# Evaluation of Sex Pheromones and Plant Essential Oils Combination as Rubber Foam Dispenser for Controlling Fruit Fly (Diptera: Tephritidae)

**Most. Mottakina Akter**

**A Thesis Submitted in Fulfillment of the Requirements for the Degree of Master of Science in Entomology**
**Prince of Songkla University**
**2020**

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**Author** Mrs. Most. Mottakina Akter

**Major Program** Entomology

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This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgment has been made of any assistance received.

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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

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## Abstract

Tephritid fruit flies are one of the most devastating pests and concerning issues across the world. Among tephritid fruit flies, *Bactrocera dorsalis* and *Zeugodacus cucurbitae* are critical in terms of damage. Male lures like methyl eugenol (ME) and cue lure (CL) along with an insecticide are commercially used to attract and kill these fruit flies, which may contribute to excessive use of pesticides and is not environmentally safe. This study aimed at male annihilation of tephritid fruit flies through trapping without insecticides. It focused on using mixed lures on rubber foam, selecting the best plant essential oil, killing tephritid fruit flies using an eco-friendly method, and testing it in laboratory conditions, as well as combining it with single and mixed lures in field conditions. Finally, investigating the best proportion of these two lures to attain a satisfying result in controlling these fly species in a mixed cropping system. Five experiments were conducted to fulfill the aims of the study.

Experiment results: i) single ME traps were more effective in attracting *Bactrocera dorsalis* than mixed lure traps. ME+CL combination worked very well compared to CL alone for *Zeugodacus cucurbitae*. Moreover, mixed lures caught more species than single lures in the field. ii) Among plant essential oils of basil, clove, citronella, eucalyptus, and basil oil performed best in terms of weathering of the essential oil in rubber foam. iii) For *B. dorsalis,* ME alone and ME mixed with basil oil gave 15.7-20.3% attractancy. For *Z. cucurbitae*, attractancy of CL mixed with basil oil (9.3% attractancy) was lower than CL alone (20.3% attractancy) and ME + CL (21.5% attractancy). iv) It was found that in both cases, mixing and separate placing of ME and CL (ME : CL), for *B. dorsalis* only, ME (100 : 0) without CL, showed the highest number trapped. However, for *Z. cucurbitae*, the highest number of trapped flies in 25

: 75 traps in both cases while separate combination was significantly different from only CL traps. v) Surprisingly, in-field test for *B. dorsalis* ME traps (5.5 ±1.0 FTD) was more effective than that mixed with basil oil (3.8 ± 0.8) and mixed lures (2.0 ± 0.0 FTD). In the case of *Z. cucurbitae,* that of both CL (0.4 ± 0.0 FTD) and ME + CL (0.4 ± 0.1 FTD) were equally effective as basil oil (ME + CL/BO) (0.2 ± 0.0 FTD).

Overall, mixed lures (ME + CL) could be a good choice in trapping *Z. cucurbitae* in mixed cropping. However, ME alone represents the most appropriate trap to catch *B. dorsalis*.

**Keywords:** *Bactrocera dorsalis,* mixed lure, plant essential oil, rubber foam, Tephritidae, *Zeugodacus cucurbitae*

**Acknowledgement**

All the admirations are for **ALLAH** (The Most Beneficent, The Most Merciful) the lord of the whole universe, for whom all my heartfelt appreciations on accomplishing this compulsory task, Who has blessed me and enabled me to have success. I pay humble gratitude to **Holy Prophet MUHAMMAD** (peace be upon him), who is the forever idol and guidance of entire humanity.

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Most. Mottakina Akter

**Table of Content**

## List of Figures

**List of Tables**

## List of Abbreviations and Symbols

| | | |
|---|---|---|
| ANOVA | : | Analysis of Variance |
| AST | : | Average Survival Time |
| APPPC | : | Asia and Pacific Plant Protection Commission |
| CABI | : | Centre for Agriculture and Bioscience International |
| CL | : | Cue Lure |
| COSAV | : | Comite de Sanidad Vegetal Del Cone Sur |
| EO | : | Essential Oil |
| EPA | : | Environmental Protection Agency |
| EPPO | : | European Plant Protection Organization |
| FTD | : | Flies per Trap per Day |
| HSD | : | Highly Significant Difference |
| IAPSC | : | Inter-African Phytosanitary Council |
| IPM | : | Integrated Pest Management |
| MAT | : | Male Annihilation Technique |
| ME | : | Methyl Eugenol |
| OAE | : | Office of Agricultural Economics |
| OIRSA | : | Organismo Intemacional Regional de Sanidad Agropecuaria |
| SIT | : | Sterile Insect Technique |
| STDF | : | Standards and Trade Development Facility |
| USDA | : | United States Department of Agriculture |

# Chapter I

# Introduction

## 1.1 Research background

Insects belong to family Tephritidae of order Diptera is commonly known as fruit flies for their close intimacy with fruits of the host plants causing enormous qualitative and quantitative losses in fruit and vegetable crops worldwide (Ekeshi and Billah, 2007). These flies are also named as peacock flies due to their strutting behavior and for their spotted and striped wing vibration. Majority of the tephritid flies are highly polyphagous in nature and infest a wide range of economically important fruits and vegetables like mango, apple, pear, peach, guava, banana, citrus, pepper, avocado, tomato, passion fruit, plum, star fruit, melon and different cucurbits (White et al., 1994; Liu et al., 2013). Besides, some fruit flies are oligophagous (Danne and Johnson, 2010). There are nearly 5,000 known species of tephritid fruit flies all over the world; almost 200 of them are considered as pests of fruits and vegetables (Georgen et al., 2011; Ekeshi et al., 2016). However, about 70 species are regarded as agriculturally invasive pests throughout the world (Clarke et al., 2005). Subfamily Dacini includes 932 defined species (Doorenweerd et al., 2018). Top three invasive fruit flies' genera are *Bactrocera, Dacus,* and *Zeugodacus* under this subfamily, which are serious pests of horticultural crops. Tephritid fruit flies caused economic losses ranged between 30% and 100% on cucurbit crops (Dhillon et al., 2005; Fabre et al., 2003; Gnanvossou et al., 2008), 40-75% on mango and other fruits (Goergen et al., 2011; Ekesi et al., 2016). They cause considerable damage to major exporting crops such as mango, avocado, cucurbits up to 40-80%.

The oriental fruit fly *Bactrocera dorsalis* (Hendel) is the most prevalent and extensively distributed species among fruit flies (Vargas et al., 1990, 2000) originated in tropical and subtropical regions of Asia. It is a phytophagous pest having a wide range of host plants consisting of over 250 fruits and vegetables (Huang and Han, 2005). *Bactrocera dorsalis* has a high dispersal ability, can move within attacked fruits and vegetables, consequently invade and adapt to a new locality very rapidly (Wan et al., 2012). Recently, this pest is getting more attention in the agricultural sector because of the high cost of production and strict biosecurity regulations (Khamis et al., 2009; Aketarawong et al., 2014). Moreover, *B. dorsalis* is a threat to the natural ecosystem and biodiversity of occupied regions as they supersede other fruit fly species in their habitat (Aketarawong et al., 2007; Zeng et al., 2018). The melon fly, *Zeugodacus cucurbitae* (Coquillett), is at the second position in prevalence and abundance among fruit fly species (Vargas et al.,1990, 2000). This species is first seen in India (Bezzi, 1913) and reported as native to Central Asia (Virgilio et al., 2010). This pest is highly prolific and infests around 125 of cucurbitaceous and solanaceous plants (Dhillon et al. 2005; Piñero et al., 2006).

These pests are mainly managed by food bait, bait sprays, parapheromones, along with insecticides since the starting of the last century. For controlling Tephritid flies, lure and kill approach based on food attractants or use of parapheromones along with an insecticide was employed as a part of Integrated Pest Management (IPM) (Canale et al., 2013). Excessive use of synthetic pesticides associated with many environmental problems such as insecticide resistance, lethal to other organisms, persistent in soil and food, and ecological pollutions was observed (Desneux et al., 2007). So, the urgent need to switch over the natural products which are environmentally friendly and less hazardous to other organisms. Plant extracts could be a potential alternative to synthetic insecticides, and many pieces of research have revealed them, with some plants which have insecticidal properties to tephritid flies (Kalemba and Kunicka et al., 2003; Benelli et al., 2012; Canale et al., 2013). They are more well-suited with surrounding components than synthetic insecticide (Isman and Machial, 2006).

Plant essential oils (EOs) are mixtures of many volatile bioactive chemicals. They have pharmacological properties like anti-fungal, anti-bacterial, anti-viral, medicinal, cosmetics and insecticidal properties (Kalemba and Kunicka et al., 2003; Amer and Mehlhorn, 2006; Bakkali et al., 2008; Benelli et al., 2013; Miresmailli and Isman, 2014) and are available in many plant families such as Apiaceae, Asteraceae, Fabaceae, Lamiaceae, Laurenceae, Poaceae, Myrtaceae Rutaceae, and Zingiberaceae (Maggi and Benelli et al., 2018). Among the tephritid fruit flies, the Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann) is the mostly studied insect with EOs (Pavlidou et al., 2004; Chang et al., 2009; Miguel et al., 2010; López et al., 2011; Benelli et al., 2012, 2013; Ghabbari et al., 2018) whereas, a few studies have been carried out with EOs on olive fruit fly, *B. oleae* (Rossi) (Canale et al., 2013; Rizzo et al., 20), Mexican fruit fly, *Anastrepha ludens* (Buentello-Wong et al., 2015, 2016), *A. fraterculus* (Brilinger et al., 2019) and Peach fruit fly *B. zonata* (Ali, 2018; El-Minshawy et al., 2018). On *B. dorsalis* and *Z. cucurbitae*, the investigation with EOs is scarce (Chang et al., 2009).

