh of which is subsequently consumed by the developing larvae (White and Elson-Harris, 1992). Fruits have been found to vary in the resources they offer to larvae, with the quality of available nutrients etc. that usually influence size, developmental time, pupal weight, adult eclosion rate and reproductive maturation time of adult flies (Khan *et al.,* 1999 and Kaspi *et al.,* 2002).

As for many herbivorous insects where selection of egg laying site depends at least partially on host plant quality (DiTommaso and Losey, 2003 and Van *et al.,* 2003). Adult fruit flies are known to make decisions about which fruit to oviposit into based on the suitability of the fruit for their offsprings' performance (Fontellas-Brandalha and Zucoloto, 2004 and Joachim-Bravo *et al.,* 2001) is one side, another fact is that the sexual behavior (including meeting, courtship and mating) of phytophagous insects may occur principally or exclusively on host plants. Males may purposefully search host plants for females simply because females are likely to visit host plant for feeding and oviposition, resulting in mixed-sex encounters and mating opportunities (Landolt and Phillips, 1997).

Adult feeding, mating and oviposition of tephritid fruit flies are considered to have close evolutionary and ecological associations with their larval host plants (Metcalf, 1990 and Drew and Hancock, 2000). According to the studies of Alies Van Sauers-Muller (2005), fruit fly infestation depends upon size and shape of the fruit specially the guava. He also found that large sized fruit showed more susceptibility. Many species of tephritid fruit fly, such as *B. dorsalis, B. cucurbitae* (Coq.), *Bactrocera minax* (Enderlein), *C. Capitata* (Wiedemann,) and *Rhagoletis* species (Rhagoletis) tend to prefer spherical shapes (Vargas *et al.,* 1991; Cornelius *et al.,* 1999; Mayer *et al.,* 2000; Drew *et al.,* 2006 and Pinero *et al.,* 2006). Preference for larger fruit models reflects fly behavior to real fruit. Within a fruit species, there tends to be a general pattern within the tephritids that female flies tend to prefer larger fruit for oviposition. Singh and Vashishtha (2002) revealed that fruit size, fruit weight and pulp/stone ratio of Indian jujube, *Ziziphus mauritiana* (Rhamnaceae) was positively correlated with fruit fly infestation. Diaz-Fleisher and Aluja (2003) postulated that females of some tephritid fruit fly species tend to deposit larger clutches in larger hosts. However, females of the Mexican fruit fly, *Anastrepha ludens* (Loew), oviposited larger clutches in peach, *Prunus persica* (Rosaceae) than in grapefruit, *Citrus paradise* (Rutaceae), despite the fact that grapefruit is a larger host (Leyva *et al.,* 1991). This result indicates that female flies are not always attracted to larger fruit when compare between the different fruit species. Thus, the preference of female flies to host fruit for oviposition may depend on factors other than fruit size. Size alone is not only important in host location by fruit flies, but so also is shape. *Anastrepha suspensa* (Loew) (Carribean fruit fly) was more attracted to natural fruit extracts on convex shaped surfaces (e.g. spheres, hemispheres, ellipsoid objects) than when the same extract was applied on a flat surface.

Wild flies displayed an absolute preference for domes, whereas laboratory bred flies laid eggs in both domes and flat surfaces (Greany and Szentesi, 1979). Spherical shapes are more attractive than cylindrical, conical or cubical shaped fruit models to *C. capitata* (Freeman and Carey, 1990 and Nakagawa *et al.*, 1978), while *A. indifferens* was more attracted to a spherical shape than a rectangle shape (Mayer *et al.,* 2000). *B. dorsalis* were more attracted to spherical shaped versus block shaped fruit models (Cornelius *et al.,* 1999).

Mohammed (2001) showed that ripe stage was the most susceptible for oviposition followed by the mature green stage. Several authors confirmed that the mature green fruit ripening stage is the most preferred stage. Jang *et al.* (1998) reported that adult female of fruit flies prefers to visit semi-ripe fruits. Drew and Lloyd (1987) emphasized that adult flies usually do not oviposit in immature or on over ripe fruits. Rauf *et al*. (2013) was conducted a laboratory experiment to determine the oviposition preference of the peach fruit fly for different fruits including guava, banana, citrus, ber, chikoo and apple under free or no choice conditions. Results showed that the guava was the most preferred host with mean pupal recovery of 318.00 ± 4.61 pupa/fruit (p/f) under free choice and 434 ± 2.64 p/f under no choice conditions, followed by banana (266.00 ± 4.5 p/f) in free choice and ber (177.00 ± 2.08 p/f) in no choice experiment whereas, apple and citrus were least preferred hosts.

Most tropical fruit flies only lay into mature fruit, but a small number can also oviposit into unripe fruit as observed by Drew *et al.* (1978). There is very limited information available on comparative host use by a fly species across fruit ripening stages (Dorji *et al.,* 2006). For example female papaya fruit fly, *B. papayae* and olive fruit fly *Bactrocera oleae* (Rossi), are reported to prefer to oviposit into the green stage of their host fruits than the mature stage (Leblanc *et al.,* 2001; Yokoyama and Miller, 2004). The available information indicating that the host fruit odors are also play an important role in the ecology of tephritid fruit flies. The odor arising from a host plant is one of the most important host location cues used by tephritid fruit flies. The host fruit odors have been found to stimulate ovarian development as noted by Aluja *et al*. (2001) and increasing mating success (Landolt, 1994, Shelly and Edu, 2007). Male fruit flies respond to volatile compounds from host and non-host plants largely because of the indirect role such chemicals play in sexual activity (Shelly and Villalobos, 2004, Keng-Hong and Nishida, 2005, Shelly and Edu, 2007 and Shelly *et al*., 2008). On the other hand, mature female flies are attracted to host plant for oviposition and tend to more sensitive to the volatile compounds of host fruit (Hernandez *et al*., 1996, Jang *et al*., 1997 and Malo *et al*., 2005). However, polyphagous flies are not equally attracted to all host odors.

In a study on host fruit volatiles as attractants, Cornelius *et al*. (2000b) concluded that odors of common guava were more attractive to female *B. dorsalis* than papaya and starfruit, whereas they were equally as attractive as strawberry, guava, orange and mango. *Cucumis sativus* odor was more attractive to female flies of *B. cucurbitae* than odors of three other host plants viz., *Cucurbita pepo* (Cucurbitaceae), *C. papaya* and *Solanum lycopersicum* (Solanaceae) (Pinero *et al*., 2006). The odors of different ripening stages of host fruit can elicit different responses from female flies. Reissig (1974) suggested that the mechanism which attracts flies to fruit may be an olfaction response to volatile compounds emanating from maturing fruit. Flath *et al*. (1990) found that organic compounds released from ripe papaya significantly enhanced attractiveness of the fruit to *B. dorsalis.* This sugges ts that female flies may use host fruit odors not only to locate suitable hosts for oviposition, but also for assessing fruit ripening.

In a nutshell, fruit preference by the tephritid flies may be driven by a range of physical stimuli, but often there is one dominant physical factor (e.g. fruit size, colour, ripeness stage etc.) influencing the flies. However, this dominant trait may vary between fruit fly species. Physical stimulus hierarchies exist in some species, for example in *A. suspensa* shape is the primary cue and colour is secondary (Greany and Szentesi, 1979). Fruit size is a more important criterion than colour and odour in host acceptance behaviour in *C. capitata* (Prokopy *et al.*, 1990), while for *B. tryoni*, *B. neohumeralis* and *B. cacuminatus* colour is secondary and odour is the primary attractive factor (Hill and Hooper, 1984). It is recognised, however, that under natural conditions the combination of all these factors may contribute in different degrees of host acceptance behaviour of tephritids (Economopoulos, 1989).

The response of tephritid flies to host fruits relies on external and internal factors. Many studies, as cited here indicating the complex relationship between female flies and their host fruits. Oviposition strategies by female flies under limiting conditions may result in eggs being placed in suboptimal conditions and hence increased pressure on their offspring. Thus, the interaction of external and internal factors which influence oviposition behavior of female flies indirectly affect their larvae. Many points are, however, not clear on the relationship between oviposition preference and larval performance in fruit flies.

2.8. Biological suppression of Tephritid fruit flies:

The biological control efforts against fruit flies of the genus *Bactrocera*, family Tephritidae have been extensive over the past half century. Its needless to state that the pest is internal feeder, externally applied conventional insecticides hardly show their efficacy against the pest. The crops on which the fruit fly infest are either fruits or vegetables and hence there remains every possiblity to remain toxic insecticidal residue in the treated fruits. Use of toxic hazardous pesticides increases cost of production and badly hamper environment. The importing countries often do not agreed to accept consignment with pesticide contaminated fruits and vegetables. In this cross roads search for alternative ways are essential.

Thus, **c**urrent social and environmental problems associated with insecticide use for fruit fly control, either by aerial or ground applications on foliage for adult control or to the soil for larvae or newly emerged adult control (Saul *et al.*, 1983 and Penrose, 1993) have motivated the search for biological alternatives, including enotmopathogenic bacteria, nematodes and fungi (Toledo, 2002). Among these biological aspects EPN has bright scope in sustainable suppression of the pest as an ecologically viable tool of IPM.

2.8.1. Entomopathogenic nematodes (EPN) in fruit fly management:

Considering importance of biological control in integrated pest management programs, and based on the positive impact of previous biological control, several scientists have renewed efforts to search for new biological control agents. Recently, ecologically compatible strategies to manage *C. capitata* populations were developed, including the use of entomopathogenic viruses, fungi, nematodes, protozoa, and bacteria as biological control agents (Castillo *et. al.*, 2000 and Lacey *et. al.*, 2001).

As the tephritid flies drop down in soil for pupation, soil inhabiting entomoparasitic nematodes have good role to play in parasitising the maggots of fruit flies. Again, after infecting their larval host they multiply in soil itself and act as an source of innoculum that have the chance for subsequest parasitisation of dropped maggots of fruit flies. Application of Entomopathogenic nematodes (EPNs) might be a viable option in an integrated fruit fly management program. Various fruit fly species including medfly were found to be susceptible to EPN (Toledo *et al.,* 2006 and Yee and Lacey, 2003).

The mature third instar larvae that are going to pupate and adult flies have been found susceptible to nematode infection, with no infection recorded for the pupae. Pupariating larvae of *C. capitata* were generally more susceptible to infection than those of *C. rosa*. Significantly more larvae of *C. capitata* were infected by *H. bacteriophora*. For *C. rosa*, the highest infectivity of larvae was obtained with *H. zealandica*. In contrast, adults of both species were highly infected by *S. khoisanae* (Ansari *et al*., 2012).

Literatures also revealed that, Entomopathogenic nematodes (EPNs) are used to control several agriculturally important insect pests of different orders in various agro-ecosystem. The infective juvenile (IJs) of EPN is microscopic having 0.5 to 1.5 mm long depending on species. The third stage juvenile of these nematodes have closed mouth and anus and cannot feed until it finds an insect host. Eighty three described EPN species have been identified (including 64 species of *Steinernema* and 8 species of *Heterorhabditis* and 1 species of *Neosteinernema*) from various insects or from the soil worldwide (Grewal *et al.,* 2001). Usually EPNs are found in soil and is activated by insect movement and then follows a gradient of CO_2 to find the insect larvae (Gaugler *et al.,* 1997) to get into the insect's blood cavity in order to kill it.

The first nematode (*S. carpocapsae*) used successfully in the control of an insect pest was reported from Australia. Commonwealth Scientific and Industrial Research Organization (CSIRO) was the first in the world to use EPNs commercially against black vine weevil in ornamentals and against currant borer moth in black currants (Georgis, 2002). Entomopathogenic nematodes in the families *Steinernematidae* and *Heterorhabditidae* have been shown to be pathogenic to a wide range of agriculturally important pests and are useful alternatives to chemical insecticides for insect control (Gaugler and Kaya, 1990).

Efficacy of EPN have been tested by several workers against different insect pests. Under field conditions *S. carpocapsae* and *H. bacteriophora* have been shown to reduce weevil densities upto 83% and 81% on plants treated with the two species, respectively (Jansson *et al.,* 1990). Allard and Moore (1989) showed that a *Heterorhabditis* spp. could cause high mortality of both adult and larvae of Coffee berry borer, *Hypothenemus hampei* (Ferrari). Richter and Fuxa (1990) reported 33-43% infection of *Spodoptera frugiperda* by *S. carpocapsae* in field corn. They also found that spraying of nematodes onto corn ears caused up to 71% infection of *S. frugiperda* and they concluded that *S. carpocapsae, S. riobrave*, and *H. megidis* have potential for controlling *S. frugiperda*.

Gaugler and Kaya (1990) mentioned that EPN of genera *Heterorhabditis* and *Steinernema* (Nematoda: Rhabditidae) have emerged as excellent insect biocontrol agents. Also it agrees with other attempts concerning the infectivity of EPN on the peach fruit fly, *Bactrocera zonata* (Attala *et al.,* 2002), the cucurbit fly, *Dacus ciliatus*, and the med fly *Ceratitis capitata* (Soliman, 2007a). Fetoh and El-Gendi (2006) and Soliman (2007b) reported that EPN were effective on the newly formed pupae than aged pupae, and used EPN to control the adults of PFF and CFF. Soliman (2007b) used sugar solutions 1% and 4% mango, guava, orange juices, agar solution and a nematode-water suspensions to control both of the peach fruit fly and the med fly. The author also concluded that Hb nematode was the best candidate for controlling fruit flies as it caused high mortality to target pests.

The high virulence of Hb nematode native isolates to the tested insects was unclear but may be able to be attributed to the rapid penetration in or rapid bacterial growth inside host’s body and ultimately kill the host. Toledo *et al.,* (2006) used Hb nematode on *Anastrepha ludens* (Diptera: Tephritidae) and mentioned that Hb nematode caused high pathogenicity. Glazer (1992) stated that *Steinernema carpocapsae* (All strains) was less effective than *Heterorhabditis bacteriophora* HP88 when applied to different lepidopteron pests according to LD_{50} and LT_{50} values.

The impact of entomopathogenic nematode (EPNs) species against *Bactrocera oleae* was very effective whenever infective juveniles (IJs) were sprayed over naturally infested fallen olives, many larvae died in the soil and *Steinernema feltiae* caused the highest overall mortality of 67.9% (Sirjani and Kaya, 2009). Nyasani and Mutua (2008) also reported that the nematode species, *Heterorhabditis indica, Steinernema karif, S. wesieri, Steinernema* spp. and *Heterorhabditis* spp. caused 96.0, 93.3, 92.0, 88.0 and 86.7% mortality in the DBM larvae within 72 hrs. Anbesse and Gebru (2008) reported that two entomopathogenic nematodes (EPN) *Heterorhabditis bacteriophora* and *Steinernema yirgalemense* caused 88.7% and 88.6% mortality to barley chafer grub *C. curtipennis*. With high concentrations of EPN wettable powders, Ebelinge and Nishida (1953) obtained effective protection against *Dacus cucurbitae, D. dorsalis* and *Ceratitis capitata* in cucumbers, watermelons and tomatoes respectively.

Fetoh *et al.* (2011) evaluated efficacy of the entomopathogenic nematodes Hb (*Heterorhabditis bacteriophora* Poinar) and Sc (*Steinernema carpocapsae*) on different stages of peach fruit fly, *Bactrocera zonata* and the cucurbit fly, *Dacus ciliatas* under laboratory condition. Mortality rates ranged from 9.3 to 42.7%, 12.7 to 52.3% for the full grown larvae of both *B. zonata* and *D. ciliatus* treated by *S. carpocapsae* nematode respectively and from 67.3 to 100%, from 46.3 to 100% for the full grown larvae of both *B. zonata* and *D. ciliatus* treated by *H. bacteriophora* nematode, respectively, whereas mortality rates of pupae ranged from 2.7 to 32.7% for the pupae of *B. zonata* treated by Sc nematode, from 1.7 to 23.3% for the pupae of *D. ciliatus* treated by Sc nematode, from 12.7 to 51.7 % for the pupae of *B. zonata* treated by Hb nematode and from 6.3 to 39.3 % for the pupae of *B. zonata* treated by Hb nematode. When IJs of *S. feltiae* were sprayed over naturally infested fallen olives, many larvae died within treated olives as well as in the soil. *S. feltiae* caused the highest overall mortality of 67.9%.

Many larvae died inside treated olives, which indicate that IJs were able to find and infect them before they exited the fruit (Sirjani *et al.*, 2009). Furthermore, the mortality rates varied from 35.0 to 78.7%, 7.7 to 50.3% for 7 days old adults of both *B.*

zonata and *D. ciliatus* treated by Sc nematode respectively, 41.7 to 90.3 and 17.0 to 67.7 % for 7 days old adults both *B. zonata* and *D. ciliatus* treated by Hb nematode respectively. From the obtained results the authors concluded that the entomopathogenic nematodes, *Heterorhabditis bacteriophora* Poinar and *Steinernema carpocapsae* (all strains) were effective on different stages of *B. zonata* and *D. ciliatus.* Hb was more virulent than Sc and the larvae and adults of *B. zonata* and *D. ciliatus* were more susceptible to the nematode infection than the pupae. Rohde *et al.* (2012) also observed that *Heterorhabditis* spp. and *S. carpocapsae* were effective in controlling larvae of *C. capitata* when applied on the soil surface, alone or in combined, with mortality rates ranging from 26 and 74%. For the range of application, *S. carpocapsae* was more efficient when applied immediately after the transfer of larvae to the soil, and 24 hours before to infestation (80 and 90% mortality respectively).

Thus, there are ample evidences of frontal technologies with regard to the present studies on the basis of which the studies were carried out and also findings of the same interpreted.

CHAPTER-3

Materials & Methods

Chapter-3

MATERIALS AND METHODS

3.1. Experimental site:

Different bio-ecological aspects of Tephritid fruit flies have been investigated exhaustively under terai and hilly agro-ecological region of West Bengal. The field experiments were conducted in the farmer's field of the agro-ecological region under consideration and at the Instructional Farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India during two consecutive years, i.e. 2013-14 and 2014-15. The laboratory investigations were carried out in the Department of Agricultural Entomology. Survey works undertaken at different locations of the northern districts of West Bengal, viz. Cooch Behar, Jalpaiguri, Alipurduar and Darjeeling. Details of the locations are as follows:

3.2. Geographical location:

The Instructional Farm, where the experiments were undertaken, is located at $26^0$19´ N and $89^0$23´ E longitude at an altitude of 43 meter above the Mean Sea Level. The experimental site falls in the district of Cooch Behar under terai agro-ecological region of the state West Bengal. The terai agro-ecological region comprises of northern parts of West Bengal that lies between $25^0$57´ to 27^0N latitude and $88^0$25´ to $58^0$54´ E longitude. The region is located in the sub-Himalayan plains comprising Kurseong, Kalimpong and Bhutan hills in the North, Bihar border in the West, Assam border in the East and Bangladesh in the South.

3.3. Soil:

The soil of the region considered to be classified into old Himalayan Pediment Plains and Teesta Flood Plains formed mostly from the Himalayan detritus. Soils of the region comprises dark brown top soil, 1-3 feet deep, sandy loam in texture, acidic in reaction (4.2-6.8), rich in raw humus content, porous, low water retention capacity, medium to high total nitrogen content, low to medium K content and poor in primary and secondary micronutrients.

3.4. Climate:

The terai agro-ecological region of West Bengal characterized by typical per humid climate with a distinct feature of high rainfall with an annual average of more than 3000 mm and relative humidity range between 95 – 65 per cent. The maximum rainfall is about 80 per cent received from the south west monsoon during the rainy months from June to September. Average maximum and minimum temperatures range between 24 to 33.2^0 C. Further the weekly mean bright sunshine hours per day remains more or less steady from the beginning of the year up to early June, i.e. just before arrival of South-West monsoon. A sharp fall has been observed thereafter which continues up to early October. Duration of bright sunny days is more than (8 hours average) during November to March. The entire area is humid and worm except having a short spell of winter during the months of December-February.

3.5. Materials required:

3.5.1. Lures and other chemicals:

i. Cue-lure (4-(p-acetophenyl)-2-butanone) (Commercial product Mfg. by Excel Crop Care Ltd.)
ii. Methyl eugenol (Mfg. by SISCO Research Laboratories Pvt. Ltd.)
iii. Food baits prepared from banana and jack fruit
iv. Formaldehyde
v. Rectified spirit
vi. Carbon tetrachloride (CCl_4)
vii. Entomoparasitic nematode (EPN), *Heterorhabditis indica* (Poinar).

3.5.2. Equipments and devices:

i. Balance
ii. Insect collection device
iii. Stereo zoom microscope with higher resolution (Magnus)
iv. SLR camera with higher specification (Canon)
v. Petriplates of different sizes
vi. Plastic trays (46 X 32 X 8 cm.)
vii. Seives of different 40 and 60 mesh
viii. Insect rearing cages
ix. Artificial fruit models (mango) of various colour
x. Natural fruits of different maturity level (mango, pumpkin)
xi. Other laboratory accessories

3.6. Determination of species diversity:

From present day IPM as well as precision crop protection point of view, accurate identification of causal agent is the first and foremost critera to make a crop protection venture successful and viable. It is equally and invariably applicable in managing the dreaded pest of several fruits and vegetables, i.e. fruit flies. Application of conventional pesticides is not effective in managing the pest becuase of its typical life history. Use of pheromone trap is one of the most important viable technology for suppresion of its population. Again pheromones are also species specific and do not usually work against non target species. So identification of species is the pre-requisite for chalking out viable IPM technology for fruit flies. Keeping this point under consideration the present study was conducted by collecting the species both by using pheromone traps, food bait as well as from infested fruits.

3.6.1. Collection of fruit flies by using methyl eugenol and cuelure trap:

The male adult fruit flies were collected by using sex attractants methyl eugenol and cue-lure from different predefined locations of northern districts of West Bengal viz. Cooch Behar, Alipurduar, Jalpaiguri and Darjeeling in the farmers' field (table-1 and fig-1). At each location, four traps of both methyl eugenol and cuelure were installed at 2 mt height from the ground level keeping at least 100 mt distance from trap to trap.

The trapped flies were counted and their numbers were recorded. Thus the flies were collected, preserved by following standard protocol and then identified. The study was continued for consecutive two years i.e. during 2014-15 and 2015-16 in the months of August-September in the plains and November-December in the hills of Darjeeling every year and the data was analyzed statistically.

3.6.2. Collection of fruit flies by using different food bait trap:

Use of parapheromone is undoubtedly an efficient technique for fruit fly collection, detection, population assessment in ecological studies, quarantine surveys, suppression and eradication programmes although this phenomena remains a complex mystery of tephritid biology. Again, in this technique only male adults are attracted. Identification of species is also remain male oriented. In addition to parapheromones, some other materials are there which act as good attractants of tephritid fruit flies.

Among them, food bait is one of the most viable alternatives for collection of both male as well as female individuals in a population.

Considering the aforesaid perspectives, in the present study the following food baits were prepared in the laboratory by collecting the respective ingredients from local markets and other necessary chemicals.

a. Banana palp + sugar (1:1)+ 5% ammonium acetate

b. Jack fruit + sugar (1:1) + 5% ammonium acetate

The collected flies were counted and their numbers were recorded and preserved by following standard protocol and then identified. The study was continued for consecutive two years i.e. during 2014 and 2015 in the months of August-September in the plains and November-December in the hills of Darjeeling every year and the data was analyzed statistically.

3.6.3. Recovery of adult fruit flies from infested fruits:

Fifty numbers of infested fallen as well as harvested fruits of pumpkin, bittergourd, bottle gourd, pointed gourd, mango and guava were collected from different locations of Cooch Behar, Alipurduar and Jalpaiguri district. For mandarin orange the fallen and infested fruits were collected from orchards of different locations (table-1) of Darjeeling district during November-December of both 2014 and 2015.

From each location, four farmers field/orchard were selected for collecting the fallen infested as well as harvested fruits. No plant protection measures were undertaken in those particular fields. The collected fruits were brought to the laboratory and kept in plastic trays (46 X 32 X 8cm) filled with sand and coco pit (1:1) (Ukey *et al.*, 2013) to facilitate pupation and adult emergence.

Fly emergence were started after 12 - 13 days and on first day 55 - 60 % flies were emerged while the rest of the flies were emerged on second day. The fruit flies were handled carefully and care was taken to prevent escaping from the rearing cages. The emerged fruit flies were provided food by hanging cotton swab soaked in 10 % honey solution for a week. The flies were allowed for sclerotization and development of body colour and killed by using chloroform.

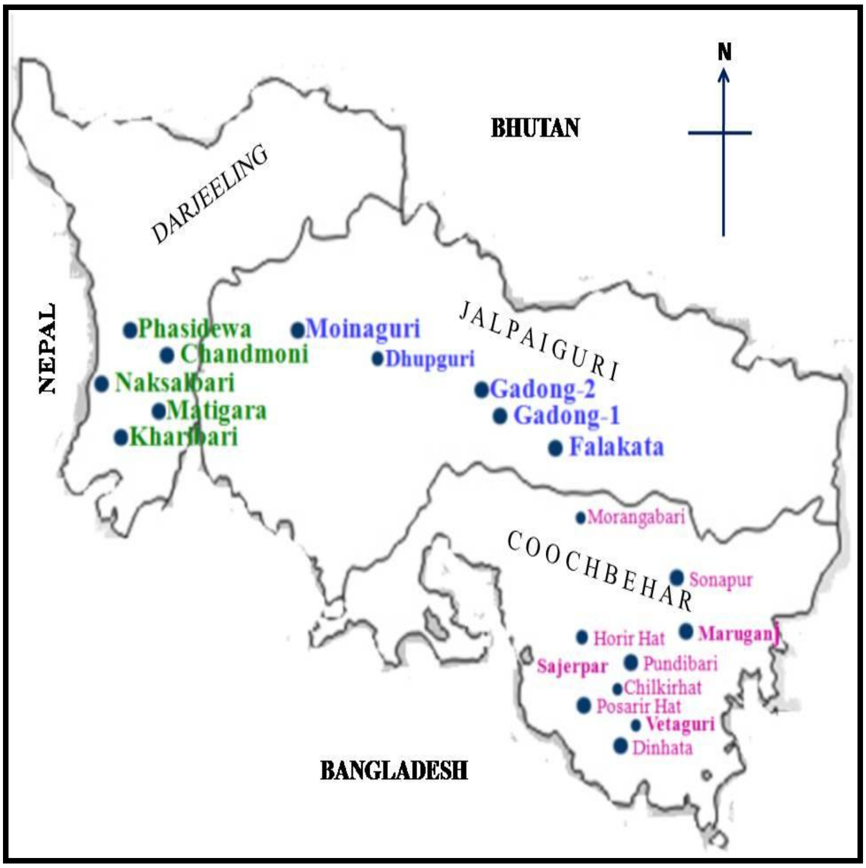
3.7. Identification of the adult flies:

The flies emerged from the damaged fruits were examined critically under stereo-binocular microscope for their morphological characteristic features and identified by following the appropriate taxonomic keys (Drew and Raghu, 2002, White and Elson-Harris, 1994 and Kapoor, 2005). From each lot, 100 flies were taken randomly for identification. Some of the specimens were also sent to Dr. V. V. Ramamurthy, National Coordinator, Network Project on Insect Biosystematics, IARI, New Delhi for proper identification.

The adult fruit flies of a particular species from each lot were recorded and preserved by following standard preservation technique. After proper identification, percent of each category of species were determined as follows:

% of a species = Number of individual of that particular species / Total number individual taken in a lot X100.

Fig-1: Survey locations at different districts of northern West Bengal. (Map not in scale).