## 1.2 Significance of the research

This research was significant importance in terms of theoretically and practically to the current research personnel, academics, students as well as farmers. The theoretical contribution of the mixed lure system and its different proportions for suppressing fruit flies in the present study pay attention to the body of research knowledge. Previous researchers work with a combination of lures on different dispensers. However, in our study, we switch into a new dispenser system, i.e. rubber foam. An extensive study of different literature could not show its usage in fruit fly.

Due to little information on proportion of lures in different combinations, we took it as a research study and the finding is satisfying, which would contribute to the research personnel and academics. Furthermore, screening of best among different plant essential oils, and its combination with single and mixed lures in field condition also provides the necessary information to the farmers as well as to the fruit fly research team.

## 1.3 Research objectives

1. To evaluate ME and CL combination in rubber foam dispenser in controlling fruit fly under field condition.
2. To screen some plant essential oils toxicity to *B. dorsalis* and *Z. cucurbitae*.
3. To evaluate the sex pheromones and basil oil combination for attraction and kill.
4. To investigate of effective lure combination (mixed and separated) for suppressing targeted multispecies pest in a mixed cropping system and finally determined the best proportion of both lures which was more effective
5. To apply sex pheromones and plant essential oils for controlling fruit fly under field condition.

# Chapter II

## Review of Literature

This section includes the related studies about importance of fruit flies, their life history and biology, different host plants, geographical distribution, various management practices, natural rubber and essential oils.

### 2.1 Importance of fruit flies as a pest

Fruit flies (Diptera: Tephritidae) are considered as one of the most destructive insect pests of fruits and vegetables in the world (Vayssiéres et al., 2008; Ekesi et al., 2009). The family Tephritidae consists of over 5,000 species of "true fruit flies" (Fletcher, 1987). Approximately 10% of tephritids are pests of fruits, vegetables and flowers (Christenson and Foote, 1960; Weems et al., 1999; Diamantidis et al., 2008) and cause annual economic losses are US$1 billion worldwide (STDF, 2010). Numerous fruit fly species constitute enormous threats to fruit and vegetable production throughout the world, causing both quantitative and qualitative losses. Due to their susceptibility to invasive tephritid species, many fruit-producing countries have imposed quarantine restrictions on the import of products from countries infested with particular fruit fly species, and/or require that fruits and vegetables undergo quarantine treatment before their importation is allowed (Vargas et al., 2008). Fast mobility, great dispersive power, high reproductive rate, movement through market chain and extreme polyphagous are among the common traits of *Bactrocera* species. Several *Bactrocera* species are well-documented invaders of horticultural crops and also rank high on quarantine lists worldwide (Clarke et al., 2005).

### 2.2 Host plants of fruit flies

Mango, papaya, melon, guava, star apple, citrus, passion fruit, luffa, oranges, plum, carambola, pumpkin, wax gourd, cucumber, and bitter gourd etc are most important host plants which females can lay eggs in fruits and decay more than 400 kinds of fruit and vegetables. Guava is the best host of *B. dorsalis* (Mann, 1990, Sharma et al., 2005, Sharma and Batra, 2007, Singh, 2008, Sharma et al., 2011, Singh and Sharma 2011, Choudhary et al., 2016). In addition to fruit crops, they are devastating to vegetables, oilseed crops and ornamental plants.

## 2.3 The importance of fruit flies in Thailand

Thailand has an area of 513,120 square kilometers (NSO, 2004). Total area under agricultural cultivation is near about 21 million ha or 40.9% (OAE, 2008). Most of the population of Thailand involved in agriculture actively or passively (Tualananda, 2000). Tropical fruits of Thailand have a good quality because of its geographical site and suitable climatic conditions (Chomchalow et al., 2008). Growing fruits and vegetables play a vital role for generating income in Thailand. Among various pest, fruit flies are the most destructive pest that affects the cultivation. Fruit flies of the family Tephritidae are measured as the most harmful, that responsible for huge economic losses in agriculture especially to array of fruits, vegetables and flowers (Diamantidis, 2008). Mainly two species of fruit fly, melon fly *Z. cucurbitae*, and Oriental fruit fly *B. dorsalis,* are of pronounced importance. Cucumber (*Cucumis sativus*) and bitter gourd (*Momordica charantia*) are two preferable vegetables to Thai people. Melon fly infestations to these crops are so common in Thailand (Ramadan and Messing, 2003). Sometimes the infestation level is so high and can cause 100% yield loss in cucurbits by melon fly (*Z. cucurbitae*) (Dhillon et al., 2005; Prabhakar et al., 2009, 2012).

Thailand is situated in a biotic alteration zone between Indochinese and sundaic plants, animals together which enrich its biodiversity. Majority of the host orchard are at roadside which helps in trapping, collecting and rearing of fruit flies. So, Thailand is more appropriate for this kind of study.

## 2.4 Oriental fruit fly (*Bactrocera dorsalis*) (Hendel) and its distribution

The Oriental fruit fly feeds on 478 types of fruits and vegetables (USDA, 2016). This species is native to Asia and spreads throughout Bangladesh, Bhutan, Cambodia, China (southern), Hong Kong, India (numerous states), Indonesia, Japan (Ryukyu Islands), Laos, Malaysia, Myanmar, Nepal, Ogasawara Islands, Pakistan, Philippines, Sri Lanka, Taiwan, Thailand, Vietnam and countries of African continent (CABI 1994, Vargas et al., 2007, Choudhary et al., 2016, Zeng et al., 2018) (Fig. 1). It is a major and polyphagous pest in Asia that infests 117 host plants (Ye and Liu, 2005). Clarke et al. (2005) reported it as a threat to agriculture across Asia-Pacific. Crop losses by *Bactrocera dorsalis* in mango (12-60%), guava (40-90%) and papaya (12-60%) have also been recorded by Allwood and Leblanc (1997). The damages on crops consist of oviposition stings on the fruit surface, fruit that drops early but also inside destruction of the fruits. *Bactrocera dorsalis* is of quarantine significance to EPPO (European Plant Protection Organization), APPPC (Asia and Pacific Plant Protection Commission), COSAV (Comite de Sanidad Vegetal Del Cone Sur), CPPC (Caribbean Plant Protection Commission), IAPSC (Inter-African Phytosanitary Council) and OIRSA (Organismo Intemacional Regional de Sanidad Agropecuaria) countries.

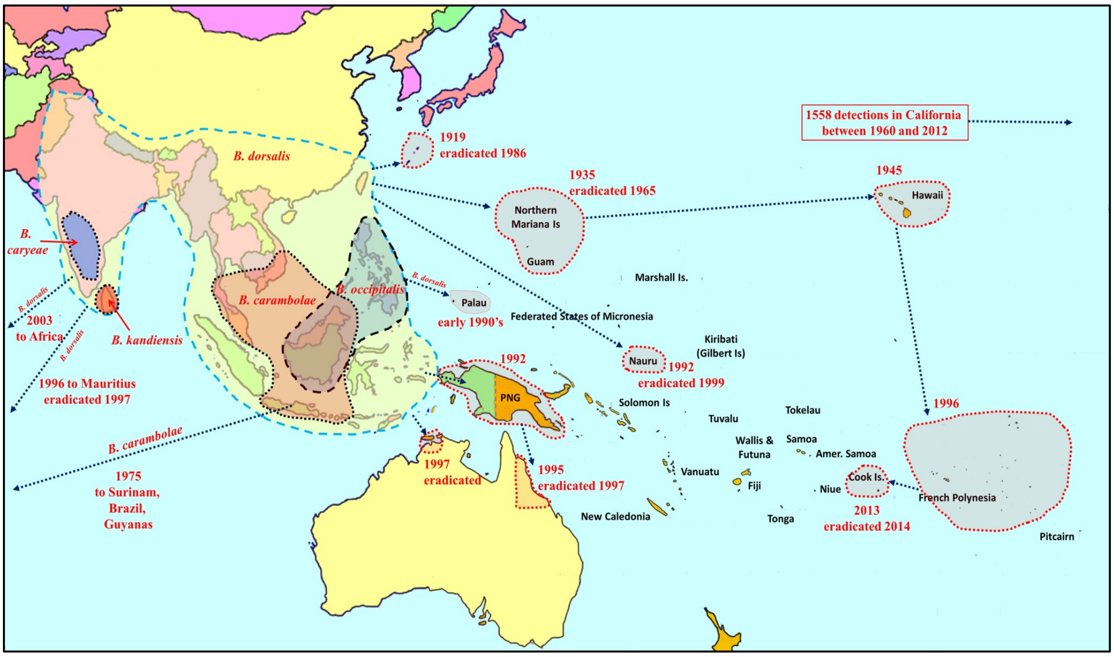



**Figure 1.** Distribution and invasion history of Oriental fruit fly *Bactrocera dorsalis* complex.
Source: Vargas et al., 2015