3.8. Intensity of fruit fly infestation:

Different districts of terai and hilly region of northern West Bengal have been selected for recording intensity of fruit fly infestation on various commercially important fruits and vegetables. Geographical location of the survey areas are as follows in tabular form:

Table-1: Geographical location of the survey area for installation of traps, collection of infested fruits etc.

1.1. Survey locations for fruits and vegetables.

District	Location	Latitude	Longitude
Cooch Behar	Chilkirhat	26^0 53′N	89^0 19′E
	Morangabari	21^0 91′N	87^0 26′E
	Posarir Hat	23^0 73′N	88^0 23′E
	Dinhata	26^0 13′N	89^0 46′E
	Vetaguri	26^0 20′N	89^0 48′E
	Maruganj	26^0 30′N	89^0 56′E
	Pundibari	26^0 41′N	89^0 38′E
	Horir Hat	26^0 39′N	89^0 49′E
	Sajerpar	26^0 29′N	89^0 19′E
Jalpaiguri	Gadong-1	26^0 55′N	89^0 10′E
	Gadong-2	26^0 54′N	89^0 17′E
	Moinaguri	26^0 57′N	88^0 82′E
	Dhupguri	26^0 58′N	89^0 00′E
Alipurduar	Sonapur	26^0 46′N	89^0 39′E
	Falakata	26^0 53′N	89^0 19′E
Darjeeling	Chandmoni	25^0 26′N	88^0 02′E
	Naksalbari	26^0 70′N	88^0 42′E
	Matigara	26^0 72′N	88^0 38′E
	Kharibari	26^0 55′N	88^0 19′E
	Phasidewa	26^0 58′N	88^0 37′E

1.2. Survey locations for mandarin orange.

District	Location	Latitude	Longitude
Darjeeling	Bomb Bosti	27^0 26′N	88^0 55′E
	Poshyore	27^0 05′N	88^0 46′E
	Lanku Valley	27^0 28′N	88^0 31′E
	Sittong	27^0 35′N	88^0 52′E
	Upper Dungra	27^0 10′N	88^0 59′E
	Lower Dungra	27^0 06′N	88^0 48′E
	Ecchey Bosti	22^0 06′N	88^0 36′E
	Mongpu	28^0 97′N	88^0 37′E
	Mongmaya	26^0 93′N	88^0 45′E

In each growing season of the crop (cucurbits, guava, citrus, mango) altogether three visits conducted during early, peak and late fruiting stage. At each location, four farmers field were selected for each vegetable and 100 fruits were taken from the harvested lot of each farmer's field randomly and then taken to the laboratory and examined critically for fruit fly infestation.

Number of infested and fresh fruits was recorded at each instance. Percent infestation was calculated as per the following:

$$\text{\% infestation} = \frac{\text{Infested fruits (No.)}}{\text{Total fruits observed (No.)}} \text{ X } 100$$

The investigation was conducted for two successive years and pooled together for final determination of intensity of infestation by fruit fly. Data obtained were analyzed by standard statistical package and presented in both tabular as well as graphical form.

3.9. Role of visual cues in host fruit selection and recognition:

Experiment was conducted in the laboratory using rearing cages. Under free choice method, artificial mango fruit model of three different colour *viz* green, yellow green and yellow were used for the study. An individual 21 to 22 days old, mated female fly of *B. dorsalis* mango were released in the rearing cages. Three different coloured artificial mango fruits were provided in the rearing cage.

Number and duration of visit by the fly on these artificial fruits were recorded from 06:00 to 16:00 hours during the day. The same experiment was repeated ten different days with individual adult female flies seperately.

The data were processed, analysed by following appropriate statistical techniques, presented in tabular form and interpreted accordingly.

3.10. Stage(s) of fruit(s) preferred by the adult fruit fly:

Free choice test was carried out under laboratory condition in the Department of Agricultural Entomology, Uttar Banga Krishi Viswavidyalaya, West Bengal, India to determine the behavior of individual female of *B. dorsalis* when three ripening stages of mango (unripe, ripe and fully ripe) of one variety were offered simultaneously in a 45 × 45 × 45 cm rearing cage. Similarly, individual female of *B. cucurbitae* was taken for the study using different maturity stages of pumpkin (10, 20 and 30 days after setting of fruit). Experiment was conducted twice during two consecutive years 2014 and 2015.

Fruits of different maturity level were placed within the cage and then newly emerged adult female flies were allowed to visit the fruits in the cage one by one. An individual of 21 to 22 days old, mated female fly was released in an observation cage with one mango per replicate each of three maturity stages (Green, ripe and fully ripe, i.e. M1, M2 and M3). Similar was the methodology in case of *B. cucurbitae* on pumpkin fruits. All fruits were bought from local farmer's field. All mangoes and pumpkin were used in experiments had been protected from wild flies by fruit bagging and had not been subjected to pesticide treatments.

Ten single fly replicates were conducted for each ripeness stage for each fruits. Fruit fly behavior observed and recorded were:

(i) Number of fly visit at hourly interval on a fruit from 06:00 to 16:00 hours.
(ii) Duration of fly visit per hour on a fruit from 06:00 to 16:00 hours.

The experiment was repeated in ten different days by using separate adult flies and conducted twice during two consecutive years 2014 and 2015. The observations obtained were analysed by following standard statistical methodology and presented in tabular as well as graphical form.

3.11. Impact of Entomopathogenic Nematodes (EPN) on dominant fruit flies:

For studying the efficacy of entomopathogenic nematode (EPN) the experiment was conducted in the laboratory of Department of Agricultural Entomology, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch behar, West Bengal, India. The experiment was conducted at 25 ± 2^0C and sandy loam soil (85% sand, 12% silt and 3% clay, pH 6.8) was used which has been autoclaved 2 weeks before use. All soil samples were heated at 80^0C for 24 hours to dry and to kill any predatory arthropods and soil borne pathogens. Only the infective juveniles of EPNs (*H. indica*) were utilised for the purpose. The *H. indica* was collected from the Department of Nematology, Tamil Nadu Agricultural Entomology (TNAU), Coimbatore, Tamil Nadu, India.

Third instar maggots of the fruit fly were taken for the study. This stage was easily differentiable since it has a "jumping" habit. Third instar maggots of fruit fly were extracted from the infested fruit which was brought from the field and poured into tray. Trays with dimension (length 46cm, breadth 30cm and depth 8cm) were taken and were filled with required amount of soil. The soil was maintained at different moisture content i.e. 5%, 10% 15% and 20%.

H. indica was extracted from foam in a petridish with water by keeping the foam for about 30 minutes and after that it was diluted in 10-20 ml of distilled water. Then 5ml of thoroughly stirred suspension containing IJs of *H. indica* was taken for counting the population to find out the average number of infective juvenile (IJs) per ml of suspension. The required amount of EPN was counted in counting disc by using nematode peaker. Different concentrations of IJs (of *H. indica*) were prepared viz. 50, 100, 150 and 250 IJs/cm^2 and added to petridishes. The soil was then treated with *H. indica* as extracted. Immediately after that fifty (50) numbers of full grown larvae were introduced in trays containing different concentrations of EPNs treated soil and trays were covered with mushlin cloth under laboratory condition. Each concentration was replicated thrice.

Control test (Untreated) was carried out at the same time in parallel with the treatments. Observations were recorded at an interval of 24hrs, 48hrs and 72hrs after treatment. Observations with regard to mortality of larvae, formation of deformed pupae and healthy adults were recorded.

The dead larvae were removed from the soil. Pupae were obtained by sieving the soi of trays. Infected pupae were turned into black colour and were treated as deformed. Percentage of the same were then determined accordingly.

However, the fresh uninfested numbers of pupae were collected in insect rearing cage for adult fly emergence and percent adult emergence withrespect to number of maggots released were also determined. Factorial design was followed to conduct the experiment. Factors were considered as follows:

a. Factor-I: Soil moisture:
   i. 5%
   ii. 10%,
   iii. 15%
   iv. 20%

b. Factor-II: Dosage of *H. indica* IJs:
   i. 250 IJs/cm^2
   ii. 150 IJs/cm^2
   iii. 100 IJs/cm^2
   iv. 50 IJs/cm^2

The experiment was conducted during summer and winter season in a year and continued for two consecutive years i.e. 2014 and 2015. The data obtained were processed, analysed by following standard statistical techniques, presented in both tabular and graphical form and interpreted accordingly.

CHAPTER-4

Results & Discussion

Chapter-4

RESULTS AND DISCUSSION

Species diversity of Tephritids under terai and hilly agro-ecological region of West Bengal have been determined after collecting sufficient quantities of specimens from different locations of northern parts of West Bengal from terai and hilly region. The locations are from the districts of Cooch Behar, Alipurduar, Jalpaiguri and Darjeeling. The agro-ecological regions have very good floral as well as faunal diversity. Several hosts of fruit flies are available here. A number of fruits and vegetables both cultivated as well as wild are found in this agro-ecological region. The findings are presented in tables and figures as follows.

4.1. Species diversity in samples collected by using traps:

For collecting tephritid specimens methyl eugenol, cuelure and bait traps were used. Adult fruit flies were also recovered from various infested fruits collected from different locations of the agro-ecological regions under consideration. Traps were installed at various locations of Cooch Behar, Alipurduar, Jalpaiguri and Darjeeling districts of northern West Bengal. Three traps of each category at each locations were installed at a time keeping at least 100 metre distance and specimens were collected after two days of installations and preserved as per standard protocol. Collections of similar category traps mixed divided by number of trap and identified one after another. Number of individuals of different categories were then tabulated and presented. The findings have also been depicted in figures.

4.1.1. Methyl eugenol trap collections:

By using methyl eugenol trap installed at four different locations of Himalayan and sub-Himalayan West Bengal four fruit fly species were recovered viz. *Bactrocera dorsalis* (Hendel), *Bactrocera zonata* (Saunders), *Bactrocera versicolor* (Bezzi) and *Bactrocera correcta* (Bezii) (Table-2). In Cooch Behar district highest catch was noted in case of *B. dorsalis* (77.28%) followed by *B. zonata* (12.32%) and *B. versicolor* (8.45%). Minimum catch was observed in *B. correcta* (1.95%). In Alipurduar district highest catch was noted in *B. dorsalis* (81.23%) followed by *B. zonata* (9.95%) and *B. versicolor* (8.30%). *B. correcta* was noted minimum (1.54%). However, in Jalpaiguri district relative abundance of *B. dorsalis, B. zonata, B. versicolor* and *B. correcta* were detected as 73.49%, 11.72%, 12.28% and 2.51% respectively. On the other hand, maximum catch was noted in *B. dorsalis* (82.96%) followed by *B. zonata* (10.95%) and *B. versicolor* (4.86%) and *B. correcta* (1.42).

Table-2: Diversity of fruit flies under terai districts of West Bengal.

District	Trap	Total Number of individuals	Species	Total number of individuals per species	Relative abundance
Cooch Behar	Methyl Eugenol	431	*B. dorsalis*	333	77.28
			B. zonata	53	12.32
			B. versicolor	36	8.45
			B. correcta	8	1.95
	Cuelure	362	*B. cucurbitae*	250	70.40
			B. diversa	55	15.20
			B. caudata	44	12.25
			B. tau	7	2.00
			B. nigrotibialis	4	0.01
			D. longicornis	1	0.003
Alipurduar	Methyl Eugenol	564	*B. dorsalis*	458	81.23
			B. zonata	56	9.95
			B. versicolor	49	8.30
			B. correcta	9	1.54
	Cuelure	395	*B. cucurbitae*	280	71.28
			B. diversa	77	19.90
			B. caudata	69	18.34
			B. tau	6	1.50
			B. nigrotibialis	4	0.01
			D. longicornis	2	0.005
Jalpaiguri	Methyl Eugenol	641	*B. dorsalis*	471	73.49
			B. zonata	75	11.72
			B. versicolor	79	12.28
			B. correcta	16	2.51
	Cuelure	510	*B. cucurbitae*	391	77.40
			B. diversa	60	11.81
			B. caudata	43	8.37
			B. tau	12	2.42
			B. nigrotibialis	3	0.005
			D. longicornis	1	0.002

Table-3: Diversity of fruit flies under hilly district of West Bengal.

District	Trap	Total Number of individuals	Species	Total number of individuals per species	Relative abundance
Darjeeling (Kalimpong)	Methyl Eugenol	493	*B. dorsalis*	409	82.96
			B. zonata	54	10.95
			B. versicolor	24	4.86
			B. correcta	7	1.42
	Cuelure	544	*B. cucurbitae*	428	78.67
			B. diversa	65	11.94
			B. caudata	33	6.07
			B. tau	11	2.02
			B. nigrotibialis	5	0.92
			D. longicornis	2	0.37

4.1.2. Cuelure trap collections:

By using cuelure trap installed at four different locations of sub-Himalayan West Bengal six fruit fly species were recovered viz., *Bactrocera cucurbitae* (Coq.), *Bactrocera diversa* (Coq.), *Bactrocera caudata* (Fab.), *Bactrocera tau* (Walker), *Bactrocera nigrotibialis* (Perkins) and *Dacus longicornis* (Wiedmann) (Table-3). Among these six species the most dominant species recorded was *B. cucurbitae* (70.40%) in Cooch Behar district. Relative abundance of *B. diversa*, *B. caudata*, *B tau*, *B. nigrotibialis* and *D. longicornis* were 15.20%, 12.25%, 2.00%, 0.01% and 0.003% respectively. In Alipurduar, Jalpaiguri and Darjeeling districts also the most dominant species was *B. cucurbitae* (71.28%, 77.40% and 78.67% respectively). Least abundance of the fly were noted in case of *B. nigrotibialis* and *D. longicornis* (0.01%, 0.005%; 0.005, 0.002 and 0.92%, 0.37% in Alipuduar, Jalpaiguri and Darjeeling district respectively).

Fig-2: Species composition of fruit flies attracted in methyl eugenol.

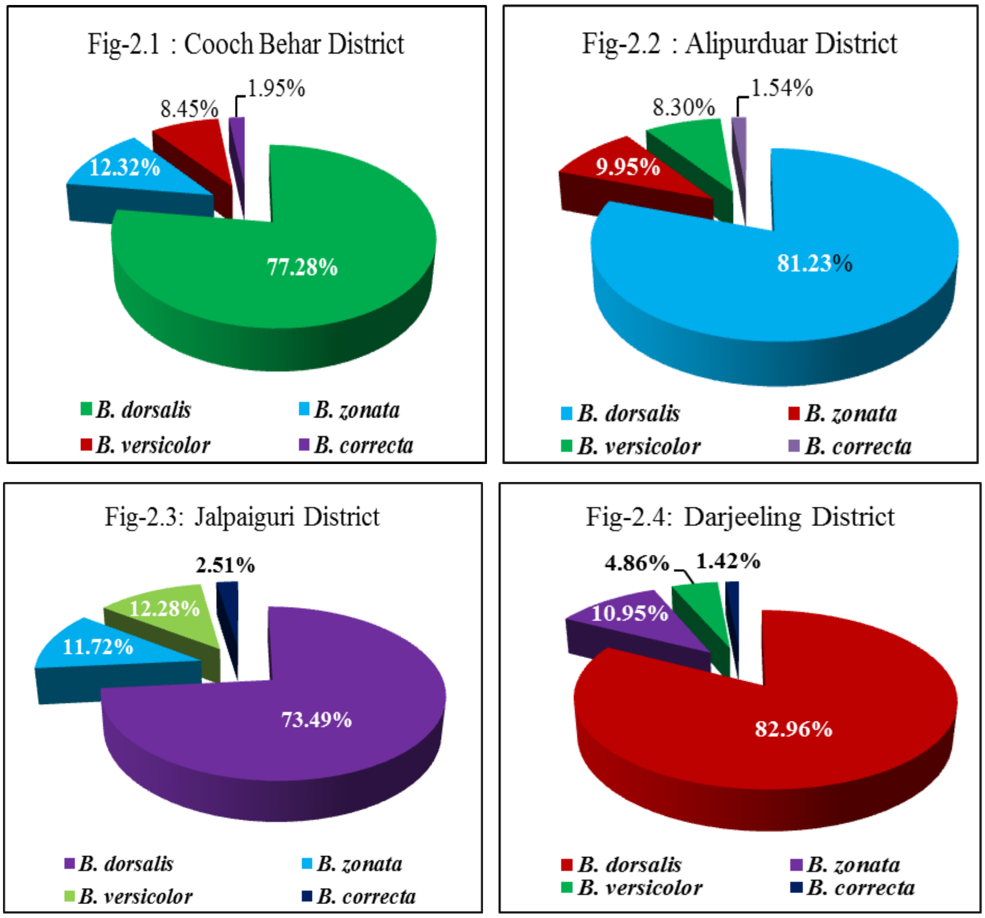
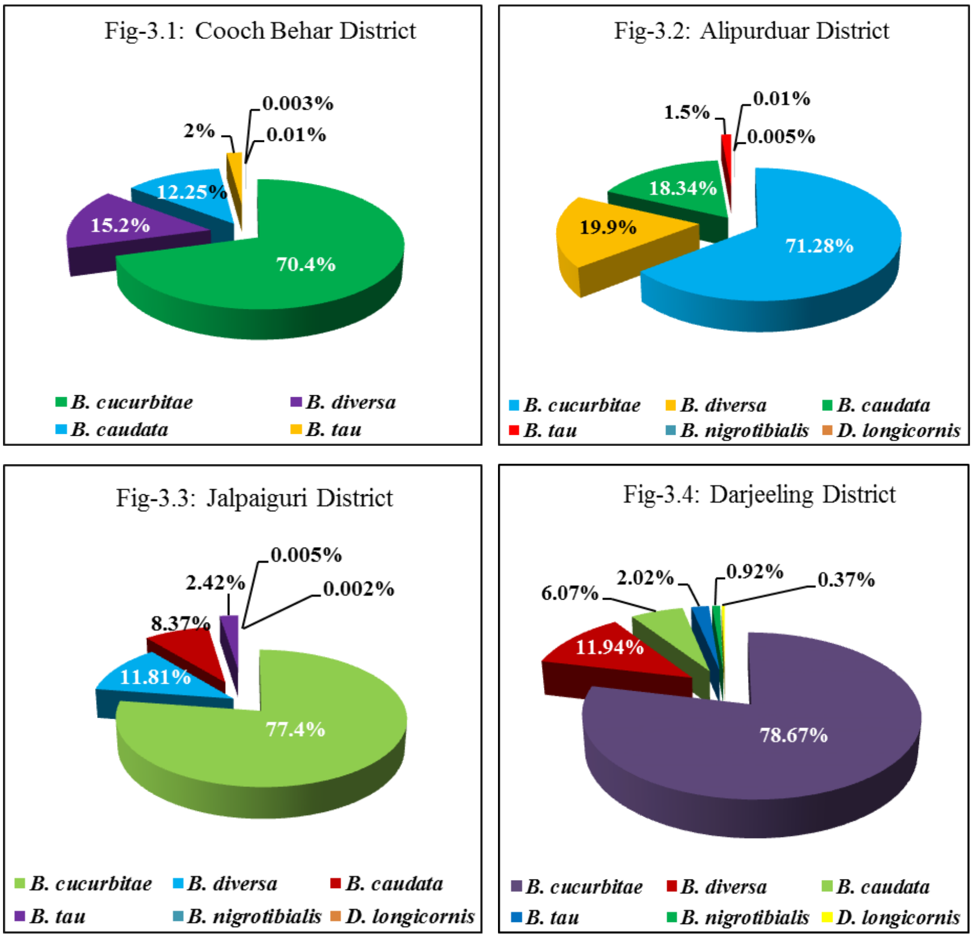


Fig-3: Species composition of fruit flies attracted in cuelure.



Thus, it appeared that a good number of tephritid flies have been found to exist in Himalayan and sub-Himalayan West Bengal. Altogether ten tephritid fruit fly species have been detected in northern tract of West Bengal by using Methyl eugenol and Cuelure trap. In all the instances Methyl eugenol collection were dominated by *B. dorsalis* whereas Cuelure collections were dominated by *B. cucurbitae.* Very meagre presence were noted in case of *B. correcta*, *B. nigrotibialis* and *D. longicornis* in these sex attractant collections.

4.1.3. Species diversity in samples collected by using food bait traps:

Like methyl eugenol and cuelure traps, food bait traps were installed at various locations of Cooch Behar, Alipurduar, Jalpaiguri and Darjeeling districts of northern West Bengal. Three traps of each category at each locations were installed at a time keeping at least 100 metre distance and specimens were collected after two days of installation and preserved as per standard protocol. Collections of similar category traps mixed and divided by number of trap and identified one after another.

The food bait used in the present study were Banana pulp + sugar (1:1) + 5% ammonium acetate, Jackfruit pulp + sugar (1:1) + 5% ammonium acetate. Number of individuals of different categories were then tabulated and presented. The findings have also been depicted in figure (Fig-4).

From food bait collection using banana pulp + sugar (1:1) + 5% ammonium acetate, the species detected and identified were *B. cucurbitae, B. dorsalis, B. correcta, B. nigrotibialis and B. minax*. Relative abundance of the flies were 45%, 35%, 10%, 5% and 5% respectively (Table-4). However, the collections were differed from district to district which may be due to difference in floral biodiversity of the locations selected. In Cooch Behar district the specimens identified were *B. cucurbitae, B. dorsalis, B. correcta,* and *B. nigrotibialis*. The dominant species was noted *B. cucurbitae* (47.61%) followed by *B. dorsalis* (38.09%), *B. correcta* (9.52%), *B. nigrotibialis* (4.76%). In Alipurduar district the relative abundance of the species identified were 41.67%, 50.00%, 8.33% in case of *B. cucurbitae, B. dorsalis* and *B. correcta* respectively. *B. nigrotibialis* was not captured by using banana as food bait trap.

In Jalpaiguri district also only three species were noted by using banana pulp food bait viz., *B. cucurbitae* (40.00%) followed by *B. dorsalis* (50.00%), *B. correcta* (10.00%). However, in Darjeeling district five species of fruit flies were captured by using banana as food bait trap. The species detected and identified were *B. cucurbitae, B. dorsalis*, *B. correcta*, *B. nigrotibialis* and *B. minax.* Here also the dominant species was *B. cucurbitae* (45.00%). It has also been noted that *B. minax* was only detected in the hills of Darjeeling district but not from the plains of Cooch Behar, Alipurduar and Jalpaiguri districts.

Table-4: Fruit fly species collected by using different food baits under terai West Bengal.

District	Food bait	Total number of individuals	Species	Total number of individuals per species	Relative abundance
Cooch Behar	Banana pulp + sugar (1:1) + 5% ammonium acetate	21	*B. cucurbitae*	10	47.61
			B. dorsalis	8	38.09
			B. correcta	2	9.52
			B. nigrotibialis	1	4.76
	Jack fruit sugar (1:1) + 5% ammonium acetate	17	*B. cucurbitae*	8	47.06
			B. dorsalis	7	41.18
			B. correcta	2	11.76
			B. nigrotibialis	0	0.00
Alipurduar	Banana pulp + sugar (1:1) + 5% ammonium acetate	12	*B. cucurbitae*	5	41.67
			B. dorsalis	6	50.00
			B. correcta	1	8.33
			B. nigrotibialis	0	0.00
	Jack fruit+sugar (1:1) + 5% ammonium acetate	26	*B. cucurbitae*	13	50.00
			B. dorsalis	10	38.46
			B. correcta	2	7.69
			B. nigrotibialis	1	3.85
Jalpaiguri	Banana pulp + sugar (1:1) + 5% ammonium acetate	10	*B. cucurbitae*	4	40.00
			B. dorsalis	5	50.00
			B. correcta	1	10.00
			B. nigrotibialis	0	0.00
	Jack fruit+sugar (1:1) + 5% ammonium acetate	31	*B. cucurbitae*	15	48.39
			B. dorsalis	12	38.71
			B. correcta	3	9.68
			B. nigrotibialis	1	3.23
Darjeeling	Banana pulp + sugar (1:1) + 5% ammonium acetate	20	*B. cucurbitae*	9	45.00
			B. dorsalis	7	35.00
			B. correcta	2	10.00
			B. nigrotibialis	1	5.00
			B. minax	1	5.00
	Jack fruit+sugar (1:1) + 5% ammonium acetate	27	*B. cucurbitae*	11	40.74
			B. dorsalis	14	51.85
			B. correcta	1	3.70
			B. minax	1	3.70

By using Jackfruit pulp + sugar (1:1) as food bait trap altogether five species of tephritid fruit flies have been captured. In Cooch Behar district three species were detected, viz. *B. cucurbitae, B. dorsalis* and *B. correcta*. Relative abundance of the flies were noted as 47.06%, 41.18% and 11.76% respectively. In Alipurduar district four species were detected. They were *B. cucurbitae* (50.00%), *B. dorsalis* (38.46%), *B. correcta* (7.69%) and *B. nigrotibialis* (3.85%). In Jalpaiguri district also these four species have been detected in varying intensity. But in Darjeeling district an additional species i.e., *B. minax* was detected although in meagre abundance (3.07%).

Here, in case of food bait trap, *B. dorsalis*, *B. correcta*, and *B. nigrotibialis* were detected in the plains of Northern West Bengal (Cooch Behar, Alipurduar and Jalpaiguri) but in hilly Darjeeling area *B. minax* was noted in addition to the aforesaid species. This additional detection of *B. minax* may be due to regional floral diversity of the survey location. In Darjeeling hills mandarin orange is an important fruit crop and *B. minax* is the pest of Rutacae family crops like mandarin orange which is also known as Chinese citrus fly.

Fig-4: Species composition of fruit flies attracted in food bait trap (Banana pulp+sugar).

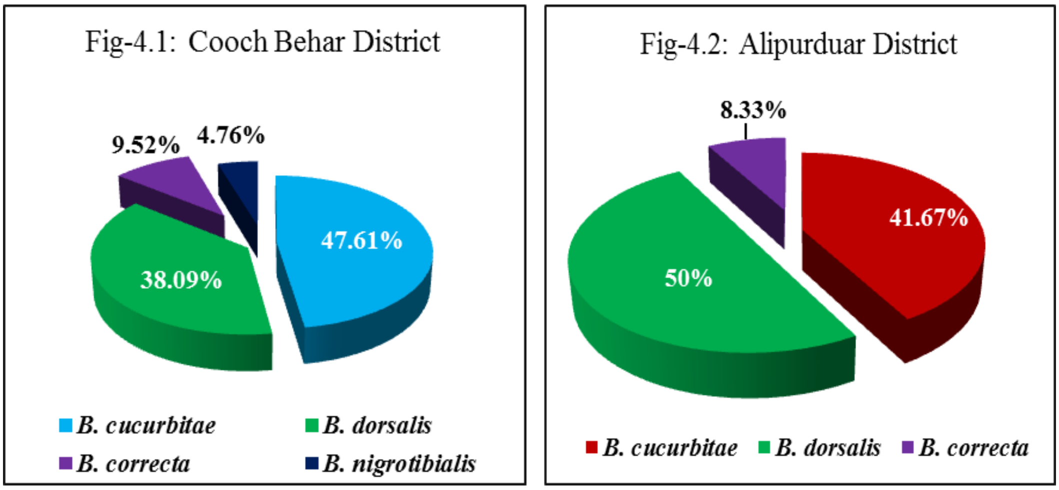


Fig-4.3: Jalpaiguri District

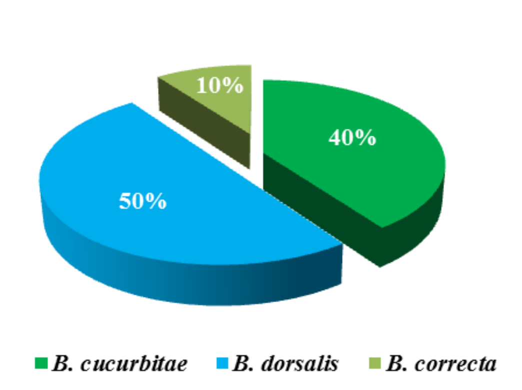
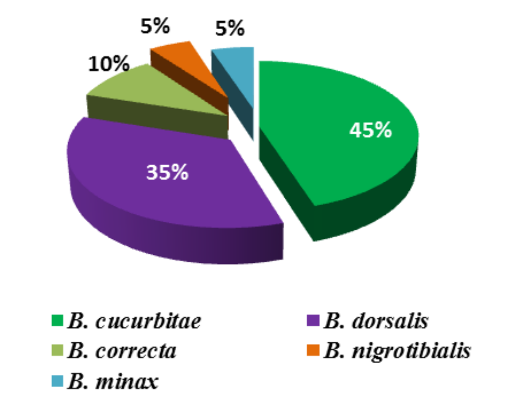


Fig-4.4: Darjeeling District



5.0. Species composition of fruit flies collected by food bait lure (Jack fruit pulp + sugar).

Fig-5.1: Cooch Behar District

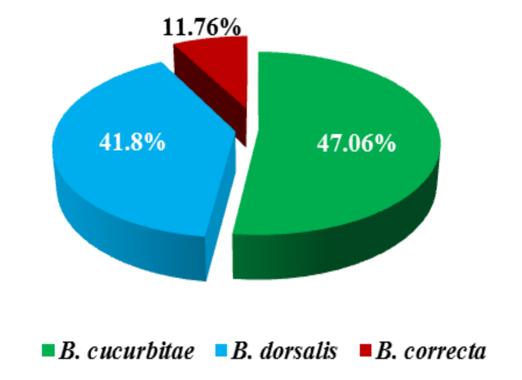


Fig-5.2: Alipurduar District

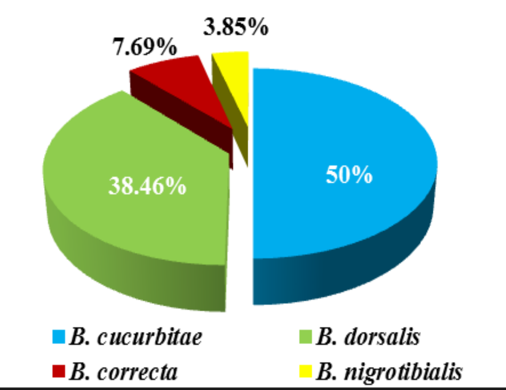


Fig-5.3: Jalpaiguri District

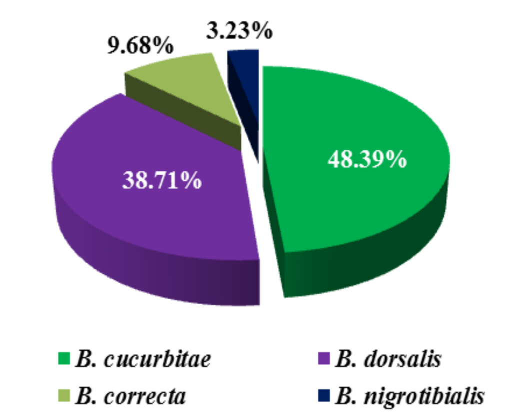
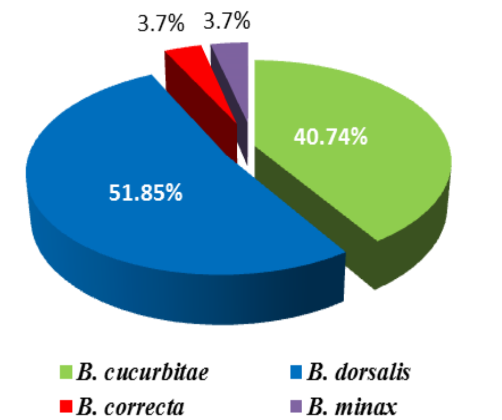


Fig-5.4: Darjeeling District



4.1.4. Species diversity in samples recovered from infested fruits:

The infested as well as fallen fruits of mango, guava, citrus, pumpkin, bottle gourd and bitter gourd were examined for the emergence of flies. Fifty number of each fruits collected from northern districts of West Bengal were brought to the laboratory of Department of Agricultural Entomology, Uttar Banga Krishi Viswavidyalaya and kept in rearing cages, provided with a bed layer having mixture of sand and cocopit (1:1) ratio was prepared at the bottom of cage to facilitate the pupation. Then infested fruits were kept on the prepared layer in rearing cages for emergence of fruit flies. After 12 - 13 days the fruit fly emergence was started and on first day 55 - 60 % flies were emerged while the rest of the flies were emerged on second day. The fruit flies were handled carefully and care was taken to prevent escaping from the rearing cages.

The emerged fruit flies were provided food by hanging cotton swab soaked in 10% honey solution for a week. The flies were allowed for sclerotization and development of body colour. The recovered flies were killed by using chloroform in killing jar. The flies emerged from the infested fruits were examined under microscope and were got identified with the help of taxonomical keys.

4.1.4.1. Species diversity in fruit fly species recovered from infested mango, guava and mandarin orange:

Data recorded on number of flies emerged from infested fruits from cages in the laboratory have been presented in Table-5. It is revealed from the presentation that from infested mango fruits three fruit fly species were recovered viz. *B. dorsalis, B. zonata and B. correcta.* Among these three species most dominant was *B. dorsalis*. Relative abundance of the *B. dorsalis* was noted as 70.73%, 70.27% and 68.75% in infested mango collected from Cooch Behar, Alipurduar and Jalpaiguri district respectively. The second dominant was *B. zonata* that showed 26.83%, 27.03% and 25.00% relative abundance in infested mango fruits collected from Cooch Behar, Alipurduar and Jalpaiguri respectively. Least dominant was the *B. correcta* in all the cases of recovery.

It appeared that the guava fruits were infested with four different fruit fly species, viz. *B. dorsalis, B. zonata, B. correcta.* and *B. versicolor*. Among them *B. dorsalis* was observed to be dominant species with highest mean percentage of flies emerged out per cage (47.82%, 52.63% and 52.38% in Cooch Behar, Alipurduar and Jalpaiguri district respectively).

Table-5: Species diversity of fruit flies recovered from infested fruits of mango, guava and citrus from northern part of West Bengal.

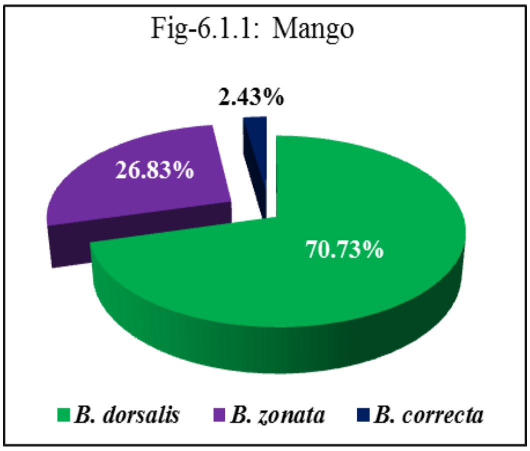
District	**Reared from infested fruits (10 fruits each)**	**Total number of individuals recovered**	**Species observed**	**Number of individual species**	**Relative abundance of the species**
Cooch Behar	Mango	41	*B. dorsalis*	29	70.73
			B. zonata	11	26.83
			B. correcta	1	2.43
	Guava	23	*B. dorsalis*	11	47.82
			B. correcta	10	43.47
			B. zonata	1	4.34
			B.versicolor	1	4.34
Alipurduar	Mango	37	*B. dorsalis*	26	70.27
			B. zonata	10	27.03
			B. correcta	1	2.70
	Guava	19	*B. dorsalis*	10	52.63
			B. correcta	8	42.11
			B. zonata	1	5.26
			B.versicolor	0	0.00
Jalpaiguri	Mango	32	*B. dorsalis*	22	68.75
			B. zonata	8	25.00
			B. correcta	2	6.25
	Guava	21	*B. dorsalis*	11	52.38
			B. correcta	9	42.85
			B. zonata	1	4.76
			B.versicolor	0	0.00
Darjeeling	Citrus	65	*B. minax*	61	93.84
			B. dorsalis	4	6.15

B. correcta was found next dominant species recorded (43.47%, 42.11% and 42.85% in Cooch Behar, Alipurduar and Jalpaiguri district respectively) followed by *B. zonata* (4.34%, 5.26% and 4.76% in Cooch Behar, Alipurduar and Jalpaiguri district respectively) (Table-5). The findings have also been depicted graphically in Fig-6.0.

In Darjeeling district infested mandarin orange was taken to recover fruit flies which is one of the most important fruit crops of northern hills of West Bengal. From fruit fly infested mandarin orange collected from Darjeeling district only two species viz. *B. minax* and *B. dorsalis* were recovered. Relative abundance of the flies were 93.84% and 6.15% respectively. Findings revealed that the most dominant species that attack darjeeling mandarin is the *B. minax.*

Fig-6.0 : Species diversity of fruit flies recovered from infested fruits of mango, guava and citrus from northern part of West Bengal.

Fig-6.1. District: Cooch Behar



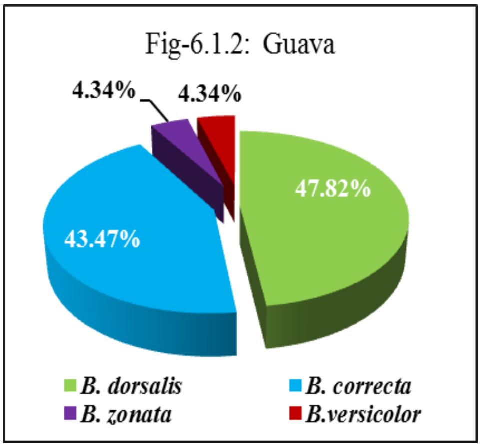
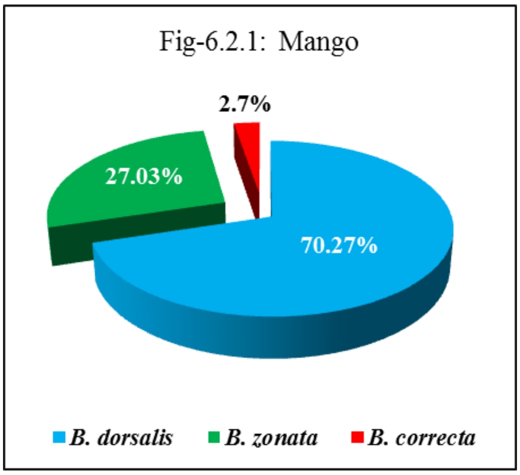


Fig-6.2: District: Alipurduar



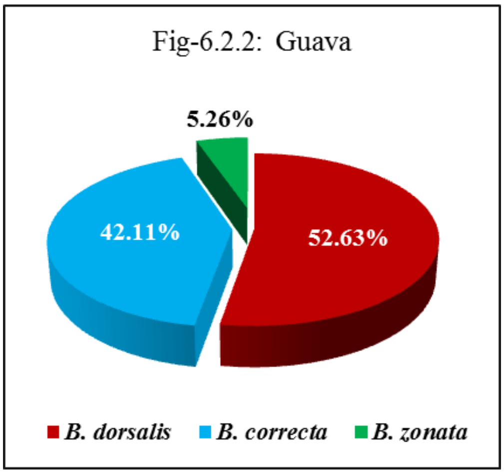
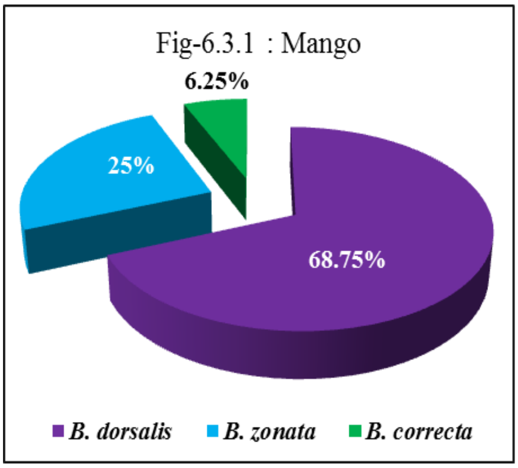


Fig-6.3: District: Jalpaiguri



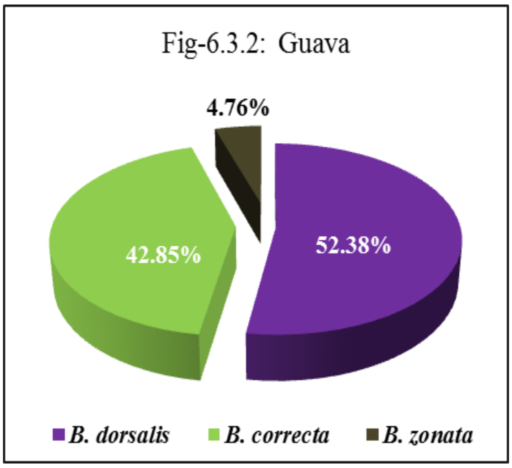
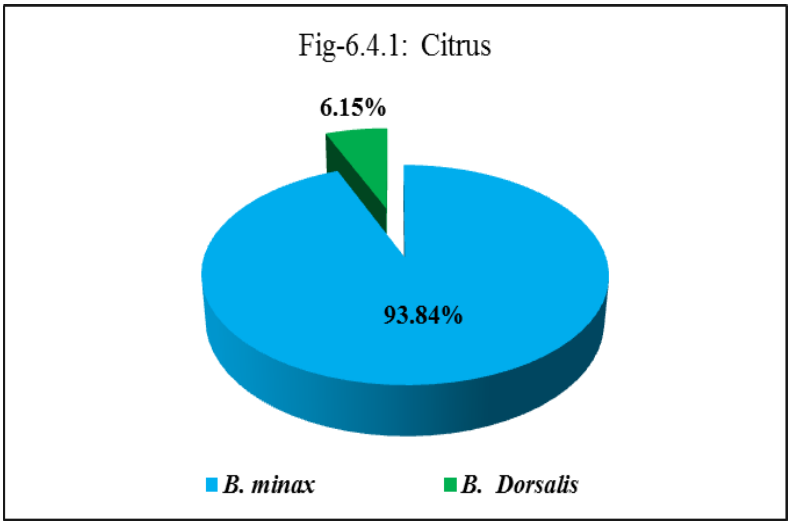


Fig-6.4: District: Darjeeling



4.1.4.2. Species diversity in fruit fly species recovered from fruits of pumpkin, bittergourd and bottle gourd:

Fruit fly infested fruits of pumpkin, bitter gourd and bottle gourd were collected from Cooch Behar, Alipurduar and Jalpaiguri districts and the adults were recovered after rearing in the laboratory by following standard technique. After identification of the recovered adults the specimens were categorized species wise for respective infested fruits. In each cages the number of adults emerged were converted into number of adults per kg infested fruits and the observations have been presented in Table-6.

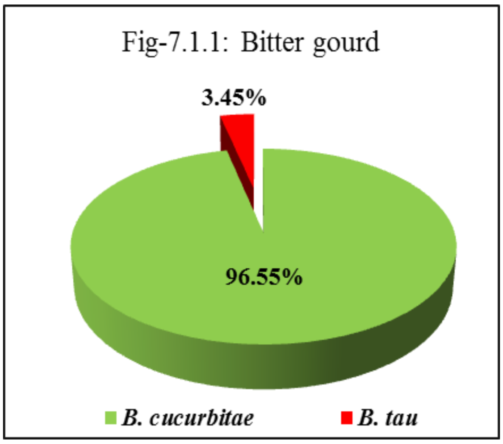
It appeared from the presentation that two species have been detected (*B. cucurbitae* and *B. tau*) from the infested pumpkin, bitter gourd and bottle gourd in each districts. The dominant species detected from these infested cucurbits is *B.*

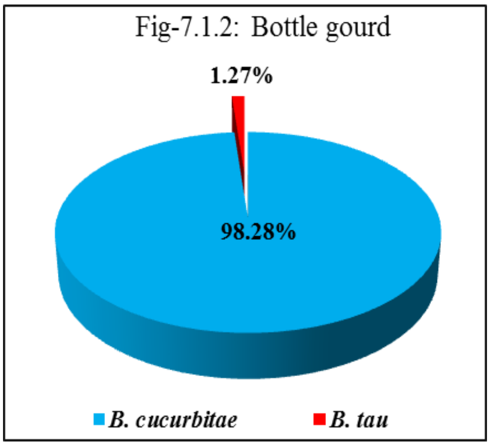
cucurbitae. Relative abundance of the *B. cucurbitae* was noted 95.38, 96.55 and 98.28% from pumkin, bitter gound and bottle gourd respectively in the samples collected from Cooch Behar district. Abundance of the *B. cucurbitae* was more or less similar in the infested cucurbitaceous fruits collected from Alipurduar as well as Jalpaiguri districts.

The least abundant fruit fly was *B. tau*. Relative abundance of *B. tau* was varied from 1.47-4.62% irrespective of infested fruits and place of collection. Maximum (4.62%) number of *B. tau* was recovered from infested pumpkin collected from Cooch Behar district and minimum (1.47%) was recovered from infested bitter gourd collected from Alipurduar district (Table-6).

Fig-7: Species diversity of fruit flies recovered from infested fruits of pumpkin, bittergourd and bottle gourd from northern part of West Bengal.

Fig-7.1: District: Cooch Behar





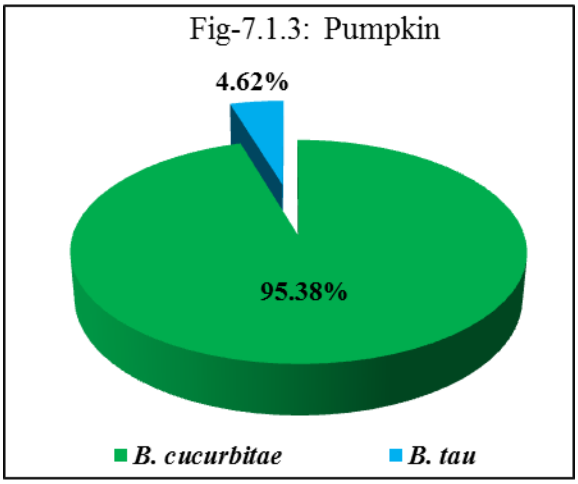
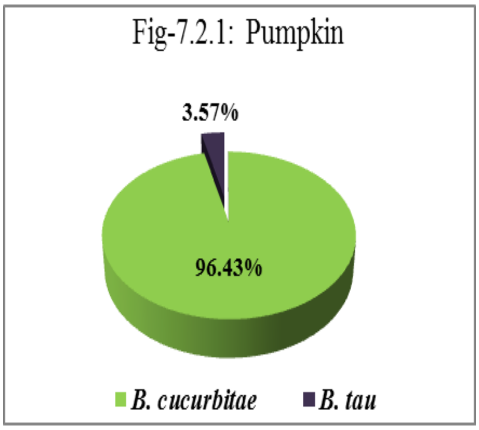
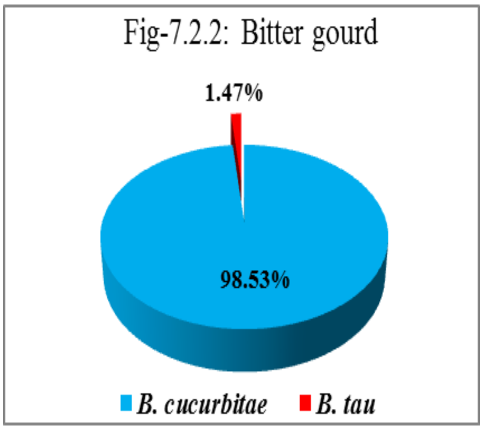


Fig-7.2: District: Alipurduar





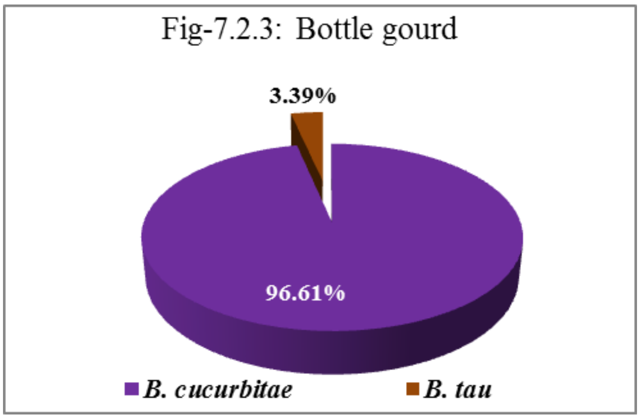
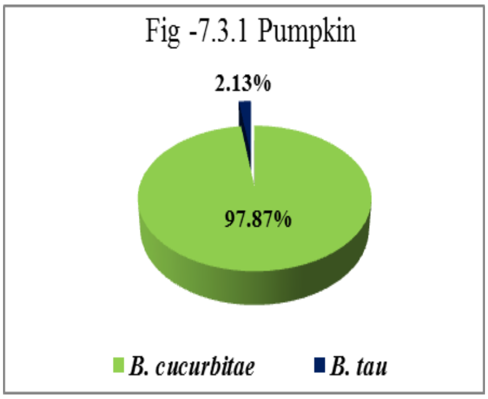
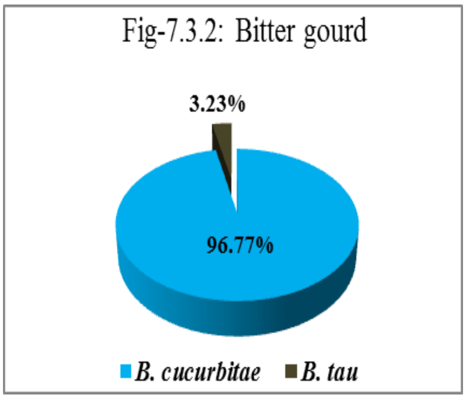


Fig-7.3: District: Jalpaiguri





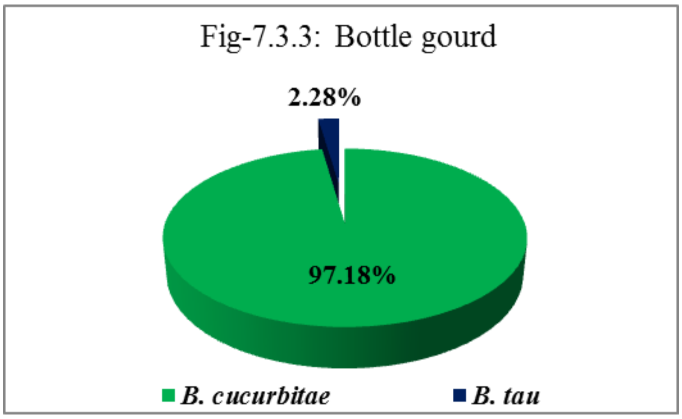


Table-6: Species diversity of fruit flies recovered from infested fruits of pumpkin, bittergourd and bottle gourd from northern part of West Bengal.

Districts	Recovered from infested fruits of	Total number of individual recovered (From 1 kg fruit)	Species	Total number of individual species	Relative abundance
Cooch Behar	Pumpkin	65	*B. cucurbitae*	62	95.38
			B. tau	3	4.62
	Bitter gourd	87	*B. cucurbitae*	84	96.55
			B. tau	3	3.45
	Bottle gourd	58	*B. cucurbitae*	57	98.28
			B. tau	1	1.72
Alipurduar	Pumpkin	56	*B. cucurbitae*	54	96.43
			B. tau	2	3.57
	Bitter gourd	68	*B. cucurbitae*	67	98.53
			B. tau	1	1.47
	Bottle gourd	59	*B. cucurbitae*	57	96.61
			B. tau	2	3.39
Jalpaiguri	Pumpkin	47	*B. cucurbitae*	46	97.87
			B. tau	1	2.13
	Bitter gourd	62	*B. cucurbitae*	60	96.77
			B. tau	2	3.23
	Bottle gourd	71	*B. cucurbitae*	69	97.18
			B. tau	2	2.82

* No. of adults emerged from 1 kg infested fruits, average of 3 rearings each year.

Authentic identification of pest species and information thereof are essential for any pest management programme. Management of fruit fly is not an exception. Several workers documented varied number of fruit fly species from all over the world as well as Indian sub-continent. Bezzi (1913) listed 39 species from India. Forty three species of fruit flies have been described under the genus *Bactrocera* from Asia, Africa, and Australia by several authors (Syed, 1969, Drew and Hooper, 1983; Munro, 1984 and Fletcher, 1987).

Kapoor (2005) reported 325 species of fruit flies occurring in the Indian subcontinent, of which 205 are from India. The author noted major pest species belong to the genus *Bactrocera* as follows: *B. cucurbitae, B.dorsalis* and *B. zonata,* while other species, such as *B. correcta, B. diversa* and *B. latifrons,* are still localized in distribution. *B. nigrofemoralis* White and Tsuruta (known as *B. nigrotibialis* in the Indian literature) was reported from south India on *Coffea canephoria* (Narayanan and Batra, 1960). It is known from South India and Sri Lanka and the only confirmed host is *Terminalia catappa* (Tsuruta and White, 2001). *B. (Tetradacus) minax* (Enderlein) is also known as *Callantra minax* and *Polistomimetes minax* (in the Indian literature) or Chinese citrus fly. It is a pest of citrus and has already been recorded from northern India as causing heavy damage to tangerine (*Citrus reticulata*).

In Indian sub-continent, the knowledge of family Tephritidae has been based largely upon the monumental monograph of Bezzi published in 1913 (Kapoor *et al.*, 1980). Agarwal and Sueyoshi (2005) noted 243 known species out of which 79 genera were from India, 41 species of 27 genera have been reported from Himachal Pradesh. Bhatia and Gupta (2003) conducted a survey during 1991-99 in six districts of Himachal Pradesh, India and reported that fruit fly (*Bactrocera cucurbitae*) on cucurbits was the most serious pests in different parts of the state. Kapoor (2004) stated that two hundred species of fruit flies were known from India. Only 35 to 40 species were so far associated directly or indirectly with their host plants. Recently, six fruit fly species were reported for the first time from Himachal Pradesh by Prabhakar *et al.* (2012). Out of 47 species of fruit flies reported from Himachal Pradesh, 13 species belong to genus *Bactrocera* and *Dacus* and majority of them are economically important pests of agricultural and horticultural crops of several countries including India.

A pictorial key for 13 species of fruit flies under 2 (two) genera viz., *Bactrocera* and *Dacus* of sub-family Dacinae (Diptera: Tephritidae) have been documented based on actual photographs of fruit flies collected from north western Himalaya of India during 2009-2010. Ukey *et al.* (2013) found different species of fruit flies such as, *B. zonata, B. dorsalis, B. correcta* and *B. verbascifoliae* infest guava in Ahmednagar District, Maharastra, India. Bhagat (2014) found a total of 48 species of fruit fly, belonging to 21genera from Jammu and Kashmir, India. These species belonging to two separate families, viz. Drosophilidae (Sub -family Drosophilinae), Tephritidae (Sub-family Dacinae, Tephritinae and Trypetinae). However, from the nearby Ladakh region, so far only two species of Tephritid are known (Chandra and Kaur, 2009).

Ukey *et al.* (2013) revealed that different species of fruit flies viz., *B. zonata, B. dorsalis, B. correcta* and *B. verbascifoliae* found to infest guava in Ahmednagar District of Maharastra, India. Among the four species, *B. dorsalis* was observed to be dominant species with highest mean number of flies 50.25 (49.95%) emerged out per cage. *B. zonata* was found next dominant species recorded 38.5 flies (31.36%) emerged out per cage. *B. correcta* recorded 24.5 flies (19.95%) emerged out per cage. Very low infestation of *B. verbascifoliae* found during that investigation which was recorded 9.5 flies (7.73%) per cage.