### 2.5 Melon fly (*Zeugodacus cucurbitae*) (Coquillett) and its distribution

*Zeugodacus cucurbitae* is native to India and so far, West of Pakistan. Based on exclusive host survey, melon fly can feed on over 125 host plants in Asia and Hawaii. They prefer cucurbitaceous plants as host in South East Asia (Fig. 2). All over the Papua New Guinea it prevails and familiar one; it is assumed to be introduced around Second World War. Melon fly founds in all the provinces of Solomon Island except Manus province (first reported in 1984). Fruits infested by melon fly, sometimes observed *Drosophila melanogaster* (vinegar fly) lays egg on the infested fruit as a scavenger

(Dhillon et al., 2005). In northern Mariana island of Common Wealth, it was seen in 1943 and annihilated in 1963 by sterile insect technique (Steiner et al., 1965; Mitchell, 1980). But it again established from nearby Guam in 1981 (Wong et al., 1989). In Nauru, it was identified in 1982 and eradicated in 1999 by male annihilation method and spraying of protein bait. Melon fly is also present in Africa (Kenya, Tanzania, Mauritius and Reunion). It causes a considerable damage of bitter gourd (95%) in Papua New Guinea, snake gourd (90%) and pumpkin (60-70%) in Solomon Island.

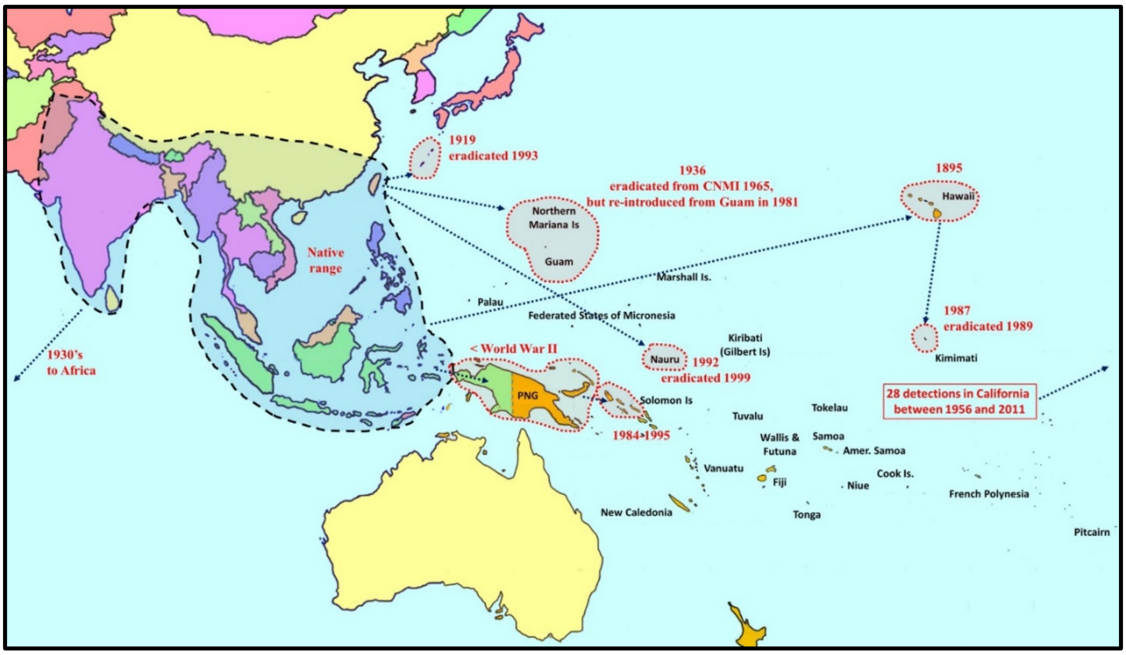



**Figure 2.** Distribution and invasion history of melon fly (*Zeugodacus cucurbitae*). Source: Vargas et al., 2015

**2.6 Biology of fruit fly**

Fruit flies have four stages in their life cycle viz. eggs, larvae (maggots), pupae and adults (Fig. 3). A short description of each of the stages of the life cycle of fruit fly is given below;

**Eggs**

The adult female at 15-35 days old after mating, they are ready to lay eggs (1-20) either singly or in cluster. For laying eggs, females prefer maturing and ripening fruit of the host plants. The eggs hatched into larvae within the fruit and it takes 2-4 days and sometimes may long up to 10 days in winter depending on the temperature. During its life span, a female can lay 400 eggs or more.

**Larvae**

According to Dhillon et al. (2005) fruit fly larvae (maggots) feed inside the fruit and in association with some bacteria enhances rotting of fruit internally, fruits become unmarketable. The larva has three instars and can totally damage the fruits (Ye and Liu, 2005). Sometimes the rotted fruit prematurely fall down from the tree. Depending on temperature this stage lasts for 5-15 days.

**Pupae**

When the fruit ripe, rot and fall down from trees, matured larvae leave the fruit by jumping and burrow 4-8 cm in the soil for pupation. In the soil, the larvae make an oval shape, dark brown, hard pupal-case, in which adult fly develops. Duration of the pupal period varies from 8-11 days.

**Adult**

The adult flies emerge from the pupae may take 8-11 days. Adult flies infest fruits by puncturing and laying eggs beneath the soft epidermis of both green and ripe fruit (Hollingsworth and Allwood, 2000).

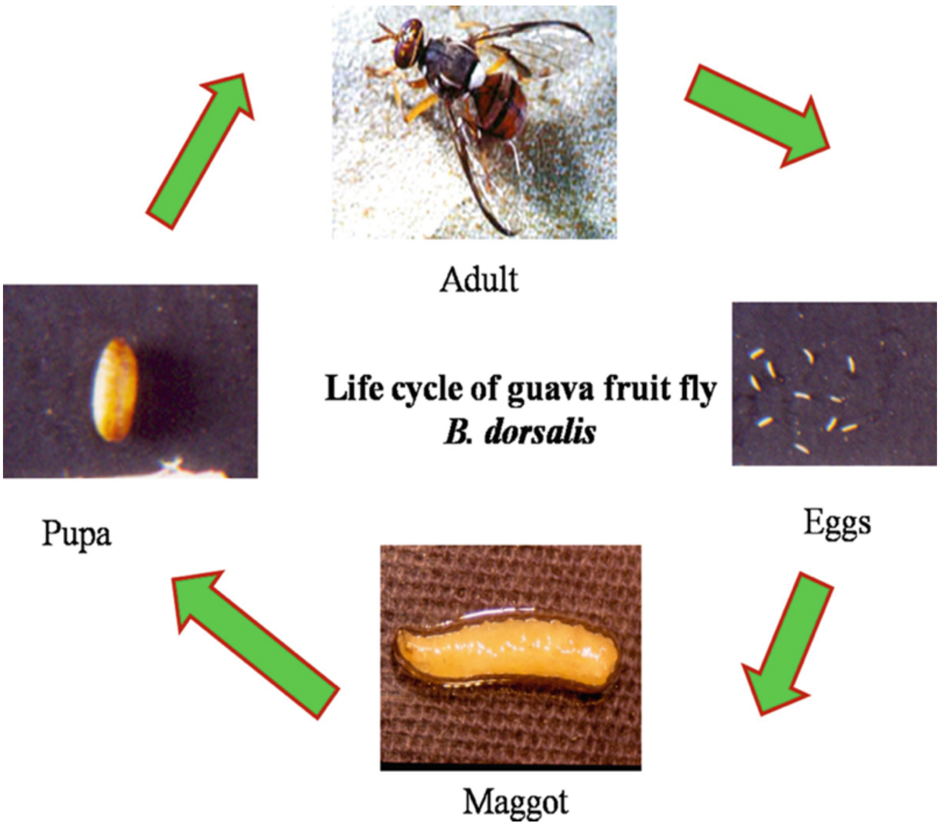



**Figure 3.** The biology of fruit fly (Pests of Guava).

Source: https://link.springer.com/chapter/10.1007/978-981-10-8687-8_15

## 2.7 Control methods of fruit fly

Control of fruit flies is more difficult than any other insect pest. Fruit fly management is very tough because the third instar larvae came out from the decaying fruits and go for the pupation in soil. Both larvae and pupae get protection in fruits and soil from surface applied insecticides (Heve et al, 2016). This pest multiplies in geometrical progression even if two or more viable females left can give a huge population in some few generations.

### 2.7.1 Cultural method

This is the most effective techniques of fruit fly control. Sanitation measures, fruit bagging and planting crops are more resistant to fruit flies, irrigation, pulverization of soil, clipping of infested parts etc. Trees with fruits that have signs of attack should be discarded immediately. Verghese et al (2004) observed low number of fruit flies infested fruits where sanitation measures practiced in the field. Cultivation of fruit fly's resistant crops is really good for good harvest e.g. some chili species, and some fruits in Thailand where fruit flies do not attack (Allwood et al., 2001). If we harvest crops early, there is a possibility to avoid attack from certain fruit flies. Bagging of fruit singly or in cluster of fruits or even whole tree is very effective against fruit flies.