The observations of the present investigation are also in corroboration with Kawashita *et al.* (2004). However, the authors not detected *D. longicornis* (Weidmann) by using Cuelure as sex attractant. Laskar *et al.* (2016) also reported the presence of nine tephritid flies from sub-Himalayan region of West Bengal which is in agreement with the present findings.

4.2. Intensity of infestation by fruit fly on some selected fruits and vegetables:

Fruit fly is one of the most important biotic stresses of several fruit and vegetable crops under northern parts of West Bengal. Intensity of fruit fly infestation on important fruit crops like mango, guava, citrus and vegetables like bitter gourd, ridge gourd and pointed gourd were determined through survey on farmers field. The fruits were collected from different location of Cooch Behar, Alipurduar, Jalpaiguri and plains of Darjeeling district. From each locations altogether one hundred (100) fruits were collected randomly at three times i.e. early, mid and late stage of the crop during 2014 and 2015. These fruits were kept in laboratory for ripening in rearing cages under observation. The ripen fruits were cut and critically examined for the presence of fruit fly maggots under magnifying lens. The fruits with maggots of fruit fly were treated as infested fruits and without maggots were treated as healthy fruits. The observations on numbers of healthy and infested fruits were recorded and then percent infestation due to fruit fly was determined.

4.2.1. Intensity of infestation by fruit flies (*Bactrocera* spp.) on Mango (*Mangifera indica* Linn.):

Observation on fruit fly infestation in mango orchards from different districts under northern part of West Bengal has been presented in table-7. It appears from the presentation that there exists significant variation in intensity of fruit fly infestation on mango during both the years of study (2014 and 2015) at different locations. Higher fruit infestation was recorded in 2014 as compared to 2015. During 2014, results showed that highest fruit infestation on mango (16.33%) by fruit flies was recorded from Chilkir hat ($26^0$53′N, $89^0$19′E) and lowest (7.33%) infestation was noted from Matigara ($26^0$72′N, $88^0$38′E). However, in the next year i.e. during 2015, the fruit infestation was recorded higher (15.00%) from Naksalbari ($26^0$70′N, $88^0$42′E) and lower (7.33%) was recorded from Pundibari ($26^0$41′N, $89^0$38′E). Pooled mean of both the years of study revealed highest average infested fruit (13.67%) at Chilkir hat localities while lowest (7.83%) at Pundibari on number basis.

The information on fruit infestation by fruit flies from different localities under northern tract of West Bengal was subjected to cluster analysis and results have been presented in table-8 and 9. In table the statistics like mean, variance, minimum value, maximum value are mentioned and the places or location which are more or less approximately similar based on have been categorised in respective clusters.

Significant differences have been detected among the clusters by performing Levens test of analysis.

Table-7: Percent fruit infestation (by number) by fruit flies on Mango during 2014 and 2015 in different parts of northern West Bengal.

Locations	2014	2015	Pooled over (2014-2015)
Chilkirhat	16.33 (4.04)	11.00 (3.32)	13.67 (3.70)
Morangabari	13.33 (3.65)	9.33 (3.05)	11.33 (3.37)
Posarir Hat	8.67 (2.94)	12.00 (3.46)	10.33 (3.21)
Dinhata	13.00 (3.61)	8.67 (2.94)	10.83 (3.29)
Vetaguri	15.33 (3.92)	8.00 (2.83)	11.67 (3.42)
Maruganj	11.33 (3.37)	13.00 (3.61)	12.17 (3.49)
Pundibari	8.33 (2.89)	7.33 (2.71)	7.83 (2.80)
Horir Hat	8.67 (2.94)	9.67 (3.11)	9.17 (3.03)
Sajerpar	11.67 (3.42)	7.67 (2.77)	9.67 (3.11)
Sonapur	11.00 (3.32)	14.00 (3.74)	12.50 (3.54)
Falakata	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)
Gadong-1	9.67 (3.11)	13.00 (3.61)	11.33 (3.37)
Gadong-2	8.00 (2.83)	8.67 (2.94)	8.33 (2.89)
Moinaguri	8.33 (2.89)	12.00 (3.46)	10.17 (3.19)
Dhupguri	8.33 (2.89)	12.00 (3.46)	10.17 (3.19)
Chandmoni	8.67 (2.94)	12.67 (3.56)	10.67 (3.27)
Naksalbari	9.33 (3.05)	15.00 (3.87)	12.17 (3.49)
Matigara	7.33 (2.71)	10.00 (3.16)	8.67 (2.94)
Kharibari	8.00 (2.83)	9.00 (3.00)	8.50 (2.92)
Phasidewa	11.67 (3.42)	12.00 (3.46)	11.83 (3.44)

* Figures within parenthesis are square root transfomed values.

During 2014, mango fruit infestation by fruit flies was significantly higher (mean value = 13.23%) in CL1 (Cluster 1) and range varied from 11.33 - 16.33% on number basis at Sonapur, Maruganj, Phasidewa, Sajerpar, Dinhata, Morangabari, Vetaguri and Chilkirhat, while lowest fruit infestation (mean value = 8.63%) was noted in CL2 (Cluster 2) and range varied from 8.00-9.66% on number basis at

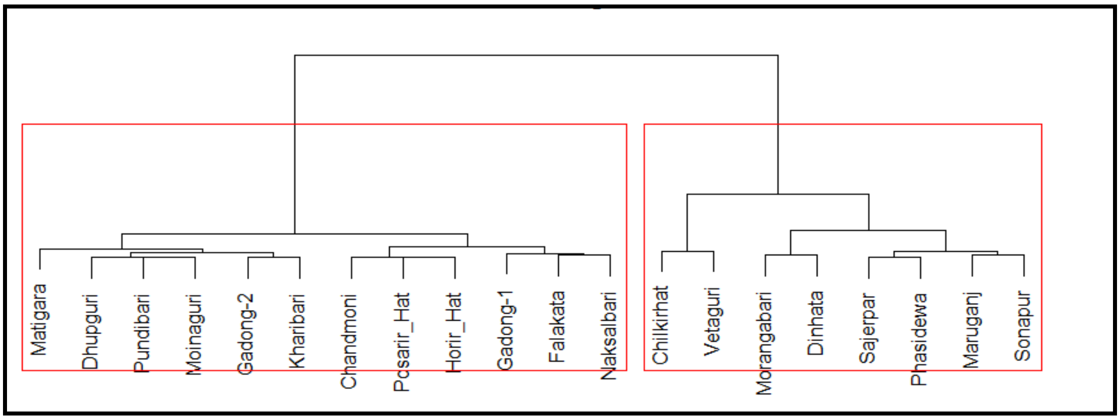
Matigara, Gadong2, Kharibari, Dhupguri, Moinaguri, Pundibari, Chandmoni, Horir Hat, Gadong-1, Posarir Hat, Falakata and Naksalbari.

Table-8: Cluster analysis of fruit fly infestation on mango in different locations during 2014.

Cluster no.	Percent fruit infestation				Name of the locations
	Mean	Variance	Min. value	Max. value	
CL1	13.23	3.855	11.33	16.33	Sonapur, Maruganj, Phasidewa, Sajerpar, Dinhata,Morangabari, Vetaguri,Chilkirhat
CL2	8.63	0.393	8	9.66	Matigara, Gadong2, Kharibari, Dhupguri, Moinaguri, Pundibari, Chandmoni, Horir Hat, Gadong-1, Posarir Hat, Falakata, Naksalbari
Levens test Statistic	9.63				
p-value	0.006				

*CL- Cluster.

Fig-8: Cluster dendogram on intensity of fruit fly infestation on Mango at different locations during 2014.



During 2015, mango fruit infestation by fruit fly was significantly higher in CL6 (15.00%) followed by CL5 (13.00%) and CL3 (12.00%) on number basis. Highest fruit infestation (15.00%) on mango was observed at Sonapur (26^0 46′N, 89^0 39′E) and Naksalbari (26^0 70′N, 88^0 42′E) and the lowest (7.83%) was recorded in

CL4 and range varied from 7.66-8.00% at different location i.e. Pundibari, Sajerpar and Vetaguri.

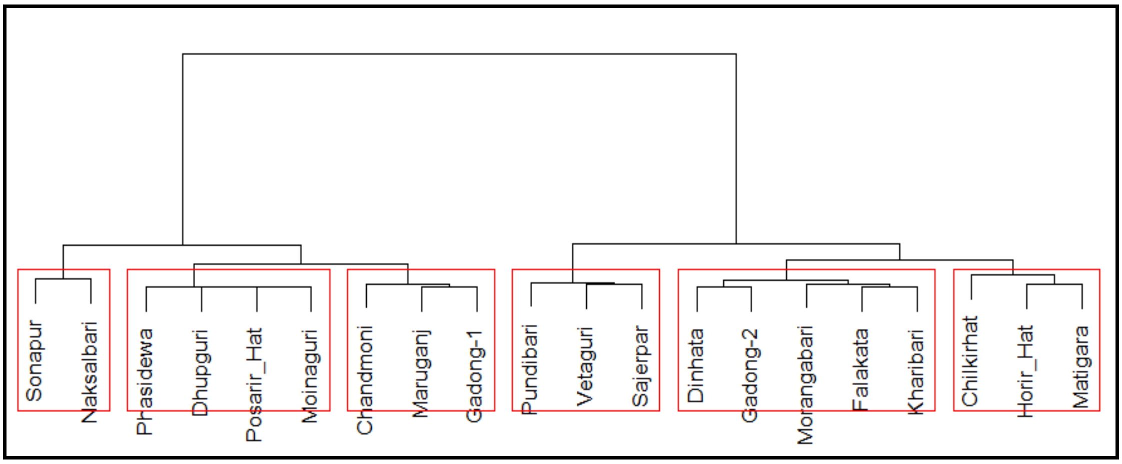
Earlier also several workers worked on fruit flies on mango across the mango growing countries of the world including India. In India, Verghese *et al.* (2002b) reported *Bactrocera dorsalis* (Hendel) as the most destructive pest occurring in homesteads of Kerala and causing 25-50% fruit loss in mango when harvested at the mature ripe stage. The extend of damage on mango was up to 80% when the pest incidence occurs in an epidemic form as reported by Abdullah (2002) and Latif (2004). Verghese *et al.* (2002a) reported 2.5 to 59.0% crop loss in mango due to *B. dorsalis* depending upon variety in Bangalore, India. The level of infestation due to *B. dorsalis* in mango was as high as 26.66% in Gujarat Sushil and Bhatt (2002) and 30% in Karnataka Babu and Virakthmath (2003). Patel and Patel (2005) reported fruit fly infestation to the tune of 16-40% on Mango in South Gujrat, India. Patel *et. al.* (2013) also reported that the highest fruit fly infestation (36.67%) was observed in ripening cum harvesting period of mango.

In the present study also the dominant species that infest mango was detected as *B. dorsalis*. Other findings with regard to intensity of infestation are also more or less corroborated with these literatures. Minor variations are may be due to locational as well as varietal variations of the study.

Table-9: Cluster analysis of fruit fly infestation on mango in different locations during 2015.

Cluster number	Percent fruit infestation				Name of the locations
	Mean	Variance	Min. value	Max. value	
CL1	10.5	0.481	10	11	Horir_Hat, Matigara, Chilkirhat
CL2	9	0.077	8.66	9.33	Dinhata, Gadong-2, Falakata, Kharibari, Morangabari
CL3	12	0.000	12	12	Dhupguri, Moinaguri, Phasidewa, Posarir Hat
CL4	7.83	0.111	7.66	8	Pundibari, Sajerpar, Vetaguri,
CL5	13	0.037	13	13	Chandmoni, Gadong-1, Maruganj
CL6	15	0.500	15	15	Sonapur, Naksalbari
Levens test Statistic	3.81				
p-value	0.04				

Fig-9: **Cluster dendogram on intensity of fruit fly infestation on mango at different locations during 2015.**



4.2.2. Intensity of infestation by fruit flies (*Bactrocera* spp.) on Guava *Psidium guajava* Kuawa.

The observations pertaining with fruit fly infestation on Guava at different districts under northern part of West Bengal have been presented in Table-10. The data revealed that infestation by fruit fly on Guava at different districts were significantly varied amongst various locations during 2014 and 2015. The percent fruit infestation on Guava varied from 9.67 - 25.00% and 8.67 - 28.33% during 2014 and 2015 respectively.

However, two years pooled mean values showed that the highest infested fruit (26.67%) on number basis was observed at Posarir hat (23^{0}73′N, 88^{0}23′E) locality and lowest (10.50%) was noted at Matigara (26^{0}72′N, 88^{0}38′E) and Kharibari (26^{0}55′N, 88^{0}19′E) area.

Table-10: Percent fruit infestation (by number) by fruit flies on guava during 2014 and 2015 in different parts of northern West Bengal.

Locations	2014	2015	Pooled over (2014-2015)
Chilkirhat	22.00 (4.69)	24.00 (4.90)	23.00 (4.80)
Morangabari	13.33 (3.65)	21.00 (4.58)	17.17 (4.14)
Posarir Hat	25.00 (5.00)	28.33 (5.32)	26.67 (5.16)
Dinhata	14.33 (3.79)	21.67 (4.66)	18.00 (4.24)
Vetaguri	21.00 (4.58	17.33 (4.16)	19.17 (4.38)
Maruganj	18.00 (4.24)	14.33 (3.79)	16.17 (4.02)
Pundibari	11.33 (3.37)	11.00 (3.32)	11.17 (3.34)
Horir Hat	15.00 (3.87)	12.67 (3.56)	13.83 (3.72)
Sajerpar	11.33 (3.37)	11.00 (3.32)	11.17 (3.34)
Sonapur	24.00 (4.90)	24.33 (4.93)	24.17 (4.92)
Falakata	12.00 (3.46)	19.67 (4.44)	15.83 (3.98)
Gadong-1	12.67 (3.56)	24.00 (4.90)	18.33 (4.28)
Gadong-2	12.00 (3.46)	12.00 (3.46)	12.00 (3.46)
Moinaguri	13.67 (3.70)	13.67 (3.70)	13.67 (3.70)
Dhupguri	13.67 (3.70)	22.33 (4.73)	18.00 (4.24)
Chandmoni	13.00 (3.61)	12.33 (3.51)	12.67 (3.56)
Naksalbari	17.67 (4.20)	11.00 (3.32)	14.33 (3.79)
Matigara	12.33 (3.51)	8.67 (2.94)	10.50 (3.24)
Kharibari	9.67 (3.11)	11.33 (3.37)	10.50 (3.24)
Phasidewa	17.67 (4.20)	17.00 (4.12)	17.33 (4.16)

* Figures within parenthesis are square root transormed values.

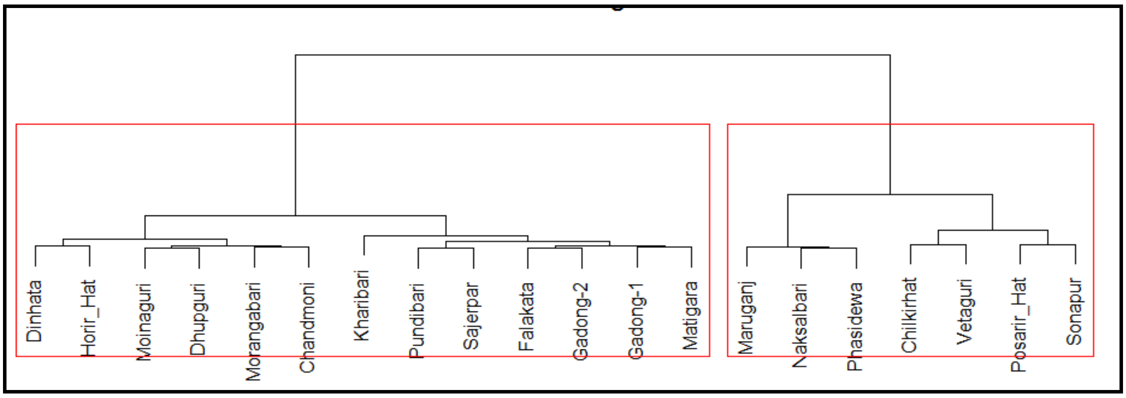
During 2014, mean values of the data indicated that highest infestation was recorded as 21.28% in CL1 (Cluster 1) followed by 12.89% in CL2 (Cluster 2). The highest percentage of fruit infestation was found to vary from 17.67 - 25.00 percent at different locations i.e. Naksalbari, Phasidewa, Maruganj, Posarir Hat, Vetaguri, Chilkirhat, Sonapur under northern part of West Bengal and the lowest infestation varied from 11.33 - 15.00% at Kharibari, Pundibari, Sajerpar, Falakata, Gadong2, Matigara, Gadong1, Chandmoni, Morangabari, Dhupguri, Moinaguri, Dinhata and Horir Hat in cluster 2.

Table-11: Cluster analysis of fruit fly infestation on guava in different locations during 2014.

Cluster number	Percent fruit infestation				Name of the locations
	Mean	Variance	Min. value	Max. value	
CL1	21.28	9.470	17.67	25.00	Naksalbari, Phasidewa, Maruganj, Posarir Hat, Vetaguri, Chilkirhat, Sonapur
CL2	12.89	2.027	11.33	15.00	Kharibari, Pundibari, Sajerpar, Falakata, Gadong2, Matigara, Gadong1, Chandmoni, Morangabari, Dhupguri, Moinaguri, Dinhata, Horir Hat
Levens test Statistic	14.32				
p-value	0.001				

*CL- Cluster.

Fig-10: Cluster dendogram on intensity of fruit fly infestation on guava at different locations during 2014.



During 2015, mean values of the data revealed that the fruit fly inflicted highest infestation (22.52%) on guava in CL1 (Cluster 1) followed by CL2 (Cluster 2) (12.15%). The highest percentage of infestation was varied from 17.33 to 28.33% at Phasidewa, Vetaguri, Falakata, Moranga, Dinhata, Dhupguri, Chilkirhat, Gadong1, Sonapur, Posarir Hat and the lowest infestation was varied from 11.00 to 14.33% at Matigara, Naksalbari, Pundibari, Sajerpar, Kharibari, Gadong2, Chandmoni, Horir Hat, Moinaguri and Maruganj.

Similar results were earlier reported by Hasseb (2007) who found 20-46% fruit fly infestation on guava. Highest crop loss of about 80% in guava was reported by Kafi (1986) and Ishtiaque *et. al.* (1999) in Pakistan. Similarly Jalaluddin *et al.* (1999)

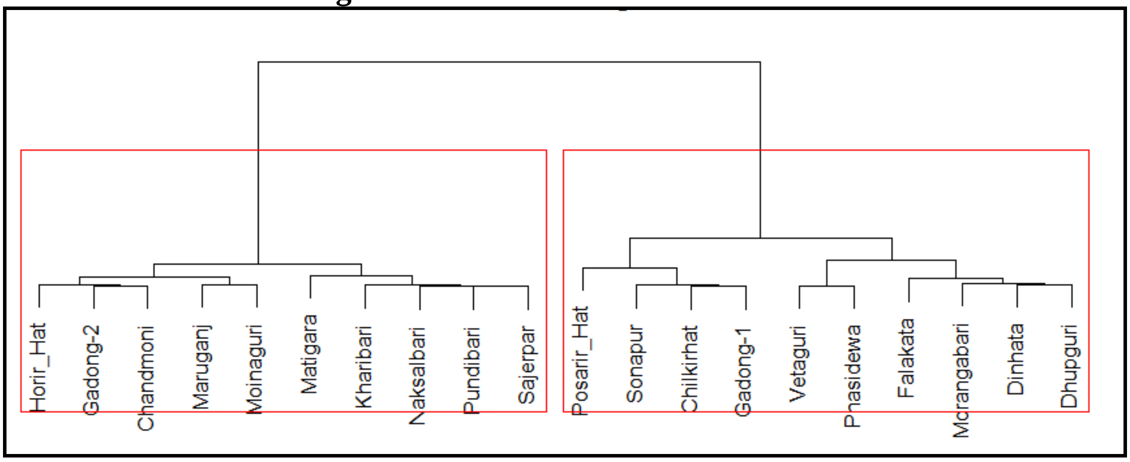
reported 60 - 80% loss in guava fruit by *Bactrocera correcta* (Bezzii) while Verghese *et al.* (2002b) reported infestation by fruits flies to guava ranged from 5-7%. In previous studies, Mehmood and Mishkatullah (2007) who found the highest percentage (80%) fruit fly infestation in guava orchards in ripening stage.

Yan-mei *et. al.* (2011) also reported that the fruit damage rate of guava was 6.67-7.33% by oriental fruit fly. Fruit loss in guava orchards infested due to fruit fly was estimated to the extent of 13.40 to 46.60% and 12.50-42.86% percent, respectively on weight basis and number basis was reported by Ukey *et. al.* (2012). Kakar, *et al.* (2014) also reported that the fruit fly infestation was varied from 3.00-49.67% on guava in Khyber Pakhtunkhawa. Thus, results of the present study are in corroboration with the previous findings.

Table-12: Cluster analysis of fruit fly infestation on guava in different locations during 2015.

Cluster number	Percent fruit infestation				Name of the locations
	Mean	Variance	Min. value	Max. value	
CL1	22.52	11.887	17.33	28.33	Phasidewa, Vetaguri, Falakata, Morangaba, Dinhata, Dhupguri, Chilkirhat, Gadong1, Sonapur, Posarir Hat
CL2	12.15	2.548	11.00	14.33	Matigara, Naksalbari, Pundibari, Sajerpar, Kharibari, Gadong2, Chandmoni, Horir Hat, Moinaguri, Maruganj
Levens test Statistic	4.85				
p-value	0.04				

Figure-11: **Cluster dendogram on intensity of fruit fly infestation on guava at different locations during 2015.**



4.2.3. Intensity of Infestation by fruit flies (*Bactrocera* spp.) on bitter gourd (*Momordicha charantia* Linn.):

The observations with regard to percent fruit infestation on bitter gourd caused by fruit flies on number basis at different localities have been presented in Table-13. The data presented in the table revealed that, fruit infestation due to fruit flies on bitter gourd were varied from 20.67- 29.00% and 19.33-29.33% during 2014 and 2015 respectively. However, highest average infested fruits (28.83%) were recorded at Posarir hat ($23^0$73′N, $88^0$23′E) in Cooch Behar district and lowest average infested fruits (20.17%) were observed at Falakata ($26^0$53′N, $89^0$19′E) in Jalpaiguri district under northern part of West Bengal at number basis.

Table-13: Percent fruit infestation (by number) by fruit flies on bitter gourd during 2014 and 2015 in different parts of northern West Bengal.

Locations	Percent fruit infestation		
	2014	2015	Pooled over (2014 and 2015)
Chilkirhat	26.33 (5.13)	26.33 (5.13)	26.33 (5.13)
Morangabari	23.67 (4.86)	25.00 (5.00)	24.33 (4.93)
Posarir Hat	28.33 (5.32)	29.33 (5.42)	28.83 (5.37)
Dinhata	25.67 (5.07)	22.67 (4.76)	24.17 (4.92)
Vetaguri	24.00 (4.90)	21.33 (4.62)	22.67 (4.76)
Maruganj	29.00 (5.93)	25.33 (5.03)	27.17 (5.21)
Pundibari	25.00 (5.00)	22.33 (4.73)	23.67 (4.87)
Horir Hat	23.33 (4.83)	23.00 (4.80)	23.17 (4.81)
Sajerpar	26.00 (5.10)	22.67 (4.76)	24.33 (4.93)
Sonapur	28.33 (5.32)	27.67 (5.26)	28.00 (5.29)
Falakata	20.67 (4.55)	19.67 (4.43)	20.17 (4.49)
Gadong-1	28.00 (5.29)	27.00 (5.20)	27.50 (5.24)
Gadong-2	22.67 (4.76)	22.67 (4.76)	22.67 (4.76)
Moinaguri	21.33 (4.62)	21.33 (4.62)	21.33 (4.62)
Dhupguri	27.67 (5.26)	26.67 (5.16)	27.17 (5.21)
Chandmoni	26.88 (5.18)	23.06 (4.80)	24.97 (5.00)
Naksalbari	26.67 (5.16)	28.33 (5.32)	27.50 (5.24)
Matigara	26.00 (5.10)	21.00 (4.58)	23.50 (4.85)
Kharibari	21.33 (4.62)	19.33 (4.40)	20.33 (4.51)
Phasidewa	26.00 (5.10)	28.33 (5.32)	27.17 (5.21)

* Figures within parenthesis are square root transformed values.

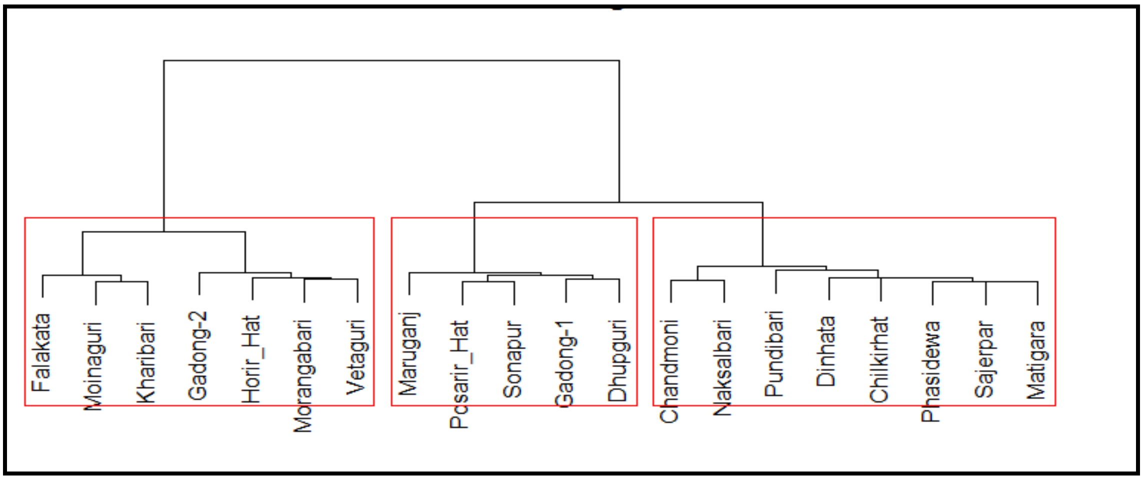
The presentations in Table-14 on cluster analysis revealed that the fruit infestation on bitter gourd was recorded significantly higher (28.41%) in CL3 (Cluster 3) followed by CL1 (Cluster 1) (26.22%). The higher percent infestation ranged from 28.00 - 29.00% on number basis at Dhupguri, Gadong, Posarir Hat, Sonapur, Maruganj and lower was ranged from 20.66 - 24.00% on number basis in CL2 (Cluster 2) at Falakata, Kharibari, Moinaguri, Gadong 2, Horir Hat, Morangabari, and Vetaguri during 2014.

Table-14: **Cluster analysis of fruit fly infestation on bitter gourd in different locations during 2014.**

Cluster number	Percent fruit infestation				Locations
	Mean	Variance	Min. value	Max. value	
CL1	26.22	0.343	25.66	26.88	Pundibari, Dinhata, Matigara, Phasidewa, Sajerpar, Chilkirhat, Naksalbari, Chandmoni
CL2	22.43	1.730	20.66	24	Falakata, Kharibari, Moinaguri, Gadong2, Horir Hat, Morangabari, Vetaguri
CL3	28.41	0.244	28	29	Dhupguri, Gadong1, PosarirHat, Sonapur, Maruganj
Levens test Static	8.45				
p-value	0.004				

*CL- Cluster.