### 2.7.2 Biological control

It is very hard to control fruit flies purely with organic methods although biological methods can reduce their number (Yoder and Wharton, 2002; Sarwar et al., 2014). Biological control of fruit flies is mainly depending on the use of predators and parasitoids. Common predators are ant and ground beetle that takes the maggot as food, adult flies get trapped in spider's web, predatory flies such as dragon fly and robber fly feed on them during their flight (Hoelmer and Kirk, 2005; Mills and Daane, 2005) in addition to other natural enemies (Riegler, 2002). Investigations similarly show that arthropods can cause considerable mortality on fruit fly pupae (Daane and Johnson, 2010; Tzanakakis, 2006). Augmentation of parasitoids is very successful. In Hawaii, an experiment revealed low level of infestation in fruits (upto 95% reduction) when larval parasitoids belonging to the families Eulophidae, Braconidae and Chalcididae were used (Allwood et al., 2001). For *Z. cucurbitae*, *Psyttalia fletcheri* (Hymenoptera: Braconidae) is a parasitoid, which have a high level of parasitism. Fungal pathogen *Metarhizium anisopliae* is an important bio-pesticide which is very active against larvae and pupae of some major *Bactrocera* sp. (*B. dorsalis* and *Z. cucurbitae*) and *Ceratitis* (*C. Capitata* (Wiedemann) and *C. cosyra* (Walker)) species in the soil (Ekeshi et al., 2007). Ekesi et al. (2002) and Dimbi (2003) proved that *M. anisopliae* have very high potential in suppressing fruit fly population. Thaochan and Ngampongsai (2015, 2018) found *M. guizhouense* inoculation reduced the mating tendency and mating range as well as Average Survival Time (AST) in treated male-female of *B. latifrons* and *Z. cucurbitae*.

**2.7.3 Sterile insect technique (SIT)**

The concept of releasing sterile males for inhibiting wild population of the same species was first introduced by Knipling (1985). This technique is suitable for regulating *Bactrocera* flies which have wide courtship behavior that can be suppressed by male lures (Benelli, 2014; Benelli et al., 2015). Male sterile fruit flies are introduced into the environment. Radiation is used for sterilization. Sterile male and wild male compete for mating with female, mating with sterile males results into no offspring. An early example sterile insect technique for eradication of oriental and melon fruit flies are from islands of Japan (Kawasaki, 1991).

**2.7.4 Chemical methods**

This method is intensively used by the farmers. DDT (dichloro-diphenyl-trichloroethane) was the first synthetic chemical insecticide, followed by organophosphate insecticides. The chemical methods to control fruit flies are done by-spraying of insecticides, trapping of adult flies and bait spray. Control of fruit fly with cover sprays begins with inorganic insecticides example lead arsenate and gradually replaced by synthetic insecticides e.g. organophosphates, chlorinated hydrocarbons and synthetic pyrethroids. Spraying of insecticides has some merits such as it is reasonable, convenient, and ensure a top level of defense against the invasion of fruit fly with permanent results (Allwood, 1997).

**2.7.5 Trapping and bait spray of fruit fly**

Trapping method of fruit fly involves practice of synthetic bait lures like male attractant. In this method a dispenser where chemical (attractant) is injected into a suitable substrate and placed into a insect collecting chamber is called trap. Bait spray is recognized as an effective technique for fruit fly control, reducing the amount of pesticide needed and found successful in many annihilation program (Roessler, 1989).

It is mostly done in the pre-oviposition period when the female flies need protein for ovarian development and egg production. They are poisoned further killed rely on this fact. Various insecticides dissolved in cane sugar and then sprayed quickly so that spray droplets retain on plant parts. Flies come, ingest and die. It was used since 1960s and been existed today as a general standard. A new bait spray preparation containing low-risk spinosad found to be as effective as Nulure-malathion bait spray in Central America and the USA against medfly (Peck et al., 2000; Vargas et al., 2002).

### 2.7.6 Pheromone for fruit flies

A pheromone is a chemical substance secreted by an organism, enhancing some physical responses in individual of the same species. Pheromones work as hormones outside the body of secreting organism to affect the behavior of the receiving individuals. Pheromone of some distinct insect species like fruit fly, gypsy moth, acrobat ant and Japanese beetle are used to trap the relevant insect for monitoring purposes, to regulate the population by misleading, to prevent mating, and to inhibit further egg laying.

Males of several fruit fly species enticed by one or more of distinct chemical compounds, named male lures. The most effective and frequently used of these lures are i) Cue lure*;* *Bactrocera* and *Zeugodacus* species males are mainly fascinated with this lure. Its chemical name is 4-(p-acetoxyphenyl)-2-butanone. It can appeal males from up to 300 m away. ii) Methyl eugenol (ME); this chemical also attracts *Bactrocera* and *Zeugodacus* species. It is commonly familiar by methoxy eugenol, and chemically it is 4-allyl-1,2-dimethyl benzene. Males are attracted up to 500m by ME.

## 2.8 Mixed Lure

Some male attractants such as ME and CL were successfully used in last 50 years for the detection and controlling of fruit flies especially for oriental fruit fly and melon fruit fly. Actually, there are no fruit fly species have been discovered that react with both ME and CL and their response is connected to the special antennal receptor sites (Metcalf and Metcalf, 1992). Although more than half of the *Dacini* spp. are non-responsive to these lures (Drew and Romig 2013). Areas where ME and CL responding fruit flies prevail; several experiments have done to evaluate the effectiveness of baits mixed with ME and CL for recognition and control of fruit flies. Mixing of lures in a wise manner could lessen the number of traps in a respective area and also the number of labors to maintain them. Besides, it may reduce the quantity of pesticides needed. Earlier investigations revealed dissimilarity in effectiveness of ME vs CL responding *Bactrocera* spp. In some study, data showed the uniform result such as if CL mixed with or adjacent to the ME it significantly reduces the trap catch of ME responding species (Hooper, 1978; Vargas et al., 2000). Hooper (1978) showed that mixing of ME to CL significantly increases the male catch (nearly doubled) of *Z. cucurbitae* than trap baited alone with CL in Taiwan. Hooper (1978) also found that there was an increase in male numbers where ME and CL applied to same traps but in separate wicks. Vargas et al. (2000) reported that attractant containing 25% CL by volume catches the same number of males of *Z. cucurbitae* as traps baited only with CL. Royer et al. (2017) found when ME and CL mixed in equal parts and placed in one trap; it significantly reduces catches of most Dacine fruit flies in Australia and Papua New Guinea. ME and CL trap catches of fruit flies with cue lure baits is connected positively with some abiotic factors such as, temperature, humidity, and rainfall (Hasyim et al., 2008).

**2.9 Properties of natural rubber**

Natural rubber shows high strength (tensile and wear) with unique resistance to weakness. It has outstanding green strength and clip that indicates the tendency to stick to itself and to other things i.e. easier to weave. One important demerits of natural rubber are moderate resistance to environmental damage by heat, light and ozone. It can withstand to cutting, chipping and tearing. Natural rubber has low heat generation which indicates long lasting of tires. It has very small pore size in order to use as dispenser so any chemical inside the foam releases very slowly in chronic wound healing. Natural rubber membrane used as a drug delivery system and it releases the drug for a specified period of time. Among different carrier systems, natural rubber membrane is safe and convenient to the patients (Henrique et al., 2006). The natural rubber foam device was showed in Fig. 4.

**Figure 4.** The natural rubber foam dispenser

## 2.10 Plant essential oils

As an essential source of bio-pesticides' plant essential oil is very popular. The global market of bio pesticides was estimated to $700.00 million and a gross international production was 45,000 tons (Tripathi et al., 2009). Recently essential oils (EOs) and bio-pesticides are becoming more reliable for bio-farming. EOs have been using as insecticides for fifty years. EOs have two main drawbacks for which they cannot give the full effort as insecticides because of its quick volatility and short residual action. Some of its good sides are safe to human being, environment-safe, synergistic with other biological control program and non-toxic to mammals. Monoterpines, sesquiterpenes and associated phenylpropenens are the active ingredient for insecticidal action of essential oils. Monoterpenes and sesquiterpenes are responsible for their repellent activity. Recently, wide application of synthetic insecticides for controlling insect pests and arthropods are becoming a threat to environment and human health. So, we have to switch on to another alternative which comes from natural products and have effectiveness and environment friendly. EOs from plant species are widely tested as well as their synergistic nature with other chemicals also evaluated for controlling insect pest.

Citronella, lemon and eucalyptus oil have been recommended by US environmental Protection Agency (EPA) as repellent ingredient for applying on skin. It became possible due to their relatively low toxicity, reasonable effectiveness and customers' choice (Katz et al., 2008). Basil oil and its three active ingredients (trans-anethole, estragole, and linalool) is very effective and $LT_{90}$ for 10% of the test chemicals is between 8 and 38 minutes on three Tephritid fruit fly species *C. capitata*, *B. dorsalis*, and *Z. cucurbitae*. It is more effective on *C. capitata* than *B. dorsalis* and *Z. cucurbitae* (Chiou et al., 2009).

EOs from three different species *Eugenia caryophyllus*, *Ocimum basilicum* and *Thymus vulgaris* were applied to Mexican fruit fly, *Anastrepha ludens* (Loew) for toxicity test. It was found that EOs of *E. caryophyllus* was more toxic than *T. vulgaris* and *O. basilicum*. The 100% mortality of adults were observed by three plant species (Buentello-Wong et al., 2016). There are a number of researches on essential oil and plant products carried out specially on stored product pests (Asian et al., 2005; Cetin and Yanikoglu, 2006; Negahban et al., 2007; Ayvaz et al., 2009). These plant oils are environmentally friendly and possess ovicidal, deterrent power against many pest species (Isman 2000; Cetin et al., 2004). Essential oil from *Mentha* species is very effective against some stored grain pest particularly *Tribolium castaneum* and *Callosobruchus maculatus* (Tripathi et al., 2000).

This research focused on the efficiency of mixed lure system with CL and ME in the rubber foam matrix and also adding some EOs for killing the fruit flies. This work will help the farmer to reduce insecticide and this method will be belonging to one part of the integrated pest management programs (IPM).

# Chapter III

# Materials and Methods

## 3.1 Efficacy of mixed lure (methyl eugenol [ME] and cue lure [CL]) systems in rubber foam

### 3.1.1 Trap container

Medium sized (750 ml), round transparent plastic containers were used to prepare the trap (Fig. 5). Four lateral holes were made in each container by 10 mm diameter as an entrance for flies in the trap. At the center of the lid, a small hole was made by aluminum wire for hang it on a tree. Outside of the trap was wrapped with the yellow color adhesive tape for increase attractancy of the insect. The 200 ml 20% of propylene glycol was used to preserve the caught flies.

### 3.1.2 Single and mixed lure (ME and CL) loading in the rubber foam

For the single lure, 100 µl of each ME or CL (Fig. 6) was taken by a 1 ml syringe and injected in the center of para rubber foam dispenser (2 × 2 × 5 cm). For the mixed lure system, 100 µl of each ME and CL injected in the same spot to para rubber foam (ME+CL). Again, 100 µl of each ME and CL were injected in separate spot at the end of para rubber foam ME/CL (Fig. 7). The prepared foam was tied inside the trap with aluminum wire.

### 3.1.3 Field testing

This experiment was conducted in completely randomized design (CRD) with four treatments viz., ME, CL, ME+CL and ME /CL. Each treatment was replicated three times with the total of 12 lures. The traps were hung at experimental field of Pest Management, Faculty of Natural Resources, Prince of Songkla University. There were fruits and vegetable crops i.e. santal, mango, rambutan, longkong, chili and luffa. The trap was set on the tree at 150 cm above the ground and distance between traps were 20 m. The caught flies in the trap were collected in each week morning and continued for 8 weeks September and October 2018.

**Figure 5.** Plastic container trap for fruit flies.


**Figure 6.** The parapheromone, methyl eugenol (A) and cue lure (B)


**Figure 7.** Two types of mixed lures ME+CL (A) and ME/CL (B)

## 3.2 Mortality test of adult flies with some plant essential oils

### 3.2.1 Insect rearing

Insects used in this study were obtained from collecting infested guava (*Psidium guajava*) and luffa (*Luffa acutangular*) from agricultural orchards at Prince of Songkla University, Hat Yai District, Songkhla Province, Thailand (latitude and longitude: 7.004658ºN, 100.503350ºE). The infested fruits with larvae were kept in a transparent plastic box (25×20×15 cm), with perforation on top of the lid for air ventilation (Figures 8 and 9). One cm layer of sterile (autoclaved and dried) sawdust was put at the bottom of the box as a medium for pupation. After adult fly's emergence, adult fruit flies were transferred to another gauge case (30×30×30 cm). To maintain the growth of the adult flies, cube sugar, yeast hydrolysate, and water *ad libitum* were provided. Adult *Bactrocera dorsalis* and *Zeugodacus cucurbitae* were identified based on the morphological characteristics described by Hardy (1973), White and Elson-Harris (1994), Drew and Hancock (1994).

Pure culture of *B. dorsalis* and *Z. cucurbitae* were further maintained in separate cages and the same food. Laboratory conditions for *B. dorsalis* and *Z. cucurbitae* adult rearing were maintained at 12: 12 h of light: dark photoperiod at 75–80% relative humidity (RH) and 27 ± 2°C temperature. To maintain the colony of flies, adult flies of both sexes of the same species kept in the same cage for mating and oviposition. For testing, only 10-15 days old adult flies were used from the culture.

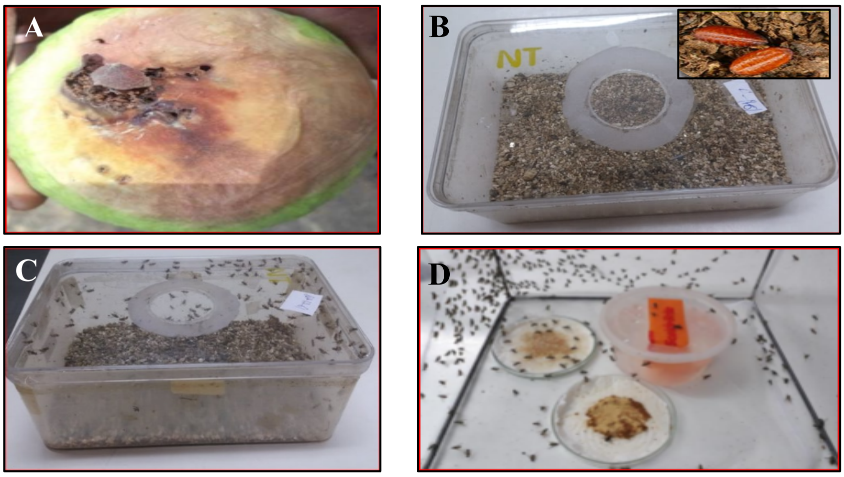



**Figure 8.** Mass rearing of *Bactrocera dorsalis*; (A) infested guava with larvae, (B) vermiculli as a substrate for pupation of larvae and pupae, (C) emergence of adult and (D) adult colony.

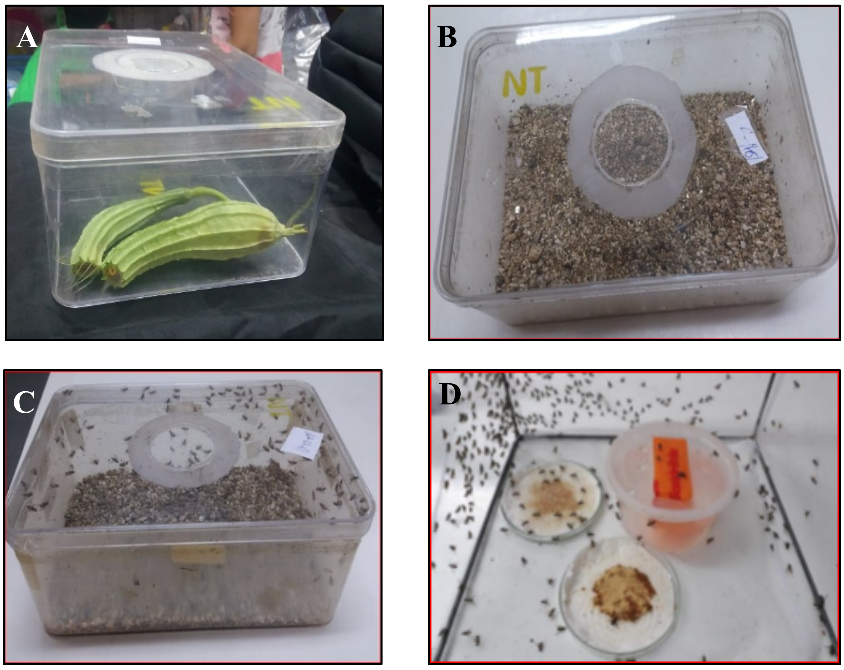



**Figure 9.** Mass rearing of *Zeugodacus cucurbitae*; (A) infested luffa with larvae, (B) vermiculli as a substrate for pupation of larvae, (C) emergence of adult and (D) adult colony.

### 3.2.2 Plant essential oils

The plant essential oils (EOs) including basil (*Ocimum* spp.), clove (*Syzygium* spp.), citronella (*Cymbopogon* spp.) and eucalyptus (*Eucalyptus* spp.) used in were commercial grades from Jiangxi Yisenyuan Plant Species Co., Ltd; China (Fig. 10).


**Figure 10.** Essential oils used in the study (A) basil oil, (B) clove oil, (C) citronella oil and (D) eucalyptus oil.

### 3.2.3 Rubber foam preparation

Para rubber foams were prepared using modified from Dunlop's technique (Calvert, 1982; Malee, 2019). The high ammonia concentrated natural latex 60% (167 g) was first stirred to remove the preservative for 5 min. The other chemical ingredients were 20% potassium-oleate solution (10 g), 50% sulphur dispersion (5 g), 50% zinc-N-diethyl dithiocarbamate dispersion (2 g), 50% zinc-2-mercaptobenzothiazole dispersion (2 g), 50% butylated hydroxytoluene (2 g), 30% diphenyl guanidine dispersion (20 g), 50% zinc oxide dispersion (20 g) and 20% sodium silicofluoride dispersion (10 g). All chemicals were added into the latex within 10 min under continuous stirring. The formed latex was poured into mold and then vulcanized using a hot air oven at 70ºC for 3 h. The prepared para rubber foams were then dipped into compounded latex to make a thin film over para foam. The para rubber foam was cut into 2×5 cm pieces and used as dispenser. All the para rubber foam dispensers were kept at the ambient temperature before bioassay.