Fig-12: **Cluster dendogram on intensity of fruit fly infestation on bitter gourd at different locations during 2014.**



On the other hand, during 2015, the extent of fruit infestation varied from 20.83 - 27.38% by number basis and significantly higher infestation (27.83%) was observed in CL1 (Cluster 1) followed by CL2 (Cluster 2) (22.81%). Higher fruit infestation of bitter gourd was varied from 25.33 - 29.33% at Morangabari, Maruganj, Chilkirhat, Dhupguri, Gadong1, Sonapur, Naksalbari, Phasidewa and Posarir Hat CL1 (Cluster 1) while lower infestation was ranged from 19.67 - 21.33% on number basis at Kharibari, Falakata, Matigara, Moinaguri and Vetaguri in CL3 (Cluster 3).

The fruit fly damage is the major limiting factor in obtaining good quality fruits and high yield of bitter gourd (Srinivasan, 1959, Lall and Singh, 1969; Mote, 1975 and Rabindranath and Pillai, 1986). Results of the present study are in agreement with Singh *et al.* (2000) who reported 31.27% damage on bitter gourd and 28.55% on watermelon in India. Kumar *et al.* (2006) found maximum 77.03% fruit fly infestation on bitter gourd. Pareek and Kavadia (1994) observed that bitter gourd is the highest preferred host. The field experiments on assessment of losses caused by cucurbit fruit fly in different cucurbits have been reported 24.7 - 40.0% and 27.3 - 49.3 % yield losses in bitter gourd and bottle gourd respectively in Nepal as reported by Pradhan (1976).

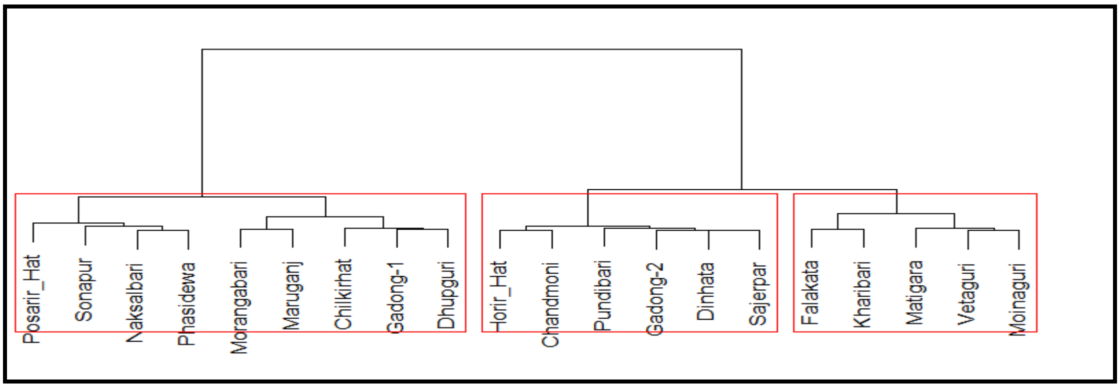
The fly has also reported to infest 95% of bitter gourd in New Guinea. 90% of snake gourd, 60 - 87% pumpkin fruits in Soloman Island has also been reported by Hollingsworth *et al.* (1997). In Pakistan, Anwar (1956) reported that up to 60% loss occurred due to fruit fly. Variations in fruit fly infestation on bitter gourd in the present study may be due to climatological of varietal differences.

Table-15: Cluster analysis of fruit fly infestation on bitter gourd in different locations during 2015.

Cluster number	Percent fruit infestation				Locations
	Mean	Variance	Min. value	Max. value	
CL1	27.38	2.083	25.33	29.33	Morangabari, Maruganj, Chilkirhat, Dhupguri, Gadong1, Sonapur, Naksalbari, Phasidewa, Posarir Hat
CL2	22.81	0.069	22.67	23.06	Pundibari, Dinhata, Gadong2, Sajerpar, Horir Hat, Chandmoni
CL3	20.83	0.630	19.67	21.33	Kharibari, Falakata, Matigara, Moinaguri, Vetaguri
Levens test Statistic	5.23				
p-value	0.02				

*CL- Cluster.

Fig-13: **Cluster dendogram on intensity of fruit fly infestation on bitter gourd at different locations during 2015.**



4.2.4. Intensity of infestation by fruit flies (*Bactrocera* spp.) on Ridge gourd (*Luffa acutangula* Linn.):

The observations with regard to percent fruit infestation on ridge gourd by fruit flies by number basis at different localities of northern West Bengal have been presented in Table-16. It appeared from the table that the intensity of fruit infestation on ridge gourd ranged from 21.33 - 34.00% and 21.67 - 29.67% on number basis during theperiod of study i.e. 2014 and 2015 respectively. During 2014, higher percent infestation was recorded 34.00% at Chilkir Hat (26^0 53′N, 89^0 19′E) and Moinaguri (26^0 57′N, 88^0 82′E) followed by 32.33%. at Horir Hat (26^0 39′N, 89^0 49′E) and Sonapur (26^0 46′N, 89^0 39′E) and the lowest infestation was recorded

21.33% at Vetaguri (26^0 20′N, 89^0 48′E). On the other hand during 2015, highest fruit infestation on ridge gourd was recorded 29.67% on at Gadong1 (26^0 55′N, 89^0 10′E) and the lowest was recorded at Falakata (21.67%). However, highest average infested fruit 30.67% was observed at Sonapur locality whereas lowest average percent infested fruit 22.17% was observed in the samples collected from Chandmoni (25^0 26′N, 88^0 02′E) area.

Table-16: Percent fruit infestation (by number) by fruit flies on ridge gourd during 2014 and 2015 in different parts of northern West Bengal.

Locations	Percent fruit infestation		
	2014	**2015**	**Pooled over (2014- 2015)**
Chilkirhat	34.00 (5.83)	26.33 (5.13)	30.17 (5.49)
Morangabari	22.67 (4.76)	23.00 (4.80)	22.83 (4.78)
Posarir Hat	27.00 (5.20)	26.67 (5.16)	26.83 (5.18)
Dinhata	28.33 (5.32)	22.67 (4.76)	25.50 (5.05)
Vetaguri	21.33 (4.62)	24.67 (4.97)	23.00 (4.80)
Maruganj	28.00 (5.29)	28.00 (5.29)	28.00 (5.29)
Pundibari	22.00 (4.69)	24.33 (4.93)	23.17 (4.81)
Horir Hat	32.33 (5.69)	24.00 (4.90)	28.17 (5.31)
Sajerpar	29.00 (5.39)	23.00 (4.80)	26.00 (5.10)
Sonapur	32.33 (5.69)	29.00 (5.39)	30.67 (5.54)
Falakata	33.33 (5.77)	21.67 (4.66)	27.50 (5.24)
Gadong-1	27.00 (5.20)	29.67 (5.45)	28.33 (5.32)
Gadong-2	22.00 (4.69)	24.33 (4.93)	23.17 (4.81)
Moinaguri	34.00 (5.83)	25.00 (5.00)	29.50 (5.43)
Dhupguri	26.67 (5.16)	29.33 (5.42)	28.00 (5.29)
Chandmoni	21.33 (4.62)	23.00 (4.80)	22.17 (4.71)
Naksalbari	27.00 (5.20)	27.67 (5.26)	27.33 (5.23)
Matigara	22.33 (4.73)	24.33 (4.93)	23.33 (4.83)
Kharibari	21.67 (4.66)	23.67 (4.87)	22.67 (4.76)
Phasidewa	28.00 (5.29)	28.67 (5.35)	28.33 (5.32)

* Figures within parenthesis are square root transformed values.

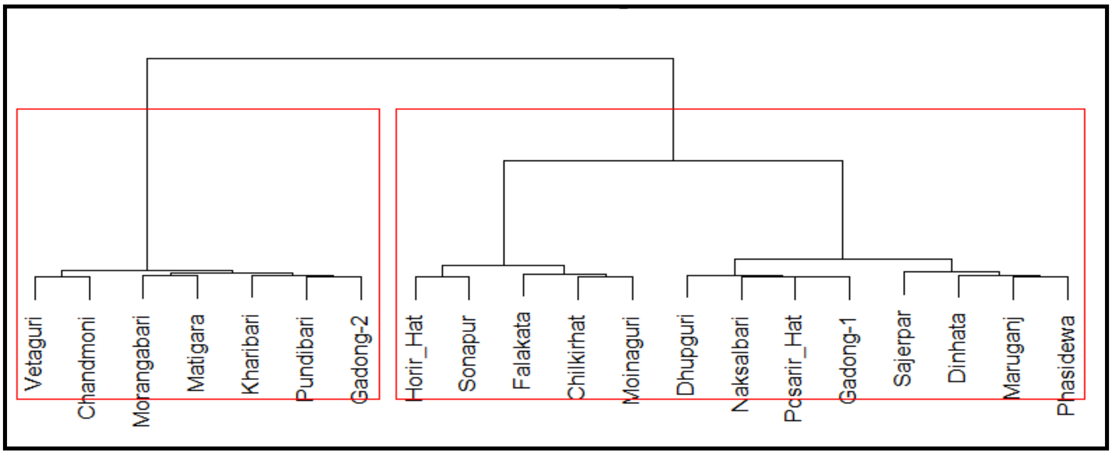
The observatations were subjected to cluster analysis and the ranges of fruit infestation was found as 22.00 - 30.03% and 22.92 - 28.43% during 2014 and 2015 (Pooled mean). However, during 2014 the fruit infestation was observed significantly higher (30.03%) in CL1 (Cluster 1) followed by CL2 (Cluster 2) (22.00%). Significantly higher infestation was noted at Dhupguri, Gadong1, Naksalbari, Posarir Hat, Maruganj, Phasidewa, Dinhata, Sajerpar, Horir Hat, Sonapur, Falakata, Chilkirhat and Moinaguri that varied from 27.00 - 34.00%. In CL2 (Cluster 2) lower infestation was recorded that ranged from 21.33-22.67% at Chandmoni, Vetaguri, Kharibari, Gadong2, Pundibari, Matigara and Morangabari.

Table-17: Cluster analysis of fruit fly infestation on ridge gourd in different locations during 2014.

Cluster number	Percent fruit infestation				Locations
	Mean	Variance	Min. value	Max. value	
CL1	30.03	8.599	27	34	Dhupguri, Gadong1, Naksalbari, Posarir Hat, Maruganj, Phasidewa, Dinhata, Sajerpar, Horir Hat, Sonapur, Falakata, Chilkirhat, Moinaguri
CL2	22	0.248	21.33	22.67	Chandmoni, Vetaguri, Kharibari, Gadong2, Pundibari, Matigara, Morangabari
Levens test Statistic	6.28				
p-value	0.02				

*CL- Cluster.

Fig-14: **Cluster dendogram on intensity of fruit fly infestation on ridge gourd at different locations during 2014.**



During 2015, fruit infestation by fruit flies on ridge gourd was significantly higher (28.43%) in CL1 (Cluster 1) followed by CL3 (Cluster 3) (24.44%). The cluster analysis showed that the higher infestation was ranged from 26.67-29.67% at Chilkirhat, Posarir Hat, Naksalbari, Maruganj, Phasidewa, Sonapur, Dhupguri and Gadong-1. Lower infestation was detected as 22.92% in CL2 (Cluster 2) that varied from 22.67 - 23.00% at Falakata, Dinhata, Chandmoni, Morangabari and Sajerpar under Cluster 2.

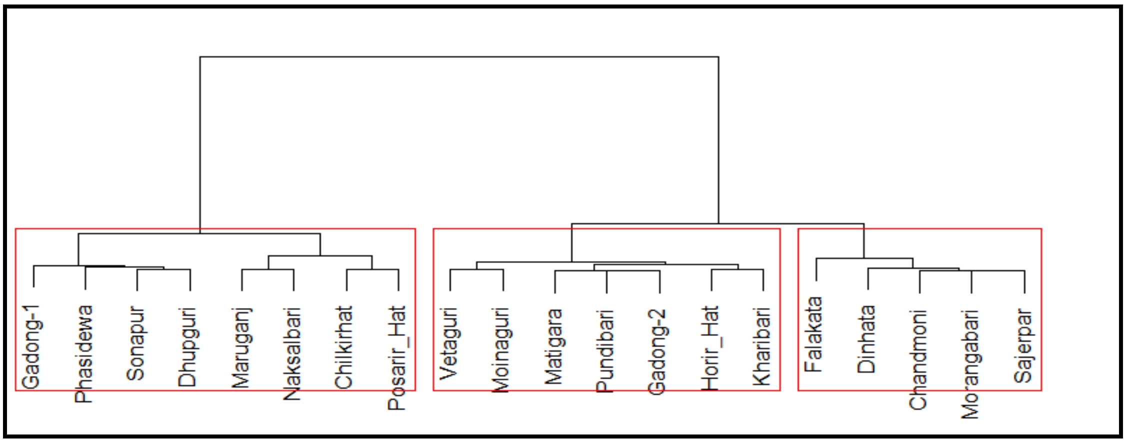
Earlier, Jakhar and Pareek (2005) observed that among the cucurbits, ridge gourd was one of the most preferred hosts by the fruit fly, bottle gourd was least preferred and bitter gourd was the moderately preferred hosts. Ghule *et al.* (2015) reported that maximum fruit fly infestation was varied from 67.5 - 70.51% on ridge gourd. However, Kumar *et al.* (2006) found maximum fruit fly infestation of 75.65% on ridge gourd and the report is also in agreement with Chaudhary and Patel (2012).

Results of the present study indicating less fruit fly infestation on ridge gourd under northern tract of West Bengal. This deviation of results from the previous studies may be due to locational variations of the study or the cultivar considered.

Table18: Cluster analysis of fruit fly infestation on ridge gourd in different locations during 2015.

Cluster number	Percent fruit infestation				Locations
	Mean	Variance	Min. value	Max. value	
CL1	28.43	1.492	26.67	29.67	Chilkirhat, PosarirHat, Naksalbari, Maruganj, Phasidewa, Sonapur, Dhupguri, Gadong-1
CL2	22.92	0.333	22.67	23.00	Falakata, Dinhata, Chandmoni, Morangaba, Sajerpar
CL3	24.44	0.185	24.00	25.00	Kharibari, Horir_Hat, Gadong2, Matigara, Pundibari, Vetaguri, Moinaguri
Levens test Statistic	5.46				
p-value	0.02				

Fig-15: **Cluster dendogram on intensity of fruit fly infestation on ridge gourd at different locations during 2015.**



4.2.5. Intensity of infestation by fruit flies (*Bactrocera* spp.) on pointed gourd (*Trichosanthes dioica* Roxb.):

The fruit infestation by fruit fly on pointed gourd has been found very less as compared to other crops studied that ranged from 2.33-10.67% and 1.00-6.33% during 2014 and 2015 respectively under northern tract of West Bengal. During 2014, the highest fruit infestation was detected as 10.67% from Chilkir Hat followed by 10.00% and 9.33% at Morangabari ($21^0$91′N, $87^0$26′E) and Sonapur (26^0 46′N, $89^0$39′E) respectively. On the other hand, lowest infestation was detected 2.33% from Gadong2 (26^0 54′N, 89^0 17′E). The data presented in table-19 also revealed that during 2015 maximum fruit infestation was 6.33% at Naksalbari (26^0 70′N, 88^0 42′E) and Phasidewa (26^0 58′N, 88^0 37′E) followed by Gadong1 (5.67%) and Chilkir hat (4.33%). However, highest average (7.50%) infested fruit was observed at Chilkir hat locality under Cooch Behar district whereas lowest average percent infestation (2.00%) was observed in samples collected from Gadong2 and Moinaguri area under Jalpaiguri district.

Table-19: Percent fruit infestation (by number) by fruit flies on pointed gourd during 2014 and 2015 in different parts of northern West Bengal.

Locations	Percent fruit infestation		
	2014	2015	Pooled over (2014-2015)
Chilkirhat	10.67 (3.27)	4.33 (2.08)	7.50 (2.74)
Morangabari	10.00 (3.16)	2.00 (1.41)	6.00 (2.45)
Posarir Hat	4.33 (2.08)	4.00 (2.00)	4.17 (2.04)
Dinhata	10.00 (3.16)	2.33 (1.53)	6.17 (2.48)
Vetaguri	8.67 (2.94)	1.67 (1.29)	5.17 (2.27)
Maruganj	9.00 (3.00)	1.67 (1.29)	5.33 (2.31)
Pundibari	10.00 (3.16)	3.67 (1.92)	6.83 (2.61)
Horir Hat	8.00 (2.83)	1.33 (1.15)	4.67 (2.16)
Sajerpar	2.67 (1.63)	1.67 (1.29)	2.17 (1.47)
Sonapur	9.33 (3.05)	2.33 (1.53)	5.83 (2.41)
Falakata	7.00 (2.65)	1.00 (1.00)	4.00 (2.00)
Gadong-1	8.67 (2.94)	5.67 (2.38)	7.17 (2.68)
Gadong-2	2.33 (1.53)	1.67 (1.29)	2.00 (1.41)
Moinaguri	2.67 (1.63)	1.33 (1.15)	2.00 (1.41)
Dhupguri	9.00 (3.00)	4.00 (2.00)	6.50 (2.55)
Chandmoni	3.00 (1.73)	3.33 (1.82)	3.17 (1.78)
Naksalbari	5.33 (2.31)	6.33 (2.52)	5.83 (2.41)
Matigara	3.00 (1.73)	3.67 (1.92)	3.33 (1.82)
Kharibari	3.33 (1.82)	3.00 (1.73)	3.17 (1.78)
Phasidewa	5.33 (2.31)	6.33 (2.52)	5.83 (2.41)

* Figures within parenthesis are square root transformed values.

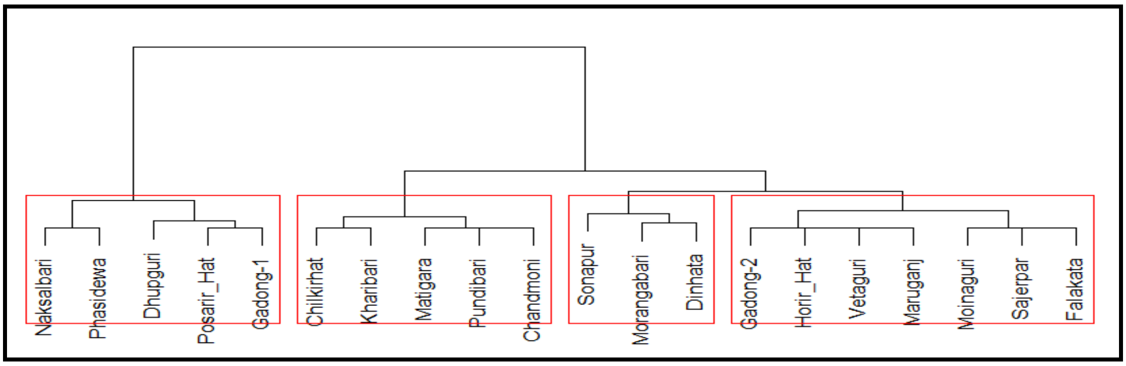
The observations with regard to fruit fly infestation on pointed gourd were subjected to cluster analysis and the results have been presented in table-20. Results presented in the table showed that significantly higher infestation (4.80%) was detected in CL3 (Cluster 3) followed by CL1 (Cluster 1) (3.11%) and CL4 (Cluster 4) (2.50%). The higher infestation was varied from 4.33 - 5.33% at Chilkirhat, Gadong1, Posarir Hat, Dhupguri, Naksalbari and Phasidewa in Cluster 3. On the other hand, lowest infestation (1.83%) was detected in CL2 (Cluster 2) and the range was varied from 1.66-2.00% at Sonapur, Dinhata and Morangabari.

Table-20: Cluster analysis of fruit fly infestation on pointed gourd in different locations during 2014.

Cluster number	Percent fruit infestation				Locations
	Mean	Variance	Min. value	Max. Value	
CL1	3.11	0.027	3.00	3.33	Chandmoni, Matigara, Pundibari, Kharibari
CL2	1.83	0.259	1.66	2.00	Sonapur, Dinhata, Morangabari
CL3	4.80	0.562	4.33	5.33	Chilkirhat, Gadong1, PosarirHat, Dhupguri, Naksalbari, Phasidewa
CL4	2.50	0.031	2.33	2.66	Gadong2, Horir Hat, Maruganj, Vetaguri, Falakata, Moinaguri, Sajerpar
Levens test Statistic	3.34				
p-value	0.05				

*CL- Cluster.

Fig-16: **Cluster dendogram on intensity of fruit fly infestation on pointed gourd at different locations during 2014.**



Cluster analysis data of 2015 presented in table-21. It appeared from the presentation that significantly higher infestation (4.59%) was detected in CL1 (Cluster 1) (6.33%) followed by CL2 (Cluster 2) (2.33%) on pointed gourd. The higher infestation was varied from 3.33 - 6.33% at different localities viz. Kharibari, Chandmoni, Matigara, Pundibari, Dhupguri, Posarir Hat, Chilkirhat, Gadong1, Naksalbari and Phasidewa. The lowest mean infestation (1.78%) was detected in CL2

(Cluster 2) that ranged from 1.33 - 2.33% at Falakata, Horir Hat, Moinaguri, Gadong-2, Sajerpar, Maruganj, Vetaguri, Morangabari, Dinhata and Sonapur.

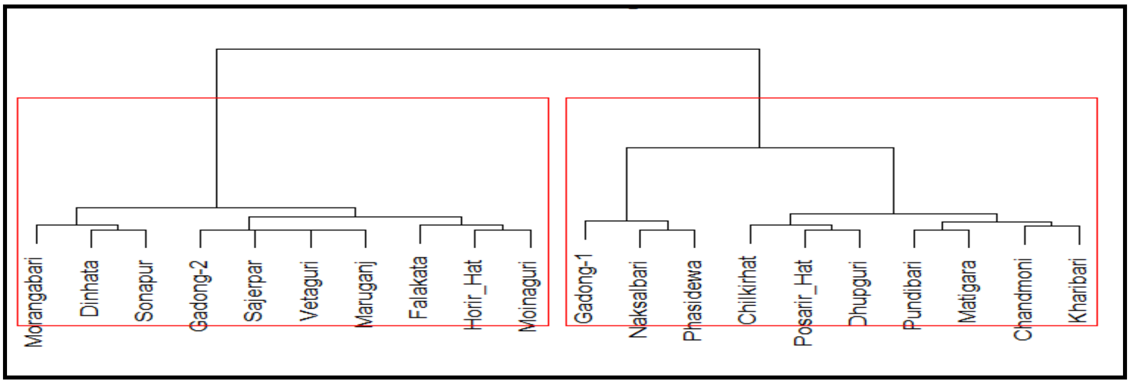
Dhillon *et al.* (2005) stated 30 - 100% yield loss due to fruit fly infestation on cucurbits depending upon species and the season. Losses without control have been estimated as 21% of fruit and 24% of cucurbits in Pakistan by Stonehouse *et al.* (1998) and 12% of fruit and 21% of cucurbits in India by Mumford *et al.* (2005). However, information with regard to fruit fly infestation on pointed gourd is meagre.

In the present study very minimum intensity of infestation have been recorded as compared to the other fruits and cucurbits studied which may be due to regional variation of conducting the experiment.

Table-21: Cluster analysis of fruit fly infestation on pointed gourd in different locations during 2015.

Cluster number	Percent fruit infestation				Locations
	Mean	Variance	Min. value	Max. value	
CL1	4.59	1.507	3.33	6.33	Kharibari, Chandmoni, Matigara, Pundibari, Dhupguri, Posarir Hat, Chilkirhat, Gadong1, Naksalbari, Phasidewa
CL2	1.78	0.184	1.33	2.33	Falakata, Horir_Hat, Moinaguri, Gadong-2, Sajerpar, Maruganj, Vetaguri, Moranga bari, Dinhata, Sonapur
Levens test Statistic	4.49				
p-value	0.05				

Fig-17: **Cluster dendogram on intensity of fruit fly infestation on pointed gourd at different locations during 2015.**



4.2.6. Intensity of fruit infestation (by fruit flies) on mandarin orange (*Citrus reticulata* Blanco.):

Fruit fly is also an key pest of mandarin orange in Darjeeling district of West Bengal. Two years observation regarding fruit fly infestation in Mandarin orange in different locations of Darjeeling district under northern West Bengal have been presented in table-22. It appeared from the table that exists variations in observation means of fruit fly infestation in mandarin orchard during both the years 2014 and 2015 at different locations. Two years mean data showed that fruit infestation was ranged from 21.22 - 41.00%. Among different locations, the highest fruit infestation was detected at Lower Dungra (41.00%) followed by Upper Dungra (37.49%), Sittong (32.60%) and Mongpu (32.35%). On the other hand, the lowest infestation was observed at Bomb Bosti (21.22%).

Table-22: Percent fruit infestation (by number) by fruit flies on mandarin orange during 2014 and 2015 in different parts of Darjeeling district (West Bengal).

Locations (Darjeeling district)	Percent fruit infestation		
	2014	2015	Pooled over (2014-2015)
Bomb Bosti	22.67 (4.81)	19.78 (4.50)	21.22 (4.66)
Poshyor	28.56 (5.39)	24.45 (4.99)	26.51 (5.20)
Ecche Bosti	33.33 (5.82)	28.76 (5.41)	31.05 (5.62)
Upper Dungra	40.00 (6.36)	34.98 (5.96)	37.49 (6.16)
Lower Dungra	43.67 (6.65)	38.34 (6.23)	41.00 (6.44)
Sittong	33.90 (5.86)	31.30 (5.64)	32.60 (5.75)
Lanku Valley	31.23 (5.63)	28.40 (5.38)	29.82 (5.51)
Mongpu	31.00 (5.61)	33.69 (5.85)	32.35 (5.73)
Mongmaya	21.79 (4.72)	23.44 (4.89)	22.62 (4.81)

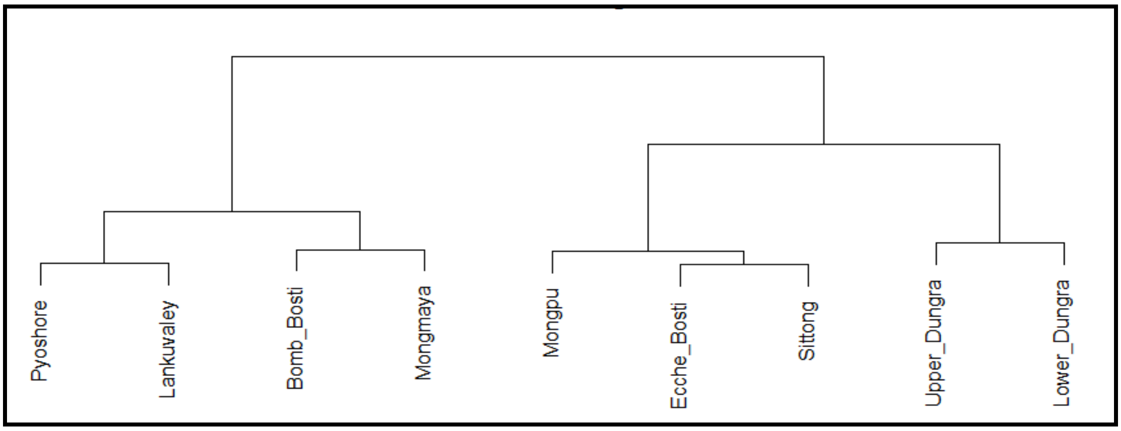
* Figures within parenthesis are square root transformed values.