### 3.2.4 Preparation of rubber foam dispenser with essential oils

A 300 µl of each plant essential oils (clove oil, basil oil, citronella oil and eucalyptus oil) loaded into a rubber foam dispenser (size 2×5 cm) by one ml syringe. Each rubber foam dispenser with each plant essential oils was weathered in the natural environment at 0, 15, 30, and 45 days, respectively, and the control was only rubber foam without a plant essential oil. Then, four foam dispensers of each plant essential oil and control were collected at respective days and used for testing with the adult flies (Fig. 11).

### 3.2.5 Fumigation toxicity bioassay

For the bioassay, 10 adult flies (5 males and 5 females; 10-15 days old) of each *B. dorsalis* and *Z. cucurbitae* were kept separately in a clear plastic container (750 ml) with some minute holes on the lid. Prepared rubber foam dispenser with respective weathering periods (0, 15, 30 and 45 days) was placed at the bottom of the container and exposed to adult flies with a maximum of 72 hours. Yeast, sugar, and water-soaked cotton were provided in the container as food. This experiment was conducted in Completely Randomized Design (CRD) with four replications for *B. dorsalis* and three replications for *Z. cucurbitae*. The mortality was observed at 1, 6, 24, 48 and 72 hrs (Fig. 12).


A B C D

**Figure 11.** Rubber foam injected with basil (A), clove (B), citronella (C) and eucalyptus (D) oils weathered at outside at 0, 15, 30, and 45 days.

A B

**Figure 12.** The observation mortality of tested flies (A) and dead flies (B).

**3.3 Attractancy test of *B dorsalis* and *Z cucurbitae* in combination of lures with best essential oil from experiment 2 at same/ different point in foam under laboratory control conditions**

**3.3.1 Attractancy test of *B. dorsalis* and *Z. cucurbitae* with lures**

One hundred µl of ME was taken and injected into a rubber foam dispenser. Likewise, the same quantity of lures was taken for the rest of the three treatments i.e. CL, ME+CL, ME/CL. This experiment was conducted in a Completely Randomized Design (CRD) with 5 treatments i.e. ME, CL, ME+CL, ME/CL and control (only foam-no chemicals) and 4 replications. Then the foams were weathered outside in natural environment for 0, 15, 30 and 45 days. Ten males of *B. dorsalis* and ten males of *Z. cucurbitae* were released in a gauge cage (30×30×30 cm). Yeast, sugar and water *ad libitum* were supplied as food. After that, a foam dispenser (previously prepared with

100 µl of lures ME) was put above the cage for 30 minutes. The highest number of attracted flies was recorded for each species. The same procedures were followed for rest of the treatments (CL, ME+CL, ME/CL).

### 3.3.2 Attractancy test of flies with lures and best essential oil at the same point of application on foam

One hundred µl of ME was loaded in to a foam dispenser and 300 µl of basil oil injected at the same point where ME was injected. The same quantity of the lures and basil oil was taken for the rest three treatments i.e. CL, ME+CL, ME/CL. This experiment was also conducted in a Completely Randomized Design (CRD) with 5 treatments i.e. ME + BO, CL+ BO, ME+CL+ BO, ME/CL+ BO and control (only foam-without chemicals) and 4 replications. Then, the foams were weathered outside in natural environment for 0, 15, 30 and 45 days. After that,ten males of *B. dorsalis* and ten males of *Z. cucurbitae* were released in a gauge cage (30×30×30 cm). After that a foam dispenser (previously prepared with 100 µl of lures ME and 300 µl of basil oil) was kept above the cage for 30 minutes. Again, the highest number of attracted males was counted for each species and for the other treatments.

### 3.3.3 Attractancy test of flies with lures and best essential oil at different point of application on foam

In a foam dispenser, 100 µl of CL were loaded at one end, and 300 µl of the basil oil were injected at the other end. The same quantity of the lures and basil oil were taken for the rest four treatment i.e. CL/BO, ME/BO, ME+CL/BO, ME/CL/BO. This experiment was conducted in a Completely Randomized Design (CRD) with 5 treatments i.e. CL/BO, ME/BO, ME+CL/BO, ME/CL/BO, and control (only foam-no chemicals) with 3 replications.  Then the foams were weathered in natural environment for 0, 15, 30 and 45 days. For the next step, a net cage was prepared where ten males of *B. dorsalis* and ten males of *Z. cucurbitae* were released. After that, a foam dispenser (previously prepared with 100 µl of lures ME and 300 µl of basil oil) was placed above the case for 30 minutes. Maximum number of attracted males were recorded for each species and for the other treatments (Fig. 13).

A B

**Figure 13.** The attractany testing of rubber foam injected lures and plant essential oils (A) and an attracting fly to the rubber foam (B)

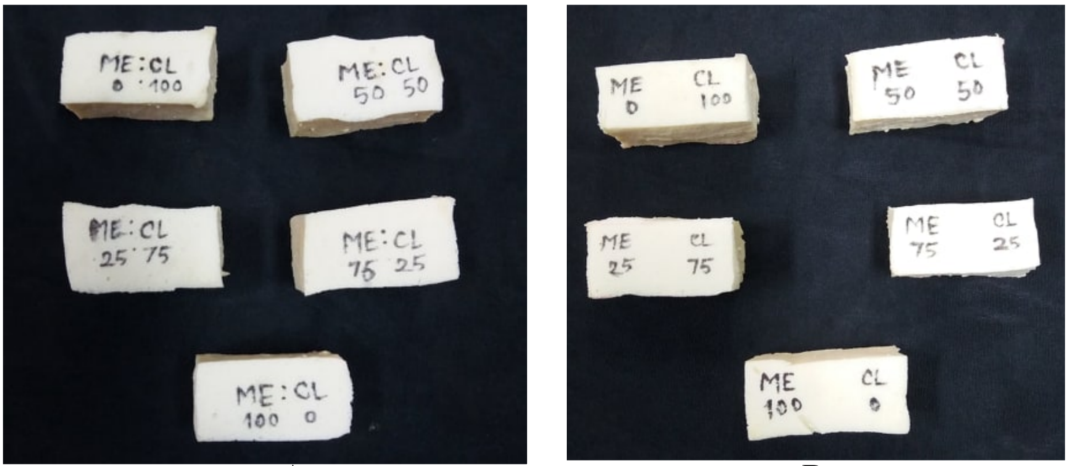
**3.4 The proportion efficiency of methyl eugenol - cue lure mixed and separated in rubber foam device for controlling tephritidae fruit fly**

**3.4.1 Study site**

This work was carried out in experimental field of Pest Management and Plant Science, Faculty of Natural Resources, Prince of Songkhla University, Songkhla, Thailand, (70º0′11.6″N 100 º 30′15.4″E) over eight weeks from August to September 2019. There were mixed fruit and vegetable crops, i.e., santal, jackfruit, mango, rambutan, long Kong, tamarind, citrus, papaya, chilli, luffa, and long yard bean at the field.

**3.4.2 Preparation of lures**

Lures were loaded in rubber foam. ME (4-allyl-1,2-dimethoxybenzene, CAS 93-15-2) and CL (4-(p-acetoxyphenyl)-2-butanone, CAS 3572-06-3) were supplied by Sigma-Aldrich, Spruce Street, Saint Louis, USA. Liquid lures were loaded in rubber foam dispenser (2 × 2 × 5 cm) by 10-100 µl of micropipette. ME-CL loaded in rubber foam in two ways. Firstly, liquid ME and CL were mixed and then loaded in rubber foam at 0 : 100, 25 : 75, 50 : 50, 75 : 25 and 100 : 0 in a total of 100 µl. Secondly, liquid ME and CL not mixed, separately loaded in rubber foam with the same proportion of the first part. So, there were five different proportions with four replications with a total of 40 traps for two combinations (Fig. 14)




A B

**Figure 14.** Treatments used in the experiments; methyl eugenol (ME) and cue lure (CL) proportions of (0: 100), (25: 75), (50: 50), (75: 25), (100: 0) mixed (A) and separated (B).

### 3.4.3 Preparation of traps

Prepared foam lures were tied in clear plastic cup traps (750 ml) hung with aluminium wire. Four lateral holes (diameter = 10 mm) were made at the upper sidewall of the trap as entry route of flies. The base portion of the trap container was wrapped by a yellow tap to attract the flies (Vargas et al., 1991). These traps are almost same with bucket traps which one is the best for year-round suppression of fruit flies (Vargas et al., 2000).

Two hundred ml of 20% propyleneglycol was poured in the trap container for caught flies preserved and identification. The clear plastic cup traps were hung about 1.5 m from the base of fruit trees that were either flowering or fruiting state (Gnanvossou et al., 2017; Royer and Meyer, 2018). For vegetables, traps were hung at the bamboo roofs. Trap to trap distance was maintained at least 4 m (as trap distance 3 m between ME and CL traps had not found interfering in trap caught) (Shelly et al., 2004). Flies were collected weekly and morphological characteristics described under Leica S8APO stereo zoom microscope (Wetzlar, Germany) by Hardy (1973), White and Elson-Harris (1994), Drew and Hancock (1994), Prabhakar et al. (2012) and Drew and Romig (2013) (Fig. 15).