The data with regard to intensity of infestation by fruit fly on mandarin orange in Darjeeling district were subjected to cluster analysis and presented in table-23. Perusal of the data indicating that mandarin orange infestation by fruit fly in different locations of Darjeeling district varied significantly and the mean infestation devided into two clusters (Cluster 1 and 2) during both the years of study i.e. 2014 and 2015. Year as source of variance was found insignificant for fruit fly infestation. Comparing infestation during both the years, the higher fruit fly infestation (36.50%) on citrus was recorded in CL2 (Cluster 2) that ranged from 31.60 - 43.67% at Mungpoo, Ecche_Bosti, Sittong, Upper Dungra and Lanku Valley. On the other hand, lowest was ranged from 21.79 - 28.56% at different locations viz. Bomb_Busty, Lower Dungra Poshyor and Mongmaya in Cluster 1.

Table-23: Cluster analysis of fruit fly infestation on mandarin orange in different locations of Darjeeling district (West Bengal) during 2014.

Cluster number	Percent fruit infestation				Locations
	Mean	Variance	Min. value	Max. value	
CL1	25.66	20.901	21.79	28.56	Bomb_Busty, Pyoshore, Mongmaya, Lankuvalley
CL2	36.5	27.661	31.60	43.67	Mungpoo, Ecche_Bosti, Sittong, Upper Dungra, Lankuvalley.
Levens test Statistic	0.33				
p-value	0.58				

Fig-18: Cluster dendogram on intensity of fruit fly infestation on mandarin orange at different locations locations of Darjeeling district (West Bengal) during 2014.

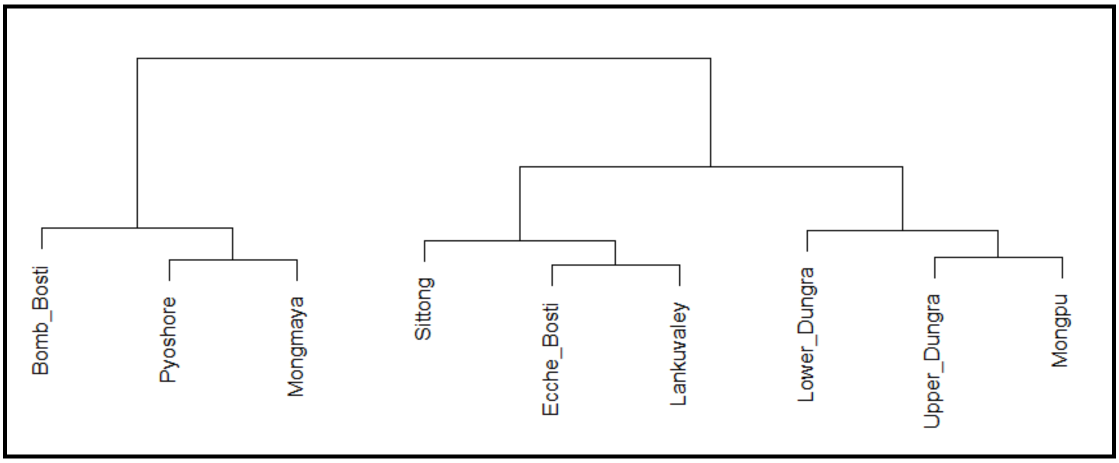


The data depicted in table-24 showed that the infestation was highest in CL2 (Cluster 2) (32.58%) ranging varied from 28.40 - 38.34% during 2015 at Lanku Valley, Sittong, Mongpu, Lower Dungra and Mongmaya. On the contrary, relatively lower infestation was observed in CL1 (Cluster 1) (22.55%) that varied from 19.78 - 24.45% at Bomb_Bosti, Ecchey Bosti, Poshyor and Upper Dungra.

Table-24: Cluster analysis of fruit fly infestation on mandarin orange in different locations of Darjeeling district (West Bengal) during 2015.

Cluster number	Percent fruit infestation				Locations
	Mean	Variance	Min. Value	Max. value	
CL1	22.55	50.112	19.78	24.45	Bomb_Bosti, Echee Bosti, Poshyor Upper Dungra
CL2	32.58	27.727	28.40	38.34	Lankuvalley, Sittong, Mongpu, Lower Dungra, Manymaya
Levens test Statistic	1.74				
p-value	0.24				

Fig-19:Cluster dendogram on intensity of fruit fly infestation on mandarin orange at different locations locations of Darjeeling district (West Bengal) during 2015.



Mandarin orange in Darjeeling district of West Bengal is badly suffering from several biotic stresses of which infestation by the fruit fly is one of the most imporatant. The fruit fly species associated with this devastation has been reported as *Bactrocera minax* (Enderlein). It is also known as *Callantra minax* and

Polistomimetes minax (in the Indian literature) or Chinese citrus fly. The pest has already been recorded from northern India as causing heavy damage to tangerine (*Citrus reticulata*). The infestation is becoming so serious that sometimes almost all fruits of a plant get damaged.

Published literature regarding the same is meagre. However, Laskar *et al.* (2016) reported 45.67% fruit infestation on mandarin orange in Darjeeling district of West Bengal. Findings of the present study also revealed considerable infestation of citrus fruits at different locations of Darjeeling district. Further exhaustive study on this aspect is needed.

4.3. Frequency and duration of visit during the day and on different stages of fruits by fruit flies:

Preference of fruit flies on different maturity stages of fruits they infest is usually found to differ. Even the intensity and duration of visit on fruits during a day vary from morning to evening hours. In the present study, the intensity and duration of visit by adult female melon fly, *B. cucurbitae* on different maturity stages of pumpkin and *B. dorsalis* on different maturity stages of mango have been conducted. Observations with regard to number of visit at hourly interval starting from 06:00 hours and continued up to 16:00 hours were recorded. The experiment was repeated 10 different days in both the cases. In each day an individual of 21-25 days old, mated female fly was taken from the culture and released in the rearing cage carefully for recording observation.

4.3.1. Frequency and duration of visits by adult female *B. cucurbitae* on pumpkin fruits of different maturity levels:

Three pumpkin fruits of different maturity levels (one each of early, mid and harvestable maturity i.e., 10, 20 and 30 days after fruit setting respectively) were provided in the rearing cage at a time before releasing the adult melon fly. Thus the fly was in a free choice condition in the cage. The observation with regard to number and duration of visit per hour on pumpkin fruits of different maturity level was recorded, analysed statistically and presented in table-25.

The mean data (±SE) revealed that the number of visit by adult mated female of melon fly at hourly interval varied significantly ($F_{0.05,\ 9,270}$=15.857) at different hours of taking observation during a day. Maximum number of visits per hour (8.53±0.60) was recorded in mid-morning hour i. e. at 09:00 to 10:00 hour followed by 6.77±0.49 at 15:00 to 16:00 hour and 5.07±0.49 at 08:00 to 09:00 hour. This phenomenon indicated that the fly remained more active during morning, mid morning as well as afternoon hours. On the other hand, the flies were in less active manner during early morning (number of visit 3.60±0.47 times at 06:00 to 07:00 hours) and mid noon hours (number of visit 2.97±0.38 and 2.27±0.26 times at 13:00 to 14:00 and 14:00 to 15:00 hours respectively) so far as the frequency of visit is concerned.

Duration of visit per hour also significantly ($F_{0.05,\ 9,270}$=10.698) varied among different hours of a day. Flies were found to spent more time (14.79±1.15 minutes per hour) on different maturity levels of fruits from 14:00 to 15:00 hours. The diurnal pattern of flies at different maturity levels was differed from early maturity stage to harvestable stage of fruits so far as visitation on pumpkin fruits is concerned.

Mean number of visits per hour by the melon fly on different maturity stages of pumpkin fruit differed significantly ($F_{0.05,2,270}$=9.842) (Table-25). Maximum number of visits (5.56±0.46) per hour was noted on M1 (10 days after fruiting) followed by 4.71±0.47 and 3.67±0.24 on M2 (20 days after maturity) and M3 (30 days after maturity) respectively. Mean duration of visits was also detected significantly ($F_{0.05,2,270}$=26.141) highest (12.17±0.73 minutes per hour) on M1(10 days after fruiting) and least was observed on M3 (7.47± 0.44 minutes per hour). However, there exits no significant interaction of hours of the day and maturity stages of the fruits on visitation by the melon fly ($F_{0.05,18,270}$=0.650 and $F_{0.05,18,270}$=0.955 respectively). Number and duration of visit per hour during the day and on different stages of pumpkin fruits have been presented in Fig-21 and 22 respectively.

Table-25: Number and duration of visits by adult female melon fly on different maturity stages of pumpkin fruits.

Hours of Visit	Number of visits (per hour) (Mean±SE)	Duration of visits (minutes per hour) (Mean±SE)
06:00 to 07:00	3.60^{de} ± 0.47	10.13^{bc} ± 1.27
07:00 to 08:00	4.47^{bcd} ± 0.55	8.99^{bcd} ± 0.78
08:00 to 09:00	5.07^{c} ± 0.49	8.06^{bcd} ± 0.73
09:00 to 10:00	8.53^{a} ± 0.60	6.09^{e} ± 0.56
10:00 to 11:00	4.90^{cd} ± 0.53	8.08^{cde} ± 0.85
11:00 to 12:00	4.27^{cde} ± 0.47	10.16^{b} ± 0.76
12:00 to 13:00	3.63^{cde} ± 0.37	10.89^{bc} ± 1.29
13:00 to 14:00	2.97^{ef} ± 0.38	13.48^{a} ± 0.96
14:00 to 15:00	2.27^{f} ± 0.26	14.79^{a} ± 1.15
15:00 to 16:00	6.77^{b} ± 0.49	7.00^{de} ± 0.60
SEM (±)	0.036	0.034
CD at 0.05	0.100	0.094
Stage of the fruit		
M_1	5.56^{a} ± 0.46	12.17^{a} ± 0.73
M_2	4.71^{b} ± 0.47	9.67^{b} ± 0.62
M_3	3.67^{c} ± 0.24	7.47^{c} ± 0.44
SEM (±)	0.020	0.018
CD at 0.05	0.061	0.051

* M_1: 10 days after fruiting, M_2 : 20 days after fruiting, M_3 : 30 days after fruiting.
(Means followed by same letters do not differ statistically by DMRT at 0.05)

Fig-20: No of visit (Mean ± SE) by female *B. cucurbitae* on three different stages of pumpkin fruit during a day.

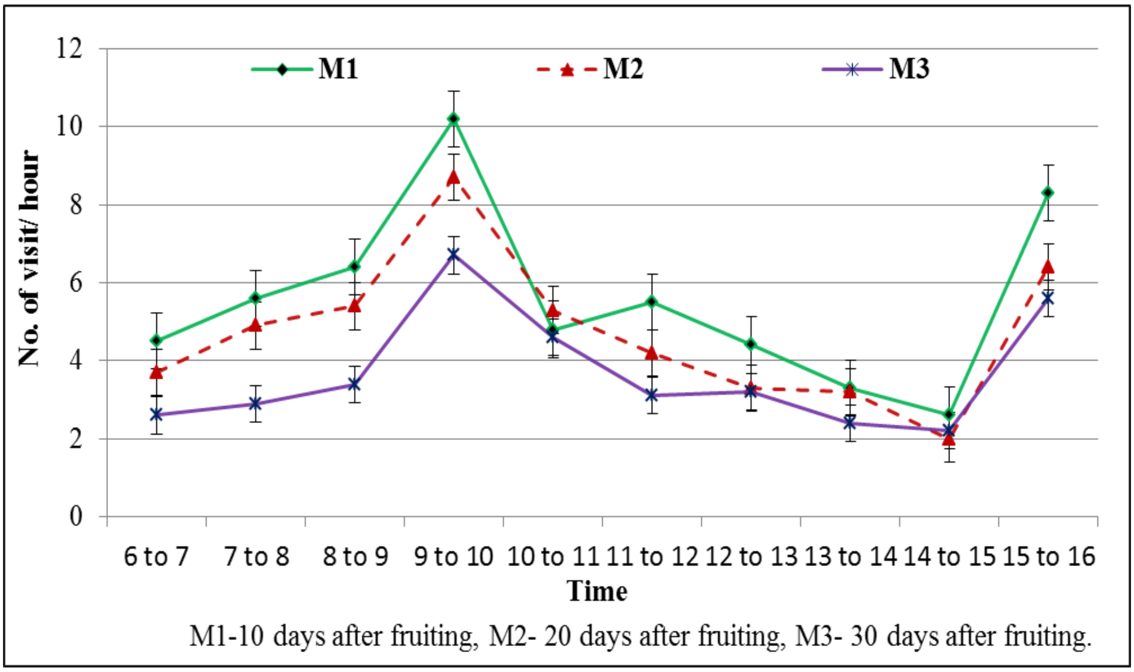
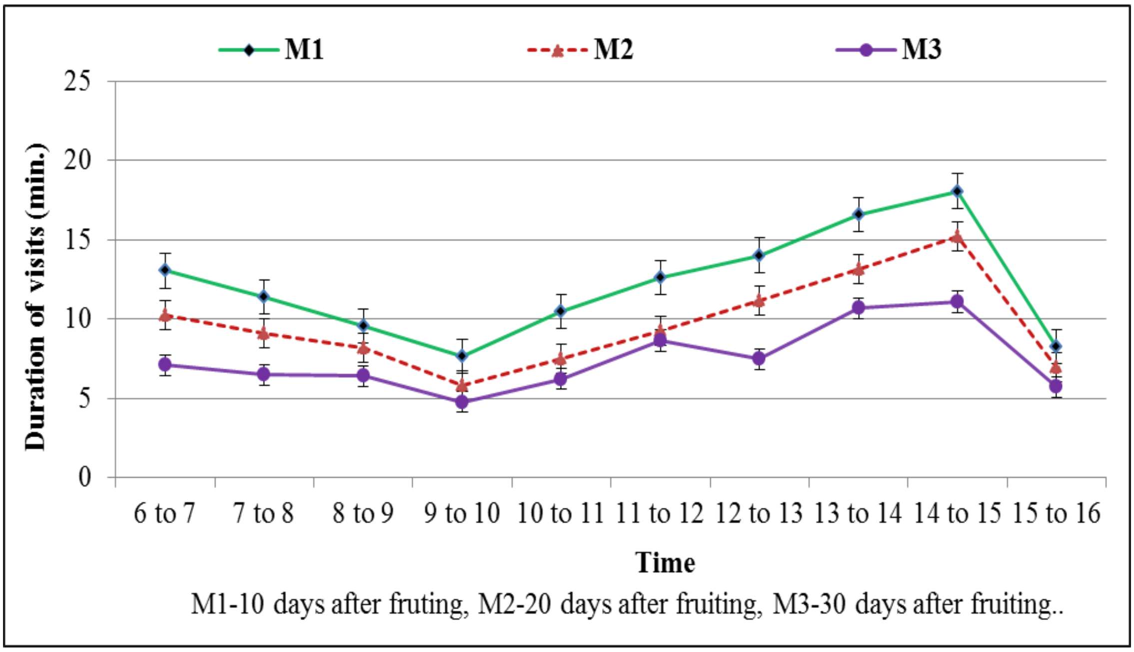


Fig-21: Duration (Mean ± SE) of hourly visitation by female *B. cucurbitae* on three different stages of pumpkin fruit during a day.



4.3.2. Frequency and duration of visits by adult female *B. dorsalis* on mango fruits of different maturity levels:

The observations with regard to number and duration of visit on different stages of mango fruits were also recorded at hourly interval starting from 06:00 hours and continued up to 16:00 hours. The experiment was repeated for 10 different days.

In each day one adult mated female fly at the age of 20-25 days old was taken and released in the rearing cage. Three mango fruits of different maturity levels (unripe, ripe and fully ripen fruits) were provided in the rearing cage at a time. Thus the fly was in a free choice condition in the cage. The observation with regard to number of visits per hour on mango fruits of different maturity level was recorded and presented in table-26.

It appeared from the table that there exist significant differences in number and duration of visits to fruit of different ripening stages across hourly time periods during the day. It has been observed that during mid morning i.e. after 08:00 hours number of visit of fly to fully ripe fruit (M_3) was higher than on unripe fruit (M_1). However, on ripe fruit (M_2) it was noted intermediate between the two (M_1 and M_3) (Fig-23). But, after late morning, there remain little differences in fly responses to different stages of fruits. The pattern of visits by flies to M_3 (fully ripen fruit) stage significantly differed from M_1 (unripe fruit) and M_2 (ripe fruit) stage of mango. The number and duration of visits both were found to vary significantly at different hours of the ($F_{0.05,9,270}$=13.713 for different hours of the day and $F_{0.05,2,270}$=47.332 for different stages of fruits) only. In case of duration of visit at hourly interval also significant differences were noted ($F_{0.05,9,270}$=20.012 for different hours of the day and $F_{0.05,2,270}$=153.02 for different stages of fruits), but there remain no significant interaction between diurnal pattern of the fly and stage of fruits ($F_{0.05,18,270}$=0.313) and duration of visit ($F_{0.05,18,270}$=0.504) by the female fly.

The mean data (±SE) showed that maximum number of visits (6.85±0.48 times per hour) was recorded at fully ripe stage (M_3) which was followed by M_2 (4.94±0.34 times per hour) and M_1 (3.23±0.19 times per hour) on unripe stage of mango fruit. Fly was also found to have more active and showed maximum number of visits (7.70±0.72 times per hour) during 9 to 10am. But after that the number of visit was gradually decreased during the day but slightly changing pattern was observed after mid day from 15:00 to 16:00 hours. it was increased up to 6.80±0.54 times per hour. The results also showed that the fly showed least number of visits (2.70±0.30 times per hour) or was less active during 14:00 to 15:00 hours. On the other hand, fully ripe stage (M_3) was found to be the most preferred stage by the fly (Table-26) followed by M_2 and M_1 stage.

Duration of visit (minutes per hour) by the adult female *B. dorsalis* during the day varied significantly on different stages of mango fruits (Fig-24). Average duration of visit was recorded highest (15.19±1.13 minutes per hour) during 14 to 15 hours of the day followed by 14.35±0.91 and 13.39± 0.91 minutes during 13 to 14 and 12 to 13 hours respectively. On the contrary, mean duration of visit was recorded minimum (7.04±0.67 minutes per hour) during 9.00 to 10 hours.

Significant difference in the duration of visits by fly to mango fruit of different ripening stage across hourly time periods during the day was also noted. The maximum time (15.37±0.35 minutes per hour) was spent by the female *B. dorsalis* fly on M_3 stage followed by in M_2 (10.38±0.54 minutes per hour) and M_1 (6.98±0.30 minutes per hour). Number of visit by the fly reflecting its diurnal pattern and it has been observed that the fly was more active during morning and afternoon hours but peak activity was recorded during mid day condition. During the observation period visitation number and duration of visits were always high on fully ripe fruit (M_1) than ripe (M_2) and unripe (M_1) stage of mango fruit.

Table-26: Number and duration of visits by adult female *B. dorsalis* on different maturity level of mango fruits.

Hours of Visits	Mean number of visits (Hourly)	Mean duration of visits (minutes per hour)
06:00 to 07:00	4.13^{d} ± 0.56	10.58^{c} ± 1.05
07:00 to 08:00	5.47^{c} ± 0.67	10.37^{c} ± 0.94
08:00 to 09:00	6.03^{bc} ± 0.63	9.93^{c} ± 0.85
09:00 to 10:00	7.70^{a} ± 0.72	7.04^{e} ± 0.67
10:00 to 11:00	5.93^{abc} ± 0.51	8.43^{d} ± 0.73
11:00 to 12:00	4.33^{d} ± 0.61	11.79^{bc} ± 0.96
12:00 to 13:00	3.80^{de} ± 0.47	13.39^{ab} ± 0.91
13:00 to 14:00	3.17^{de} ± 0.39	14.35^{a} ± 0.91
14:00 to 15:00	2.70^{e} ± 0.30	15.19^{a} ± 1.13
15:00 to 16:00	6.80^{ab} ± 0.54	8.05^{de} ± 0.74
SEM(±)	0.034	0.024
CD at 0.05	0.095	0.066
Stage of the fruit		
M1	3.23^{c} ± 0.19	6.98^{c} ± 0.30
M2	4.94^{b} ± 0.34	10.38^{b} ± 0.54
M3	6.85^{a} ± 0.48	15.37^{a} ± 0.35
SEM(±)	0.019	0.013
CD at 0.05	0.052	0.040

M1- Unripe, M2-Ripe, M3-Fully ripen

* Means followed by similar letters do not differ statistically by DMRT at 0.05.

Fig-22: No of visits (Mean ± SE) by female *B. dorsalis* on three maturity stages of mango fruit during a day.

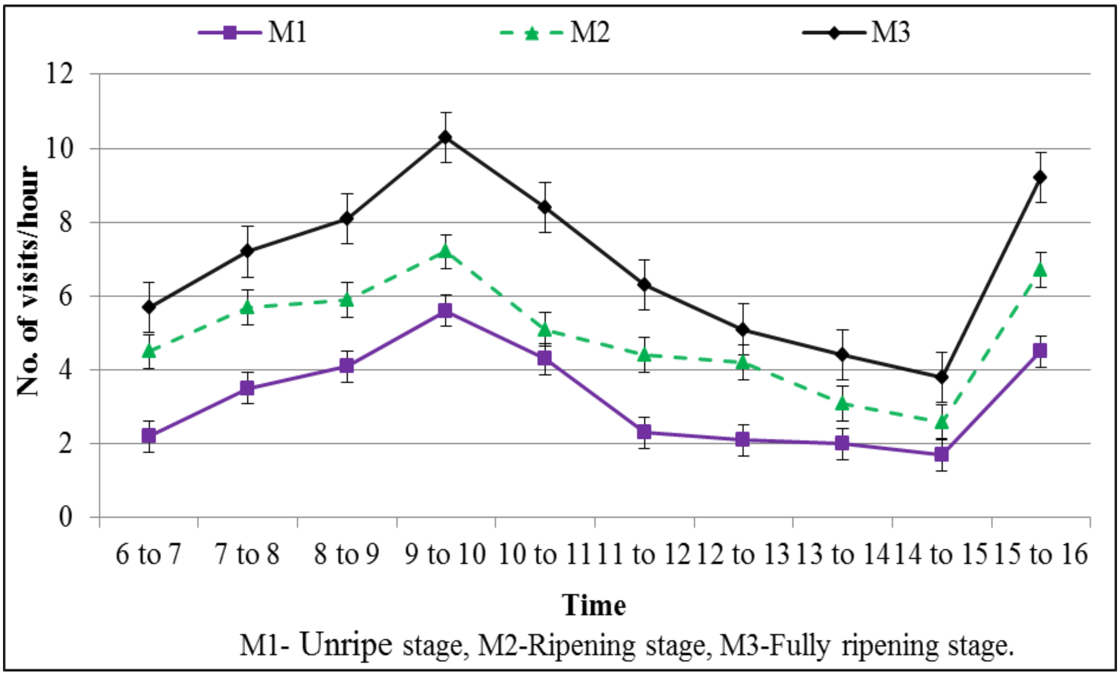
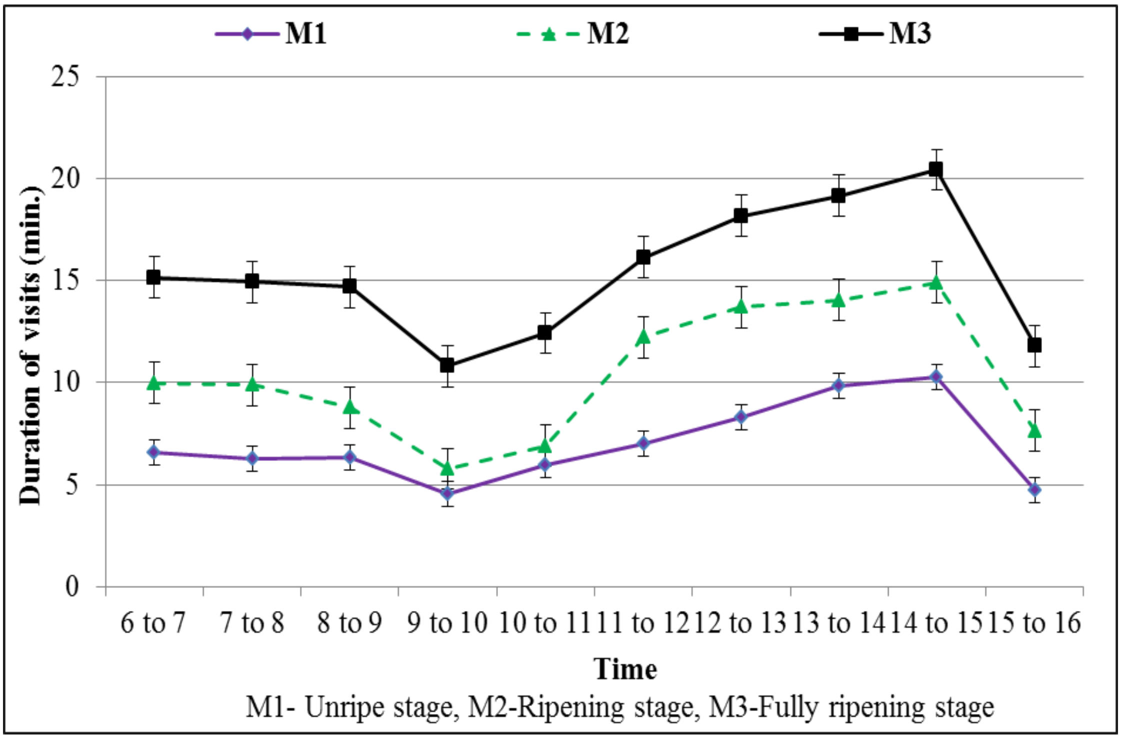


Fig-23: Duration (Mean ± SE) of hourly visitation of female *B. dorsalis* on three maturity stages of mango fruit.



4.4. Number and duration of visit by adult female *B. dorsalis* on different stages of artificial mango fruits:

Three artificial mango fruit at different maturity stages were taken to determine the role of visual cues in host fruit recognition and selection by *B. dorsalis*. The artificial fruits were bought from local market. Colour of the fruits were green (resembling unripe stage and indicated as M_1), yellow green (resembling ripe stage and indicated as M_2) and yellow (resembling fully ripening stage and indicated as M_3). Different coloured artificial fruits of each ripening stage of mango were placed in a insect rearing cage and then an individual 21-25 days old, mated female fly was released in the rearing cage. Observation on number and duration of visit on different fruits at hourly interval was recorded during the day. The experiment was repeated 10 days taking adult female fly separately. The data with regard to the same have been presented in table-27.

It appeared from the presentation that the mean (±SE) number of visits (at hourly interval) of the fly on different coloured artificial fruit were varied significantly ($F_{0.05,9,270}$=14.612) during the day. Maximum number of visit (8.83 ± 0.66) was recorded during 09-10 hours followed by 8.20± 0.84 times during 08-09 hours. On the contrary, minimum number of visit (3.23± 0.42 per hour) was noted during 14-15 hours followed by 3.50 ± 0.41 times per hour during 12-13 hours. This phenomenon reflects the intensity of activity of fly during the day and thus it may be inferred that the fly become more active during mid morning and afternoon hours than the mid noon hours. Number of visit at hourly interval were found significantly variable ($F_{0.05,2,270}$=32.421) among different coloured artificial fruits. On yellow couloured artificial (resembling fully ripe) fruit (M_3) it was noted greater than the green coloured artificial (resembling unripe) fruit (M_1). However, yellow green coloured artificial (resembling ripe) fruit (M_2) was detected in between the two (Fig-25 and 26).