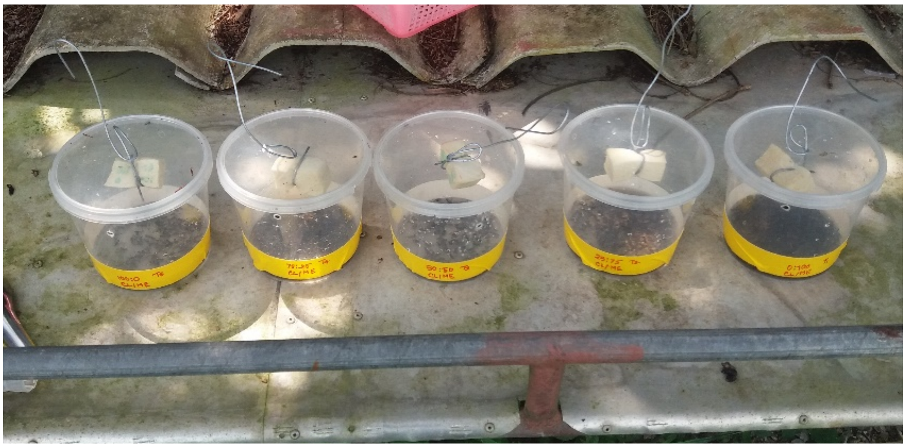


**Figure 15.** Trapped flies in the plastic container trap

**3.5 Efficiency of best mixed lure and best essential oil controlling fruit flies in field condition**

**3.5.1 Efficiency of best mixed lure and best essential oil controlling fruit flies in field condition**

This experiment was conducted on three fruit orchards at Koh Yor, Songkhla, Thailand (Fig. 16). This experiment had seven selected treatments and three replications from previous experiments. The treatments were ME, CL, ME + CL, ME/BO, CL/BO, ME + CL/BO and control (only foam-no chemicals) (Fig. 17). The total number of traps were 21 for each orchard. The lure 100 µl and essential oil 300 µl were taken and injected in the foam dispenser for these treatments. The experimental design was conducted in the randomized completely block design (RCBD) with seven treatments and three replications (orchards). After that the trap with only lures, 200 ml of 20% propylene glycol was supplied for collecting the attracted flies while ME/BO, CL/BO, ME + CL/BO traps were not supplied with 20% propylene glycol as EO causing death of flies at the same time. Trap were hung in three different locations and the distance between two traps will be considered 3-4 m (Fig. 18). Trapped flies were counted from each trap at every two weeks for a total of eight weeks during August to September, 2019. Finally, species were separated and identified in the laboratory based on the morphological characteristics described under Leica S8APO stereo zoom microscope (Wetzlar, Germany) by Hardy (1973), White and Elson-Harris (1994), Drew and

Hancock (1994), Prabhakar et al. (2012) and Drew and Romig (2013). As the environmental factors like temperature, relative humidity, rainfall, etc. have some effects on catching of flies in traps meteorological data were taken during the test from the meteorology department.


**Figure 16.** Study location of three different orchards at Koh Yor, Songkhla.


**Figure 17.** Treatments used in the experiments; ME, CL, ME + CL, ME/BO, CL/BO, ME + CL/BO, and control


**Figure 18.** The plastic container trap and trapped flies in the field test

**3.6 Data analysis**

The weekly number of caught flies in each trap was counted and converted as flies per trap per day (FTD). These discrete data were log-transformed and analyzed by using SPSS 11.0 program for Windows (SPSS, 2001). Boxplots were made for easy understanding of the performance of the treatments (Experiments 3.1, 3.3, 3.4 and 3.5). For experiment 3.2, percentage mortality was calculated after data transform. ANOVA and mean Tukey's Highly Significant Difference test compared mean comparison among treatments with a probability level of 0.05%.

# Chapter IV

# Results and Discussion

## 4.1 Efficacy of mixed lure (methyl eugenol [ME] and cue lure [CL]) systems in rubber foam for tephritid fruit fly control

In the mixed crops at the department field, all lures i.e. ME, CL, ME+CL and ME/CL showed effectiveness to attract tephritid fruit flies. Significantly, the maximum flies were caught with ME traps at 1st, 3rd, 4th, 5th and 6th week and number ranged from 102.33-251.60 flyies/trap/week (Table 1).

**Table 1.** The average number of total trapped fruit flies (mean ± SE) in single and mixed lure systems (flies/trap/week) caught in ME, CL, ME+CL and ME/CL traps.

| Weeks | Mean number of caught flies (flies/trap/week) * | | | | CV (%) | F value |
|---|---|---|---|---|---|---|
| | ME | CL | ME+CL | ME/CL | | |
| 1st | 251.60 ± 113.54a | 14.33 ± 4.91b | 34.67 ± 16.51ab | 156.67 ± 54.23a | 120.21 | ** |
| 2nd | 106.00 ± 41.04a | 4.33 ± 2.02a | 31.67 ± 16.76a | 75.00 ± 16.62a | 99.12 | ns |
| 3rd | 102.33 ± 28.90a | 15.67 ± 8.74b | 39.33 ± 12.55ab | 86.67 ± 14.84a | 73.99 | ** |
| 4th | 207.33 ± 57.00a | 15.00 ± 4.62b | 124.67 ± 42.83a | 112.67 ± 35.05a | 80.39 | ** |
| 5th | 158.67 ± 29.45a | 26.00 ± 6.43b | 99.33 ± 26.64a | 106.00 ± 36.83a | 65.33 | ** |
| 6th | 126.67 ± 27.44a | 27.00 ± 14.36b | 100.67 ± 37.26ab | 80.00 ± 8.39ab | 63.09 | ** |
| 7th | 76.00 ± 1.53a | 43.67 ± 26.17a | 84.00 ± 15.57a | 56.00 ± 9.54a | 44.49 | ns |
| 8th | 40.67 ± 21.14a | 24.33 ± 16.50a | 63.67 ± 4.91a | 52.33 ± 11.35a | 58.75 | ns |

*The data were transformed by log (x+0.5) before analyses. Mean in the same row with different letters are significantly different by Tukey's HSD test ($P<0.05$).

There was significant difference among single and mixed lures for *B. dorsalis*. Maximum number of traps catches was found in ME traps ranged from 31.00-210.33 flies/trap/week (Table 2). Mixed lures were less effective for catching *B. dorsalis* ranged from 16.67-128.33 flies/trap/week and not significantly different from ME trap (Table 2). Mixing or placing CL adjacent to ME, decreases its effectiveness (Hooper 1978, Shelly et al., 2004). Although, *B. dorsalis* was not CL responsive species. Few numbers of this species were caught in CL lure. It might be inappropriate in handling during injecting the lures (Table 2).

**Table 2.** The average number of trapped (mean ± SE) of *Bactrocera dorsalis* (flies/trap/week) caught in ME, CL, ME+CL and ME/CL traps

| Weeks | Mean number of caught flies (flies/trap/week) * | | | | CV (%) | F value |
|---|---|---|---|---|---|---|
| | ME | CL | ME+CL | ME/CL | | |
| 1st | 210.33 ± 101.04a | 2.67 ± 1.67c | 19.00 ± 8.66bc | 128.33 ± 44.91ab | 133.74 | ** |
| 2nd | 71.67 ± 27.82a | 0.00 ± 0.00b | 16.67 ± 8.33ab | 56.33 ± 11.78a | 104.37 | ** |
| 3rd | 74.67 ± 23.81a | 0.33 ± 0.33b | 26.33 ± 13.96a | 76.33 ± 16.17a | 92.56 | ** |
| 4th | 154.00 ± 54.53a | 0.33 ± 0.33b | 47.33 ± 14.62a | 86.33 ± 29.46a | 104.81 | ** |
| 5th | 136.67 ± 22.26a | 0.00 ± 0.00b | 59.00 ± 21.39a | 91.67 ± 37.22a | 87.84 | ** |
| 6th | 109.00 ± 23.64a | 0.00 ± 0.00b | 65.67 ± 23.18a | 58.67 ± 8.09a | 80.89 | * |
| 7th | 62.00 ± 2.00a | 0.00 ± 0.00b | 53.00 ± 7.09a | 45.67 ± 8.84a | 67.47 | ** |
| 8th | 31.00 ± 15.63ab | 0.00 ± 0.00b | 43.33 ± 2.03a | 37.00 ± 11.72a | 81.37 | * |

*The data were transformed by log (x+0.5) before analyses. Mean in the same row with different letters are significantly different by Tukey's HSD test ($P$<0.05).

Regarding to *Z. cucurbitae*, the 1.00-10.67 flies/trap/week (Table 3). Among eight weeks, the combinations trap of ME+CL and ME/CL caught *Z. cucurbitae* and it was not significantly different from the CL alone trap (Table 3). The other work reported that the CL added to ME increased the effectiveness of CL and catches more *Z. cucurbitae* than CL alone traps (Hooper, 1978; Shelly et al., 2004).