Duration of visit by the fly on different coloured artificial mango fruit at hourly interval during the day were also varied significantly ($F_{0.05,9,270}$=20.039). From table-27 it is revealed that maximum time per hour (10.95 minutes) was spent by the fly on a fruit during 12.00-13.00 hours followed by 10.71, 9.99 and 9.67 minutes per hour during 13.00-14.00, 14.00-15.00 and 11.00.12.00 hours respectively. On the other hand, minimum time were spent by the fly during 09.00-10.00 hours followed by 5.07, 5.77 and 6.47 minutes per hour during 8.00-9.00, 15.00-16.00 and 10.00-11.00 hours respectively. Duration of visit per hour on different coloured artificial mango fruits were also varied significantly ($F_{0.05,2,270}$=60.471). More time per hour was spent on yellow colored artificial fruit (9.66±0.39 minutes) and least time was spent on green coloured artificial fruit (5.65±0.25 minutes per hour) indicating preference of yellow colour as compared to green.

Table-27: Number and duration of visits by adult female *B. dorsalis* on different maturity level of mango fruits.

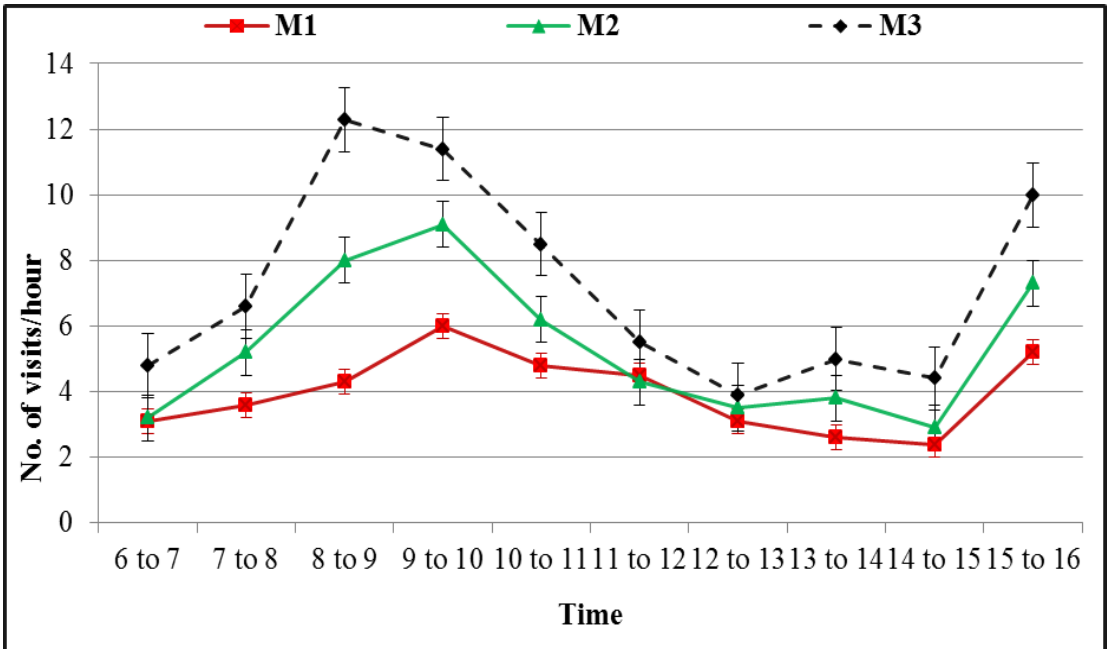
Hours of Visit	Mean number of visit (hourly)	Mean duration of visit (minutes per hour)
06:00 to 07:00	3.70^{ef} ± 0.53	8.11^{bc} ± 0.63
07:00 to 08:00	5.13^{cd} ± 0.55	7.59^{cd} ± 0.57
08:00 to 09:00	8.20^{ab} ± 0.84	5.07^{f} ± 0.42
09:00 to 10:00	8.83^{a} ± 0.66	4.93^{f} ± 0.48
10:00 to 11:00	6.50^{bc} ± 0.57	6.47^{de} ± 0.54
11:00 to 12:00	4.77^{de} ± 0.48	9.67^{ab} ± 0.79
12:00 to 13:00	3.50^{ef} ± 0.41	10.95^{a} ± 0.81
13:00 to 14:00	3.80^{def} ± 0.44	10.71^{a} ± 0.71
14:00 to 15:00	3.23^{f} ± 0.42	9.99^{a} ± 0.67
15:00 to 16:00	7.50^{ab} ± 0.68	5.77^{ef} ± 0.64
SEM (±)	0.037	0.027
CD at 0.05	0.103	0.075
Stage of the fruit		
M1	3.96^{c} ± 0.38	5.65^{c} ± 0.25
M2	5.35^{b} ± 0.26	8.47^{b} ± 0.35
M3	7.24^{a} ± 0.23	9.66^{a} ± 0.39
SEM (±)	0.020	0.015
CD at 0.05	0.060	0.046

M1- Green coloured artificial fruit model,
M2- Yellow green coloured artificial fruit model,
M3- Yellow coloured artificial fruit model.
*Means followed by same letters do not differ statistically by DMRT at 0.05.

Fruit flies damage fruits by puncturing and laying eggs under the soft skin in both mature and green fruits (Hollingsworth *et al.*, 1997). Earlier, several workers reported their findings with regard to preference of colour and stages of fruits in different parts of the worlds including India. Jang *et al.* (1998) reported that adult female of fruit flies prefers to visit semi-ripe fruits. In a study by Lopéz-Guillén *et al.*

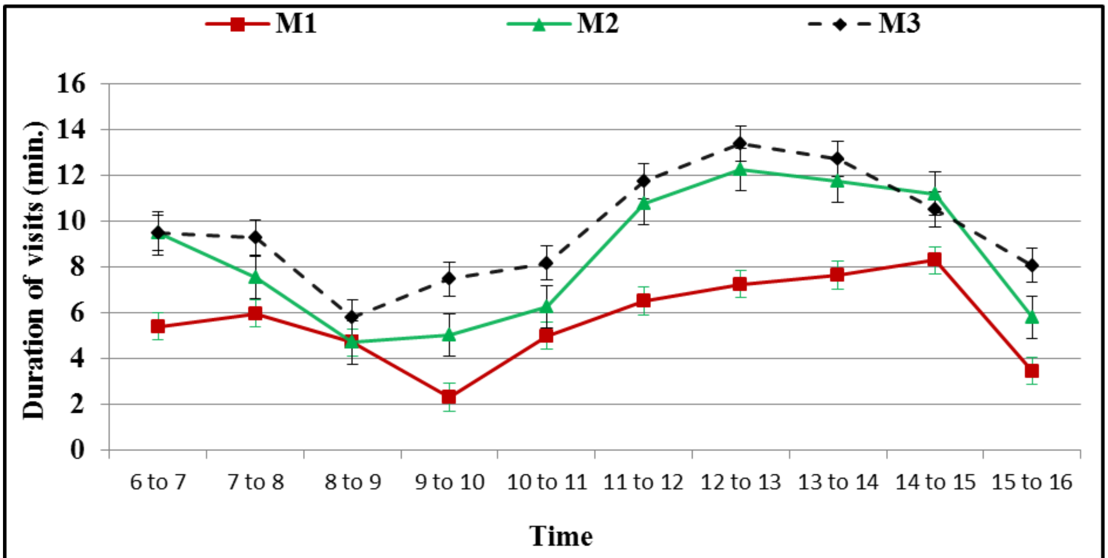
(2009) found that *Anastrepha obliqua* (Diptera: Tephritidae) were more attracted to lime-green, orange and yellow spheres than to red, black or white spheres.

Fig-24: No of visits (Mean ± SE) by female *B. dorsalis* on three artificial coloured mango fruits model during a day.



M1- Green, M2- Yellow green, M3- Yellow

Fig-25: Duration (Mean ± SE) of hourly visitation of female *B. dorsalis* on three maturity stages of artificial mango fruit model.



M1- Green, M2- Yellow green, M3- Yellow

De Oliveira *et al.* (2015) also reported that the fruit fly, *Anastrepha fraterculus* mostly preferred ripening stage over green-ripe and green stage of guava. Similar results were earlier found in others studies (Uchoa-Fernandes *et al.*, 2002). Ripening of fruits causes many fruit physiological and chemical changes (Bashir and Abu-Bakr, 2003; Yashoda *et al.*, 2007), which helps to provide favourable host environments for developing tephritid larvae (Leyva *et al.*, 1991). The effects of fruit ripening are largely consistent across fruit flies, for example increasing ripeness of mango is beneficial to larvae of both *A. suspensa* (Hennessey and Schnell, 2001) and *B. dorsalis* (Rattanapun *et al.*, 2009). Visual cues are important in short range (i.e. within one meter) host finding for *R. pomonella* (Prokopy and Roitberg, 1984) and this fly shows a higher propensity to lay eggs in fruit models whose sizes are similar to their natural hosts (Prokopy and Bush, 1973).

In the present study, variation in diurnal pattern of dominant fruit fly of northern part of West Bengal viz. *B. cucurbitae* was detected at hourly interval round the day with regard to number of visit on fruit per hour. The results are more or less in agreement with the previous findings and essence thereof. The same is true with respect to choice of fruit by the adult female melon fly. However, degree of preference of extend of variation in diurnal activity deviated from some studies which may be due to difference in the test insect or the fruits used for the study.

4.5. Impact of Entomopathogenic Nematode (EPN), *Heterorhabditis indica* (Poinar) on *B. cucurbitae* (Coq.):

It is very tough to manage tephritid fruit fly infestation on fruits and vegetables. These are internal feeder and it remain concealed during most of the period of its life cycle. Use of conventional pesticides do not usually show good results in suppressing its population. The tephritid flies drop down in soil from the infested fruits after being full fed for pupation. In this perspective, soil inhabiting entomoparasitic nematodes have good role to play for parasitising the maggots of fruit flies. Again, after infecting their larval host will multiply in soil itself and will act as a source of innoculum that have the chance for subsequent parasitisation of dropped maggots of fruit flies.

Considering this environmentally viable scope of Entomopathogenic nematodes (EPNs) in managing tephritid fruit flies in the present study the most dominant species of *Bactrocera* spp. like *B. cucurbitae* was selected for evaluating the efficacy of *Heterorhabditis indica* at different concentrations in laboratory condition. Role of soil moisture on it efficacy was also taken into account. The mature third instar larvae that are going to pupate were taken for the study.

For studying the impact of selected concentrations of the EPNs on mature maggots (third instar) of *B. cucurbitae* at different moisture level i.e. 5%, 10%, 15% and 20% altogether five treatments were taken at each level of soil moisture. Third instar maggots were taken for determining the efficacy of EPN concentration of IJs (infective juveniles) which were used in different treatments were as follows:

T_1 : 250 IJs/cm^2

T_2 : 150 IJs/cm^2

T_3 : 100IJs/cm^2

T_4 : 50 IJs/cm^2 and

T_5 : Control

The observations with regard to larval mortality, formation of deformed pupa, and emergence of adults were recorded at 24, 48 and 72 hours after treatment (HAT) at different moisture concentration.

4.5.1. Effect of different concentrations of *H. indica* on mature maggots of *B. cucurbitae* (Coq.) at different moisture levels 24 hours after treatment.

After treatment of soil in tray maintaining selected moisture levels the mature maggots were released. Observations with regard to larval mortality, deformed pupa and adult emergence in different treatment were recorded analysed and presented in table-28. It revealed from the data that the mean mortality percentage varied significantly at different EPN (*H. indica*) concentration in different moisture level. After 24 hours of treatment, two years pooled means mortality percentage varied from 2.67 -21.33%, 1.58-25.33%, 3.11-16.00% and 2.00-16.67% percent at 5%, 10%, 15% and 20% moisture level respectively. The highest larval mortality percentage was recorded 21.33% in T_1 (250 IJs/cm^2) followed by 18.00% in T_3 (100 IJs/cm^2) in the year 2014 (Table-28). In the next year (2015) the highest mortality **(**22.67%) was observed in treatment T_2. All the treatments exhibited significantly higher deformed pupae than the control (T_5). On the basis of pooled mean data, it showed highly significant percentage larval mortality i.e. 21.33% in T_1 (250 IJs/cm^2) and T_2 (150 IJs/cm^2) followed by 18.00% in $T_{3.}$ Both the treatments showed significantly higher mortality than all other treatments at 5% moisture content in soil.

At 10 percent moisture during 2014 the highest mortality of third instar larva of *B. cucurbitae* was recorded 22.67% in T_1 (250 IJs/cm^2) and was significantly higher than the other treatments. During 2015, the highest percent mortality of larvae (28.00%) was noticed in T_1 followed by 20.00% in T_2 and T_3. However, all the treatments were significantly superior in reducing the number of larvae over untreated control. With reference to the pooled mean (2014 and 2015), at 10 percent moisture level T_1 (250 IJs/cm^2) provided highest percent mortality (25.33%) followed by T_2 and T_3 (20.00%). In this case also, all the treatments were found to be significantly superior over untreated control.

Table-28: Impact of different concentrations of *H. indica* under different soil moisture on mortality percentage of mature maggots of *B. cucurbitae* 24 hours after treatment (HAT).

Treatment	Dose	5% moisture level			10% moisture level			15% moisture level			20% moisture level		
		2014	2015	Pooled	2014	2015	Pooled	2014	2015	Pooled	2014	2015	Pooled
T1	**250 IJs/cm^2**	21.33 (4.62)	21.33 (4.62)	21.33 (4.62)	22.67 (4.76)	28.00 (5.29)	25.33 (5.02)	13.33 (3.65)	18.67 (4.32)	16.00 (3.98)	16.00 (4.00)	17.33 (4.16)	16.67 (4.08)
T2	**150 IJs/cm^2**	20.00 (4.47)	22.67 (4.76)	21.33 (4.62)	18.67 (4.32)	21.33 (4.62)	20.00 (4.47)	16.00 (4.00)	16.00 (4.00)	16.00 (4.00)	16.00 (4.00)	14.67 (3.83)	15.33 (3.92)
T3	**100 IJs/cm^2**	14.67 (3.83)	21.33 (4.62)	18.00 (4.24)	13.33 (3.65)	26.67 (5.16)	20.00 (4.47)	10.67 (3.27)	17.33 (4.16)	14.00 (3.74)	12.00 (3.46)	10.67 (3.27)	11.33 (3.37)
T4	**50 IJs/cm^2**	13.33 (3.65)	16.00 (4.00)	14.67 (3.83)	10.67 (3.27)	20.00 (4.47)	15.33 (3.92)	8.00 (2.83)	12.00 (3.46)	10.00 (3.16)	12.00 (3.46)	10.67 (3.27)	11.33 (3.37)
T5	**Control**	4.00 (2.00)	1.33 (1.15)	2.67 (1.63)	2.67 (1.63)	0.5 (0.71)	1.58 (1.26)	2.67 (1.63)	2.67 (1.63)	3.11 (1.63)	1.33 (1.15)	2.67 (1.63)	2.00 (1.41)

* Figures within parenthesis are square root transformed values.

Factors	Dose	Moisture	Dose X Moisture
SEM (±)	0.151	0.135	0.302
CD at 0.05	0.426	0.381	0.851

D - Dose M - Moisture

When soil moisture was maintained at 15 percent, all the treatments were almost effective in exhibiting the higher percent mortality on mature maggots with regard to untreated control.

Maximum mortality rate (16.00%) was noticed in T_2 (150 IJs/cm^2) during 2014, but in the next year during 2015, the maximum mortality (18.67%) was observed in T_1 (250 IJs/cm^2) followed by 17.33% in T_3 (100 IJs/cm^2). Percent mortality on maggots for these two years were analyzed by pooled, and the highest mortality (16.00%) was observed in T_1 and T_2 (table-28). At 20 percent moisture level, the two years pooled data indicated that the efficacy of *H. indica* was significantly higher in T_1 (16.67%) followed by T_2 (15.33%) on mature maggots of fruit flies.

4.5.2. Effect of different concentrations of *H. indica* on mature maggots of *B. cucurbitae* at different moisture levels 48 hours after treatment.

In this case, the mature maggots of *B. cucurbitae* were released in the soil after 48 hours of treament with *H. indica*. Maximum efficacy of the entomopathogenic nematode (*H. indica*) was observed at 5 percent moisture level as compared to the other moisture levels. At 5% moisture level, the pooled mean data (table-29) indicated that the maximum mortality of maggots was recorded in T_1 (66.67%) followed by T_2 (61.67%) and T_3 (54.00%). In 10 percent moisture content of the soil T_1 with 250 IJs/cm^2 was found to be highly significant (69.33%) mortality than the other treatments. On the other hand, lower mortality was recorded in T_5 (control) (2.00%) followed by T_4 (44.67%). In case of 15% moisture level, the mean mortality percentage was increased in a parallel manner with the increase in EPN concentration. On the basis of two years pooled data, the highest mortality percentage of maggots was obtained in T_1 (51.33%) followed by T_2 (44.67%) and T_3 (36.67%). At 20% moisture level, higher mortality (40.00%) was recorded in T_1 as compared to others treatments.

Table-29: Impact of different concentrations of *H. indica* under different soil moisture on mortality percentage of mature maggots of *B. cucurbitae* 48 hours after treatment (HAT).

Treatment	Dose	5% moisture level			10% moisture level			15% moisture level			20% moisture level		
		2014	2015	Pooled	2014	2015	Pooled	2014	2015	Pooled	2014	2015	Pooled
T1	250 IJs/cm^2	68.00 (55.55)	65.33 (53.93)	66.67 (54.82)	62.67 (52.34)	76.00 (60.67)	69.33 (56.37)	49.33 (44.62)	53.33 (46.92)	51.33 (45.76)	40.00 (39.23)	40.00 (39.23)	40.00 (39.23)
T2	150 IJs/cm^2	56.67 (48.83)	66.67 (54.74)	61.67 (51.75)	50.67 (45.38)	62.67 (52.34)	56.67 (48.83)	44.00 (41.55)	45.33 (42.32)	44.67 (41.94)	34.67 (36.02)	40 (39.23)	37.33 (37.66)
T3	100 IJs/cm^2	48.00 (43.85)	60.00 (50.77)	54.00 (47.29)	46.67 (43.09)	61.33 (51.55)	54.00 (47.27)	36.00 (36.78)	39.33 (38.84)	36.67 (38.81)	28.00 (31.95)	34.67 (36.07)	31.33 (34.04)
T4	50 IJs/cm^2	45.33 (42.32)	48.00 (43.85)	46.67 (43.09)	37.33 (37.66)	52.00 (46.15)	44.67 (41.94)	33.33 (35.26)	36.00 (36.87)	34.67 (36.07)	26.67 (31.09)	33.33 (35.26)	30.00 (33.21)
T5	Control	12.00 (20.27)	4.00 (11.54)	8.00 (16.43)	1.33 (6.63)	2.67 (9.40)	2.00 (8.13)	5.33 (13.35)	5.33 (13.35)	5.33 (13.35)	2.67 (9.94)	4.00 (11.54)	3.33 (10.52)

* Figures within parenthesis are arch sine transformed values.

Factor	Dose (D)	Moisture (M)	D X M
SEM (±)	0.900	0.805	1.801
CD at 0.05	2.536	2.268	5.071

D - Dose M - Moisture

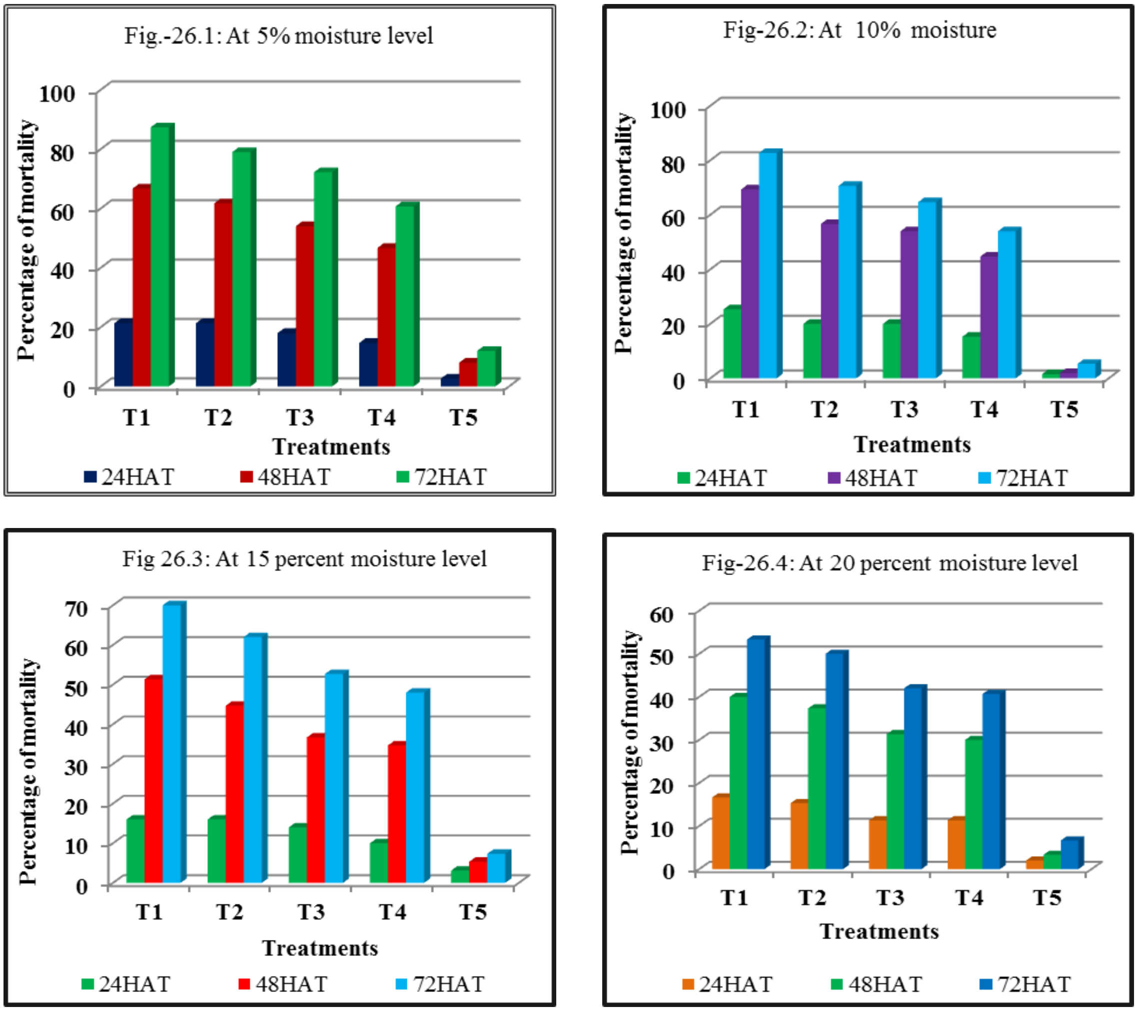
4.5.3. Effect of different concentrations of *H. indica* on mature maggots of *B. cucurbitae* at different moisture levels 72 hours after treatment.

The pooled mean data of the study conducted during 2014 and 2015 with regard to larval mortality after treating the soil with IJs of *H. indica* (EPN) in different moisture level are presented in table-30. Among the treatments, mortality percentage of larvae 72 hours after treatment at 5% soil moisture varied from 12.00% to 87.33%. The maximum mortality percentage of larvae was recorded in treatment T_1 (87.33%) followed by T_2 (79.00%) and T_3 (72.17%). At 10 percent soil moisture, the results showed that the maximum mortality was observed in T_1 (82.67%) followed by T_2 (70.67%) and T_3 (64.67%) on the basis of two years pooled data. However, at 15% and 20% moisture level, the mortality was recorded to be maximum in T_1 (70.00% and 53.33% respectively). Overall result revealed that the mean percentage mortality of larvae was observed to be maximum at 5% moisture level than at 10, 15 and 20% moisture level.

4.5.4. Effect of *H. indica* on pupation of *B. cucurbitae* maggots at different soil moisture:

The results presented in table-31 revealed that *H. indica* is effective in causing formation of deformed pupae of melon fly, *B. cucurbitae* (Coq.). However, this efficacy varied in different moisture content of soil where the maggots were allowed to pupate. Lower efficacy of the entomopathogenic nematode was recorded at 20% moisture content followed by 15%, 10% and 5% moisture content of soil. On the basis of pooled data at 5% moisture level, the percent deformed pupae varied from 2.67% to 10.67% at different concentrations of *H. indica*. The highest percentage of deformed pupae i.e. 12.00% was recorded in T_2 (150 IJs/cm^2) followed by 10.67% in T_3 (100 IJs/cm^2) and T_4 (50 IJs/cm^2) during 2014 (table-31). However, during 2015 the highest percent of deformed pupae (10.67%) was observed in treatment T_4. All the treatments exhibited higher percentage of deformed pupae than the control (T_5).

Fig-26: Larval mortality of *B. cucurbitae* (Coq.) at different concentration of *H. indica* in different soil moisture.



(HAT- Hours after treatment)

Table-30: Impact of different concentrations of *H. indica* under different soil moisture on mortality percentage of mature maggots of *B. cucurbitae* 72 hours after treatment (HAT).

Treatment	Dose	5% moisture level			10% moisture level			15% moisture level			20% moisture level		
		2014	2015	Pooled	2014	2015	Pooled	2014	2015	Pooled	2014	2015	Pooled
T1	250 IJs/cm^2	85.33 (67.48)	89.33 (70.94)	87.33 (69.15)	77.33 (61.57)	88.00 (69.73)	82.67 (65.40)	66.67 (54.74)	73.33 (58.91)	70.00 (56.79)	53.33 (46.92)	53.33 (46.92)	53.33 (49.91)
T2	150 IJs/cm^2	74.00 (59.34)	84.00 (66.42)	79.00 (62.73)	65.33 (53.93)	76.00 (60.67)	70.67 (57.21)	60.00 (50.77)	64.00 (53.13)	62.00 (51.94)	49.33 (44.62)	50.67 (45.38)	50.00 (45.00)
T3	100 IJs/cm^2	64.00 (53.13)	80.33 (63.67)	72.17 (58.16)	61.33 (51.55)	68.00 (55.55)	64.67 (53.21)	49.33 (44.62)	56.00 (48.45)	52.67 (46.53)	40.00 (39.22)	44.00 (41.54)	42.00 (40.38)
T4	50 IJs/cm^2	61.33 (51.55)	60.00 (50.77)	60.67 (51.16)	48.00 (43.85)	60.00 (50.77)	54.00 (47.29)	44.00 (41.55)	52.00 (46.15)	48.00 (43.85)	37.33 (37.66)	44.00 (41.55)	40.67 (39.62)
T5	Control	17.33 (24.60)	6.67 (14.96)	12.00 (20.27)	5.33 (13.35)	5.33 (13.35)	5.33 (13.35)	8.00 (16.43)	6.67 (14.96)	7.33 (15.71)	6.67 (14.96)	6.67 (14.96)	6.67 (14.96)

* Figures within parenthesis are arch sine transformed values.

Factor	Dose (D)	Moisture (M)	D X M
SEM (±)	0.851	0.761	1.702
CD at 0.05	2.396	2.143	4.793

D - Dose M - Moisture

On the basis of two years (2014 and 2015) pooled mean data, higher deformed pupae of melon fly (10.67%) were noted in T_4 (50 IJs/cm^2) followed by 8.67% in T_2 (150 IJs/cm^2) and T_3 (100 IJs/cm^2) which are statistically at par with each other. During 2014 at 10 percent soil moisture the highest percentage (12.00%) of deformed pupae of melon fly was recorded in T_2 (150 IJs/cm^2) and was significantly higher than the other treatments. During 2015, the highest percentage (13.33%) of deformed pupae was noticed in T_3 followed by 10.67% in T_4. All the treatments were found significantly superior in reducing the formation of healthy pupa of melon fly over untreated control. With reference to the pooled mean (2014 and 2015), at 10 percent moisture level T_3 (100 IJs/cm^2) provided highest (11.33%) deformed pupa followed by 10.67% in T_2 (150 IJs/cm^2).

At 15% soil moisture, all the treatments exhibited almost similar efficacy in forming deformed pupae over control. Maximum percent of deformed pupae (16.00%) was noticed in T_2 (150 IJs/cm^2) during 2014, but in the next year during 2015, the maximum deformed pupa (12.00%) was observed in T_3 (100 IJs/cm^2) followed by 9.33% in T_4 (50 IJs/cm^2). Percentage of deformed pupa for these two years was pooled together and highest deformed pupa (13.33%) was observed in T_3. However, at 20% moisture level, the maximum deformed pupa (9.33%) was noticed in T_1 (250 IJs/cm^2) followed by 8.00 in T_3 (100 IJs/cm^2) during 2014. In 2015, the maximum percentage of deformed pupa (6.67%) was noticed in T_1 (250 IJs/cm^2). On the other hand, 4.00% and 2.67% were recorded in T_2 and T_4 respectively. On the basis of two years pooled data, in T_1 with 250 IJs/cm^2 provided higher percentage of deformed pupae but was significantly higher than all other treatments.

4.5.5. Effect of treatments on adult emergence of *B. cucurbitae* (Coq.) at different moisture levels of soil:

The informations obtained from the study have been presented in table-32 showed that the use of EPN, *H. indica* was found to be effective in suppressing life cycle of *B. cucurbitae.* The mean mortality percent increased in a parallel manner with the increase in EPN concentrations (50, 100, 150 and 250 IJs/cm^2) respectively. Emergence of adult fruit flies were exhibited in the inverse order with the concentration of *H. indica.*

Table-31: Effect of *H. indica* under different soil moisture on the formation of deformed pupa of *B. cucurbitae*.

Treatment	Dose	5% moisture level			10% moisture level			15% moisture level			20% moisture level		
		2014	2015	Pooled	2014	2015	Pooled	2014	2015	Pooled	2014	2015	Pooled
T1	**250 IJs/cm^2**	6.67 (2.58)	4.00 (2.00)	5.33 (2.31)	6.67 (2.58)	4.00 (2.00)	5.33 (2.31)	13.33 (3.65)	8.00 (2.83)	10.67 (3.27)	9.33 (3.06)	6.67 (2.58)	8.00 (2.83)
T2	**150 IJs/cm^2**	12.00 (3.46)	5.33 (2.31)	8.67 (2.94)	12.00 (3.46)	9.33 (3.06)	10.67 (3.27)	16.00 (4.00)	6.67 (2.58)	11.33 (3.37)	6.67 (2.58)	4.00 (2.00)	5.33 (2.31)
T3	**100 IJs/cm^2**	10.67 (3.27)	6.67 (2.58)	8.67 (2.94)	9.33 (3.06)	13.33 (3.65)	11.33 (3.37)	14.67 (3.83)	12.00 (3.46)	13.33 (3.65)	8.00 (2.83)	4.00 (2.00)	6.00 (2.45)
T4	**50 IJs/cm^2**	10.67 (3.27)	10.67 (3.27)	10.67 (3.27)	6.67 (2.58)	10.67 (3.27)	8.67 (2.94)	8.00 (2.83)	9.33 (3.06)	8.67 (2.94)	6.67 (2.58)	2.67 (1.63)	4.67 (2.16)
T5	**Control**	2.67 (1.63)	2.67 (1.63)	2.67 (1.63)	2.67 (1.63)	1.33 (1.15)	2.00 (1.41)	4.00 (2.00)	2.67 (1.63)	3.33 (1.83)	2.67 (1.63)	2.67 (1.63)	2.67 (1.63)

* Figures within parenthesis are square root transformed values.

Factor	Dose (D)	Moisture (M)	D X M
S.Em	0.172	0.154	0.344
CD	0.484	0.433	0.969

D - Dose M - Moisture

The adult emergence of fruit flies from pupae was significantly higher in untreated control (T_5) because there was no treatment. The adult emergence percentage was significantly higher i.e. 85.33%, 91.33%, 89.33% and 90.33% respectively at 5%, 10%, 15% and 20% moisture level on the basis of two years pooled means (2014 and 2015) in control (T_5). At 5% moisture level, adult emergence noticed during 2014 was 80.00% and during 2015 percent adult emerged was 90.67 in T_5 (Untreated control). On the basis of pooled mean, the higher (85.33%) adult emergence was recorded in T_5 which was followed by 28.00% in T_4 and 20.00% in T_3. But very less emergence percentage was observed in T_1 (7.33%) which may be due to higher percent mortality of maggots.

Similar trend was observed and recorded in case of 10% moisture level of soil. Maximum adult emergence (91.33%) was also noticed in T_5 (Untreated control). The adult emergence was significantly higher in T_4 (35.33%) and T_3 (24.00%) but lesser than T_5. At 15% moisture level, in T_4 with 50 IJs/cm^2 48.00% adult emergence was obtained during 2014 and 41.33% adult emergence was obtained during 2015. On the basis of pooled data, the adult emergence was obtained 44.67% in T_4 followed by 34.00% in T_3 but lesser than 89.33% in T_5 (Untreated control).

On the basis of pooled mean data (Table-32), the adult emergence percentage was very less in T_1 (39.33%) followed by 44.67% in T_2. But the adult emergence percentage was very high in T_5 (90.33%) respectively because there was no biotic stress in that treatment. But the emergence percentage was very less in T_1 because this treatment was found to be very pathogenic and virulent regarding the mortality of fruit flies larvae.

Table-32: Effect of *H. indica* under different soil moisture on adult emergence of *B. cucurbitae*.

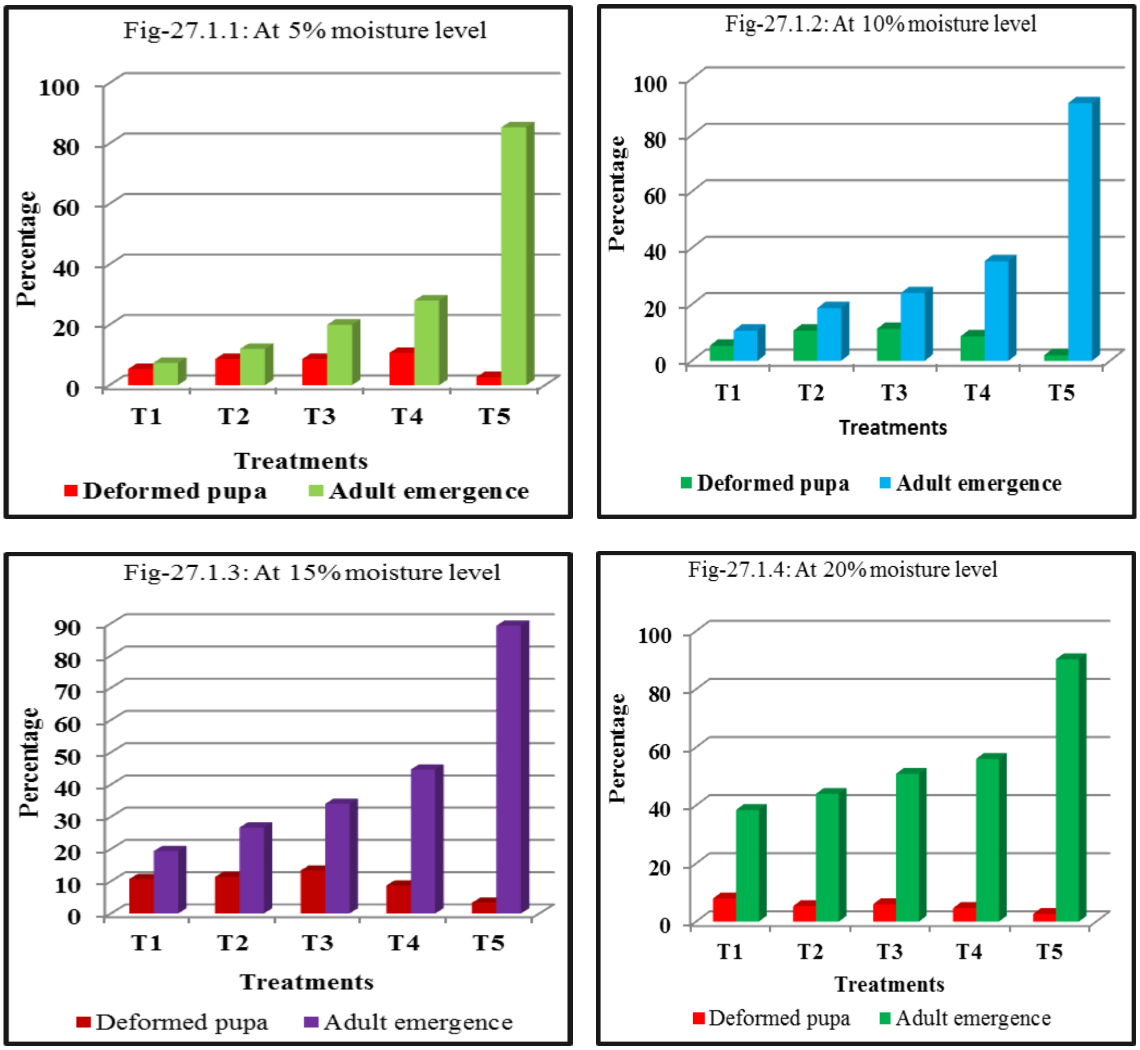
Treatment	Dose	5% moisture level			10% moisture level			15% moisture level			20% moisture level		
		2014	2015	Pooled	2014	2015	Pooled	2014	2015	Pooled	2014	2015	Pooled
T1	**250 IJs/cm^2**	8.00 (2.83)	6.67 (2.58)	7.33 (2.71)	16.00 (4.00)	5.33 (2.31)	10.67 (3.27)	20.00 (4.47)	18.67 (4.32)	19.34 (4.40)	38.67 (6.22)	40.00 (6.32)	39.33 (6.27)
T2	**150 IJs/cm^2**	13.33 (3.65)	10.67 (3.27)	12.00 (3.46)	22.67 (4.76)	14.67 (3.87)	18.67 (4.32)	24.00 (4.90)	29.33 (5.42)	26.66 (5.16)	44.00 (6.63)	45.33 (6.73)	44.67 (6.68)
T3	**100 IJs/cm^2**	25.33 (5.03)	14.67 (3.83)	20.00 (4.47)	29.33 (5.42)	18.67 (4.32)	24.00 (4.90)	36.00 (6.00)	32.00 (5.66)	34.00 (5.83)	51.33 (7.16)	52.00 (7.21)	51.67 (7.19)
T4	**50 IJs/cm^2**	28.00 (5.29)	28.00 (5.29)	28.00 (5.29)	45.33 (6.73)	25.33 (5.03)	35.33 (5.94)	48.00 (6.93)	41.33 (6.43)	44.67 (6.68)	56.00 (7.48)	53.33 (7.30)	54.67 (7.39)
T5	**Control**	80.00 (8.94)	90.67 (9.52)	85.33 (9.24)	92.00 (9.59)	90.67 (9.52)	91.33 (9.56)	88.00 (9.38)	90.67 (9.52)	89.33 (9.45)	90.67 (9.52)	90.00 (9.45)	90.33 (9.49)

* Figures within parenthesis are square root transformed values.

Factor	Dose (D)	Moisture (M)	D X M
S.Em	0.766	0.685	1.531
CD	2.156	1.928	4.312

D - Dose M - Moisture

Fig-27.1: Impact of *H. indica* (Poinar) on pupation and adult emergence of *B. cucurbitae* (Coq.) at varied soil moisture level.



It has been shown that *H. indica* concentration and soil moisture levels are important factors for influencing larval and pupal mortality for many tephritid species (Neuenschwander *et al.*, 1981, Alyokhin *et al.*, 2001 and Dimou *et al*, 2003). Result from this study confirms that this also to be the case with *B. cucurbitae*. The relationship between the concentration of IJs (infective juveniles) of EPN and soil moisture is not simple, with a significant interaction between these two variables in the present experiments. The obtained results emphasized that the entomopathogenic nematode could be used successfully in suppressing fruit fly population in the agro-ecosystem. The non-feeding, third-stage infective juvenile of nematodes (IJs) is the only stage that survives outside of the host. The infective juvenile carries cells of symbiotic bacteria in its intestine. When the IJ finds a suitable host, it invades and enters into the host's heamocoel through the natural openings and releases the bacteria that kill the host within 48 hours. These bacteria produce antibodies that prevent other micro-organisms from colonizing the cadavers of host. Furthermore, serving as a food source for nematodes, the bacteria digests the host tissues, thereby providing suitable nutrients for the nematode growth and development (Ehlers, 2001).

Earlier studies also confirmed that the entomopathogenic nematodes were effective and virulent on the larvae and pupae of fruit flies. This is in the same trend with Gaugler and Kaya (1990) who mentioned that EPN of genera *Heterorhabditis* and *Steinernema* (Nematoda: Rhabditidae) have emerged as an excellent insect biocontrol agents. Also it agrees with other attempts concerning the infectivity of EPN on the peach fruit fly, *Bactrocera zonata* (Attala *et al.*, 2002), the cucurbit fly, *Dacus ciliatus* (Fetoh and El-Gendi, 2006), peach fruit fly, *Bactrocera zonata* and the med fly *Ceratitis capitata* (Soliman, 2007a). Fetoh *et al.* (2011) also reported that mortality rates ranged from 67.3 to 100%, from 46.3 to 100% for the full grown larvae of both *B. zonata* and *D. ciliatus* treated by *H. indica* nematode whereas mortality rates of pupae ranged from 12.7 to 51.7 % and 6.3 to 39.3% for the pupae of *B. zonata* and *D. ciliatus* treated by same nematode.

Entomopathogenic nematodes are lethal parasites of soil dwelling insects that are being used for the biological control of several agricultural and ornamental crops in many countries (Hominick, 2002 and Grewal *et al.*, 2005). The success of nematode applications for insect control in soil depends on the IJ's ability to disperse and persist in the soil until it can locate a host (Kung *et al.*, 1991 and Koppenhöfer *et al.*, 1995). Their dispersal and persistence in the soil, in turn, depends upon many abiotic environmental factors, such as soil moisture, temperature, and soil texture (Ames, 1990 and Kung *et al.*, 1991). Of these, the moisture condition of soil have been recognized as one of the most important factors in the soil environment affecting survival, virulence and persistence of nematodes (Curran, 1993). For instance, nematodes may become dormant at very low soil moisture; on the other hand they may not be able to move freely at very high soil moistures (Grant and Villani, 2003).

It is therefore, evident from this study that the EPN, *H. indica* established in the host at low soil moisture. However, the etomopathogenic nematodes could establish in host at 5% and above soil moistures, though their establishment rate varied. At 5% soil moisture, insect mortality was found to be maximum for *H. indica*. However, at 5% and above soil moisture, 87.33% mortality of insect larvae was recorded in *H. indica* species studied. Taken as a whole, our study clearly indicates that establishment of EPNs vary markedly with different soil moistures.

The results of present study are in agreement with Yadav and Lalramliana (2012) who observed that the soil moistures had significant effect on the establishment of *H. indica.* The author also noted that with regard to the mortality of insect larvae at 72 hr exposure time, only *H. indica* was able to cause insect mortality (33.33%) followed by *S. thermophilum* (40.50%) at 5% soil moisture. Also, Koppenhöfer *et al.* (1995) studied the effect of different soil moistures on pathogenicity of *S. carpocapsae* and *S. glaseri* and observed that considerable establishment of IJs of *S. carpocapsae* occurred at 4-5% moistures, however, nematodes establishment declined at the highest soil moisture studied (19%). In the present study, *H. indica* could not establish at above 20% soil moisture and its highest establishment was observed at 5% soil moisture.

CHAPTER-5

Summary & Conclusion

Chapter-5

SUMMARY AND CONCLUSION

Species diversity:

Fruit flies were collected by using different kinds of traps like methyl eugenol, cuelure and food bait traps at Cooch Behar, Alipurduar, Jalpaiguri and Darjeeling district of northern part of West Bengal. Fruit flies have also been collected from the infested fruits. Observation with regard to species diversity of tephritid specimen revealed that maximum catch was obtained by using methyl eugenol and cuelure trap. In all the instances methyl eugenol collections were dominated by *B. dorsalis* (77.28% Cooch Behar, 81.23% Alipurduar, 73.49% Jalpaiguri and 82.96% Darjeeling district) whereas cuelure collections were dominated by *B. cucurbitae* (70.40% Cooch Behar, 71.28% Alipurduar, 77.40% Jalpaiguri and 78.67% Darjeeling district.

In case of food bait trap by using Banana pulp + sugar (1:1) and Jack fruit pulp + sugar (1:1), *B. dorsalis, B. correcta* and *B. nigrotibialis* were detected in the plains of Northern part of West Bengal (Cooch Behar, Alipurduar, Jalpaiguri) but *B. minax* was noted only in Darjeeling district. However, in case of species diversity recorded from infested fruit (mango, guava and mandarin orange) *B. dorsalis* was observed to be dominant species in Cooch Behar, Alipurduar and Jalpaiguri district and in Darjeeling district only two species were recovered viz. *B. dorsalis* and *B. minax.* Among them, *B. minax* was found to be dominant species recovered from mandarin orange. *B. cucurbitae* dominated in the recovered specimens from infested cucurbitaceous vegetables viz., pumpkin, bitter gourd and bottle gourd.

Intensity of infestation:

Studies on intensity of infestation by fruit fly (*Bactrocera* spp.) on different fruit and vegetables revealed that highest fruit infestation on mango was found to be 13.67% at Chilkir hat locality while lowest i.e. 7.83% at Pundibari. Intensity of infestation on guava was noted highest (26.67%) at Posarir hat locality ($23^0$73′N, 88^0 23′E) and lowest (10.50%) at Matigara ($26^0$72′N, $88^0$38′E) and Kharibari ($26^0$55′N, $88^0$19′E) area.

On bitter gourd 28.83% fruit infestion was recorded at Posarir hat ($23^0$73′N, 88^0 23′E) in Cooch Behar district and 20.17% at Falakata ($26^0$53′N, $89^0$19′E) in Jalpaiguri district. Intensity of infestation on Ridge gourd was ranged from 22.17% (at Chandmoni, $25^0$26′N, $88^0$02′E) to 30.67% (at Sonapur, $26^0$46′N, $89^0$39′E). Again, on pointed gourd minimum infestation was detected that ranged from 2.00-7.50%.

Considerable intensity of fruit fly infestation on mandarin orange was noted in Darjeeling district. The study revealed highest infestation (41.00%) at Lower Dungra locality and lowest was observed at Bom Bosti (21.22%).

Role of visual cues in host plant selection and identification of the preferred stage(s) of fruit(s):

Observation on frequency and duration of visit by adult female *B. Cucurbitae* revealed that maximum number of visits per hour (8.53 ± 0.60) was recorded in mid-morning i.e., 09.00 to 10:00 hours, which indicates that the fly remain more active during morning, mid morning as well as afternoon hours. Flies spent more time (14.79 ± 1.15 minutes per hour) on different maturity level of fruits from 14:00 to 15:00 hours. Mean duration of visits was highest on M_1 (10 days after fruiting) and least on M_3 (7.47 ± 0.44 minutes per hour).

In mango, it was found that the maximum number of visits (6.85± 0.89 times per hour) was recorded at fully ripe stage (M_3) followed by M_2 (4.94 ± 0.77 times per hour) and M_1 (3.23 ± 0.63 times per hour) on unripe stage of mango fruit. Fly was found to be more active and showed maximum number of visits (7.70 ± 0.72 times per hour) during 09:00 to 10:00 hours. The maximum time (15.37 ± 0.35 minutes per hour) was spent by the female *B. dorsalis* fly on M_3 stage followed by M_2 (10.38 ± 0.54 minutes per hour) and M_1 (6.98 ± 0.30 minutes per hour). However, during the observation period visitation number and duration of visits were always high on fully ripe fruit (M_1) was found to be the most preferred stage by the fly followed by M_2 and M_1 stage.

Duration of visit by the fly on different coloured artificial mango fruit model varied significantly ($F_{0.05,2,270}$=60.471). More time per hour was spent on yellow coloured artificial fruit model (9.66± 0.39 minutes per hour) and least time was spent on green coloured artificial fruit (5.65 ± 0.25 minutes per hour) indicating preference of yellow colour as compared to green.

Entomopathogenic nematodes, *H. indica* in managing fruit fly:

Analysis on impact of entomopathogenic nematode, *H. indica* on *B. cucurbitae* revealed that maximum percentage of mortality of third instar maggots at 24 hours after treatment was found maximum at T_1 (250 IJs/cm^2) at 5% and 10% moisture level of soil. At 15% moisture level, the maximum mortality percentage was observed in T_1 (250 Ijs/cm^2) and T_2 (150 IJs/cm^2) (16.00% on pooled basis). At 20% moisture level, the maximum percentage mortality was found in T_1 followed by T_2. At 48 hrs. after treatment, the maximum efficacy of *H. indica* was observed at 5% moisture level in T_1 (66.67%). In 10% moisture content in T_1 with 250 IJs/ cm^2, 69.33% mortality of maggots was noted. At 15% moisture level, highest mortality obtained in T_1 (51.33%). At 20% moisture level, higher mortality was recorded in T_1 (40.00%). Lower mortality was recorded in T_5 (Control) (3.33%). However, at 72 hours after treatment, the maximum mortality percentage of larvae was observed to be maximum at 5% moisture level then at 10, 15 and 20% moisture level concentration.

Effect of *H. indica* on pupation of *B. cucurbitae* maggots revealed that the higher percentage of deformed pupae was recorded in T_4 (50 IJs/cm^2) (10.67%) at 5% moisture level. At 10% moisture level, higher percentage of deformed pupae was recorded in T_3 (100 IJs/cm^2) (11.33%). At 15% moisture level, highest percentage of deformed pupae was recorded in T_3 (100 IJs) (13.33%). At 20% moisture level, the maximum deformed pupa was observed in T_1 (250 IJs/cm^2). On the basis of effect of treatments on adult emergence of *B. cucurbitae* at different moisture levels of soil it was observed that at 5% moisture level, the highest percentage of adult emergence was noticed in T_5 (Untreated Control) because there was no biotic stress in that treatment.

Thus, it may be concluded that a diverse number of tephritid fruit fly species are present in the terai and hilly agro-ecological region of West Bengal. The flies cause serious yield reduction in several vegetable and fruit crops grown commercially under the region. In the present study it has also been reconfirmed that certain visual cues as well as stages of fruit maturity affect fruit preference by the fly. Again, for sustainable management of this dreaded insect pest, entomoparasitic nematode, *Heterorhabditis indica* which is an obligate parasite having symbiotic association with bacteria have been found effective. It produced a good larval mortality, suppressed fruit fly population and hence appeared promising as a good biological tool for future IPM.

CHAPTER-6

Future Scope of Research

Chapter-6

FUTURE SCOPE OF RESEARCH

It is needless to state that, past always envisage the future and throws light on the lacunae, short comings etc. if there is any, in previous findings. Therefore, present study would act as spectacles to see the future inventions. In the present investigation, quite a substantial information regarding not only the species diversity of tephritid fruit flies and extent of fruit infestation of different cucurbitaceous vegetables viz., bitter gourd, ridge gourd, pointed gourd and some important fruits viz. mango, guava and citrus but also preference of different stages of fruits and impact of *H. indica*. Species composition of tephritid fruit flies that infest and likely to infest the cucurbitaceous vegetables and some fruits in northern tract of West Bengal have been determined through rearing of infested fruits as well as using sex attractants like Cuelure and Methyl eugenol as well as recovering from infested fruits.

The information would be very much helpful in formulating sustainable and biorational management programme befitting for the agroecological region under consideration.

However, several others aspects need to be studied further so as to refine or upgrade the present status of information with regard to the tephritid fruit flies. Some of the important aspects are stated hereunder:

- More number of vegetable as well as fruit crops may be tried so as to assess the species composition of fruits flies that infest cucurbits in the agro-ecological region under consideration. Other food baits may also be utilised for that purpose.
- Other alternate hosts of the flies need to be investigated and for that purpose recovering of adult flies from fruits of wild plants may also be undertaken.
- More emphasis is needed to be given on effective entomopathogenic nematodes for sustainable and effective management of the fly. Other EPNs may also be tried in addition to *H. indica*.
- Some more promising attractants and poison baits etc. which are selective and safe to non-target organisms may be explored.
- Cost benefit ratio of these alternative technologies need to be calculated.

- Ovipositional preference by the female gravid adult flies need to be assessed.
- Studies on non-chemical management modules are needed to be explored further in the agro-ecological region under consideration.

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Chapter-7

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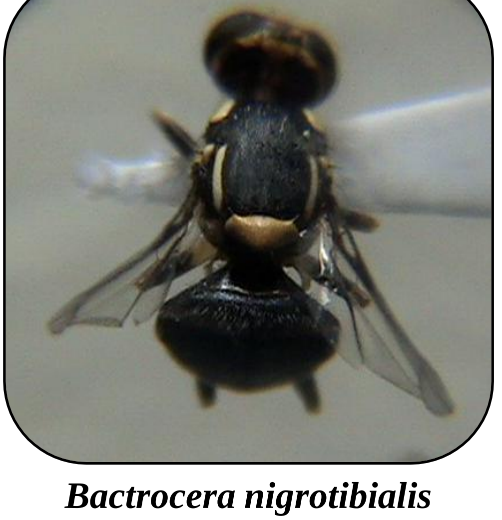
PLATE-8
Species diversity of fruit flies

Bactrocera cucurbitae

Bactrocera dorsalis

Bactrocera correcta



Bactrocera nigrotibialis

Bactrocera diversa

PLATE-9
Species divesity of fruit flies

Bactrocera minax

Bactrocera versicolor

Bactrocera zonata

Bactrocera caudata

Bactrocera tau

PLATE-1
Use of traps for fruit fly collection

Food bait trap

Cuelure trap

Methyl eugenol trap

Methyl eugenol trap

PLATE-2

Collection of infested fruits for fly recovery

Collection of bitter gourd

Infested bitter gourd

Collection of ridge gourd

Infested ridge gourd

Collection on pointed gourd

Infested maturity stage of pointed gourd

PLATE-3

Collection of infested fruits for fly recovery

Collection of guava fruit

Infested Guava

Fruit fly infesting ridge gourd

Infested bottle gourd

Infested tender bottle gourd

Infested tender pumpkin

PLATE-4

Collection of infested fruits for fly recovery

Bearing mandarin orange plant

Harvested Mandarin orange

Infested mandarin orange

Maggots from infested fruit

PLATE-5

Preference of fruit fly on mango fruit models


Green model


Yellow green model


Yellow model

Study on preference of different colored mango fruit model in insect rearing cage

PLATE-6

Different stages of pumpkin fruits

10 days after fruiting

20 days after fruiting

30 days after fruiting

Study on preference of fruit fly on different maturity stages of pumpkin fruit in insect rearing cage

PLATE-7

Extraction and application of *H. indica* on fruit fly maggots

Foam within vial containing
IJs of *H. indica*

Extraction of IJs in water

Healthy maggots before
release

Release of maggots in treated soil

PLATE-10

Impact of *H. indica* on fruit fly maggots

Infected maggots 24 hours after releasing in EPN treated soil

Infected maggots 48 hours after releasing in EPN treated soil

Infected maggots 72 hours after releasing in EPN treated soil

Healthy and infected fruit fly pupae