**Table 3.** The average number of trapped (mean ± SE) of *Zeugodacus cucurbitae* (flies/trap/week) caught in ME, CL, ME + CL, ME/CL traps

| Weeks | Mean number of caught flies (flies/trap/week) * | | | | CV (%) | F value |
|---|---|---|---|---|---|---|
| | ME | CL | ME+CL | ME/CL | | |
| 1st | 0.00 ± 0.00b | 6.00 ± 0.58a | 4.00 ± 1.53a | 1.67 ± 1.20ab | 96.38 | ** |
| 2nd | 0.00 ± 0.00a | 1.00 ± 0.58a | 1.67 ± 0.88a | 0.00 ± 0.00a | 127.29 | ns |
| 3rd | 0.00 ± 0.00a | 7.00 ± 4.04a | 4.67 ± 3.18a | 2.67 ± 1.67a | 134.34 | ns |
| 4th | 0.00 ± 0.00b | 7.00 ± 3.06ab | 37.33 ± 17.64a | 3.00 ± 0.58ab | 172.81 | ** |
| 5th | 0.00 ± 0.00b | 10.67 ± 3.76a | 19.67 ± 9.60a | 3.00 ± 0.58ab | 132.18 | ** |
| 6th | 0.00 ± 0.00b | 6.67 ± 1.45a | 12.00 ± 7.21a | 9.00 ±7.51ab | 130.55 | * |
| 7th | 0.00 ± 0.00c | 6.67 ± 1.33a | 7.33 ± 1.86a | 1.67 ± 0.33b | 99.02 | ** |
| 8th | 0.00 ± 0.00b | 3.67 ± 1.45a | 3.67 ± 1.45a | 3.67 ± 1.67a | 93.19 | * |

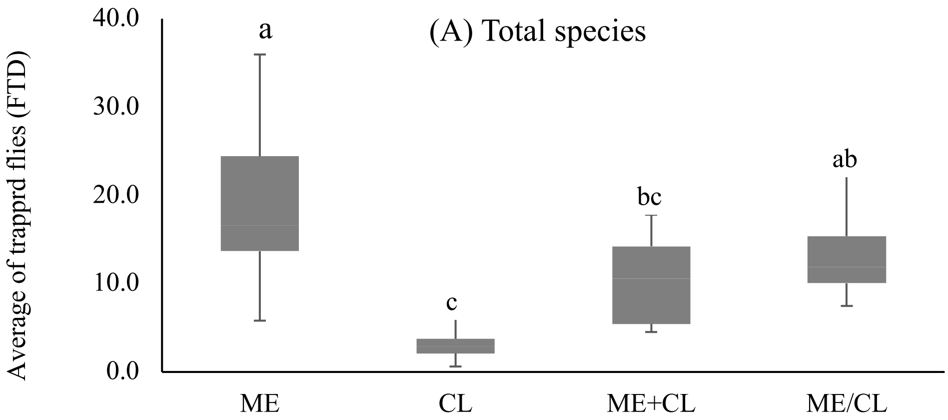
*The data were transformed by log (x+0.5) before analyses. Mean in the same row with different letter are significantly different by Tukey's HSD test (P<0.05).

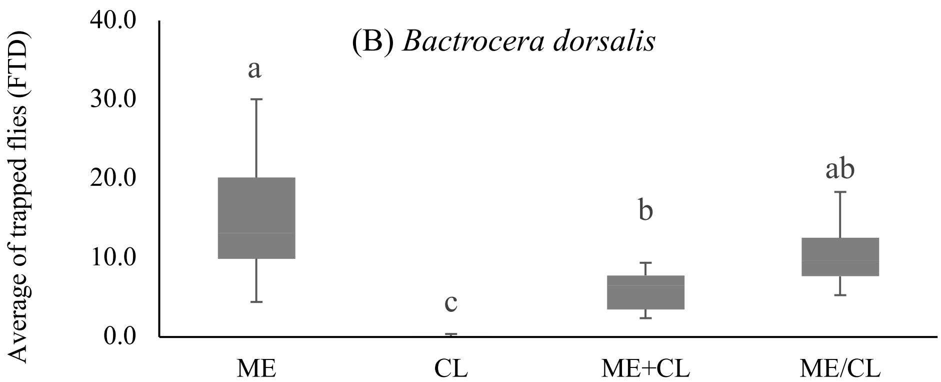
For an easy and clear understanding of the performance of four the treatments, the data were plotted on boxplot and analyzed. There was a highly significant variation among treatments ($F_{3,32} = 9.427$; $P<0.01$) for total species trapped. The highest mean trapped flies were found in ME traps (19.1 ± 3.5 FTD) followed by ME/CL traps (12.9 ± 1.7 FTD). The mean trapped flies by ME + CL traps were (10.3 ± 1.7 FTD), and in CL traps were 3.0 ± 0.6 FTD (Fig. 19A).

For *B. dorsalis*, there was a highly significant variation ($F_{3,32} = 91.813$; $P<0.01$) with trap catches. ME traps trapped the maximum number of flies (15.1 ± 2.9 FTD), which statistically followed by the ME/CL traps (10.36 ± 1.4 FTD). ME + CL traps catches were (5.9 ± 0.9 FTD). A little fly was trapped in CL traps due to interference (0.06 ± 0.05 FTD) (Fig. 19B).

In the case of *Z. cucurbitae*, opposite and statistically different trend observed ($F_{3,32} = 11.698$; $P<0.01$). ME + CL traps caught the highest mean number of flies (1.6 ± 0.6 FTD) but with CL traps (0.8 ± 0.1 FTD). While CL traps were statistically same with ME/CL traps (0.4 ± 0.1 FTD). ME traps were empty (Fig.19C).

Moreover, the single ME trap caught four species of fruit flies i.e. *B. carambolae*, *B. correcta*, *B. dorsalis* and *B. umbrosa*. The single CL trap caught six species of fruit flies i.e. *B. albistrigata*, *B. caudata*, *B. dorsalis*, *B. nigrofemoralis*, *Z. cucurbitae* and *Z. tau*. Interestingly, ME+CL or ME/CL combination caught nine species of fruit flies (Table 4 and Fig. 20). The combination of ME and CL in the same traps found more effective of caught flies' species than single traps (Hooper, 1978, Shelly et al., 2004).



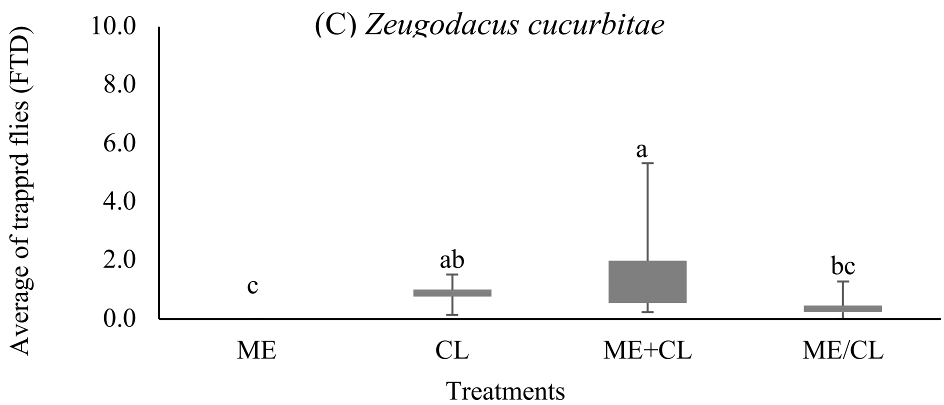




**Figure 19**. Boxplot analysis of average caught flies of total species (A), *Bactrocera dorsalis* (B), and *Zeugodacus cucurbitae* (C) to rubber foam with methyl eugenol (ME) and cue lure (CL) mixed (ME + CL), and separated (ME/CL). Mean with different letter were significantly different by Tukey's HSD test (*P*<0.05).

**Table 4.** Nine species of fruit flies caught in the traps of rubber foam with methyl eugenol (ME) and cue lure (CL) mixed (ME + CL), and separated (ME/CL).

| Species | ME | CL | ME+CL | ME/CL |
|---|---|---|---|---|
| *Bactrocera albestrigata* | - | ✓ | ✓ | ✓ |
| *B. carambolae* | ✓ | - | ✓ | ✓ |
| *B. caudata* | - | ✓ | ✓ | ✓ |
| *B. correcta* | ✓ | - | ✓ | ✓ |
| *B. dorsalis* | ✓ | ✓ | ✓ | ✓ |
| *B. nigrofemoralis* | - | ✓ | ✓ | ✓ |
| *B. umbrosa* | ✓ | - | ✓ | ✓ |
| *Zeugodacus cucurbitae* | - | ✓ | ✓ | ✓ |
| *Z. tau* | - | ✓ | ✓ | ✓ |

Previous studies suggest an asymmetry in the effectiveness of ME+CL mixture for ME- versus CL-responding species. The single and mixed CL placed immediately adjacent to ME, reduces trap capture of ME-responding species (Hooper, 1978; Vargas et al., 2000). However, ME/CL combinations have been found to be equally attractive as ME for *B. dorsalis* (Vargas et al., 2012).

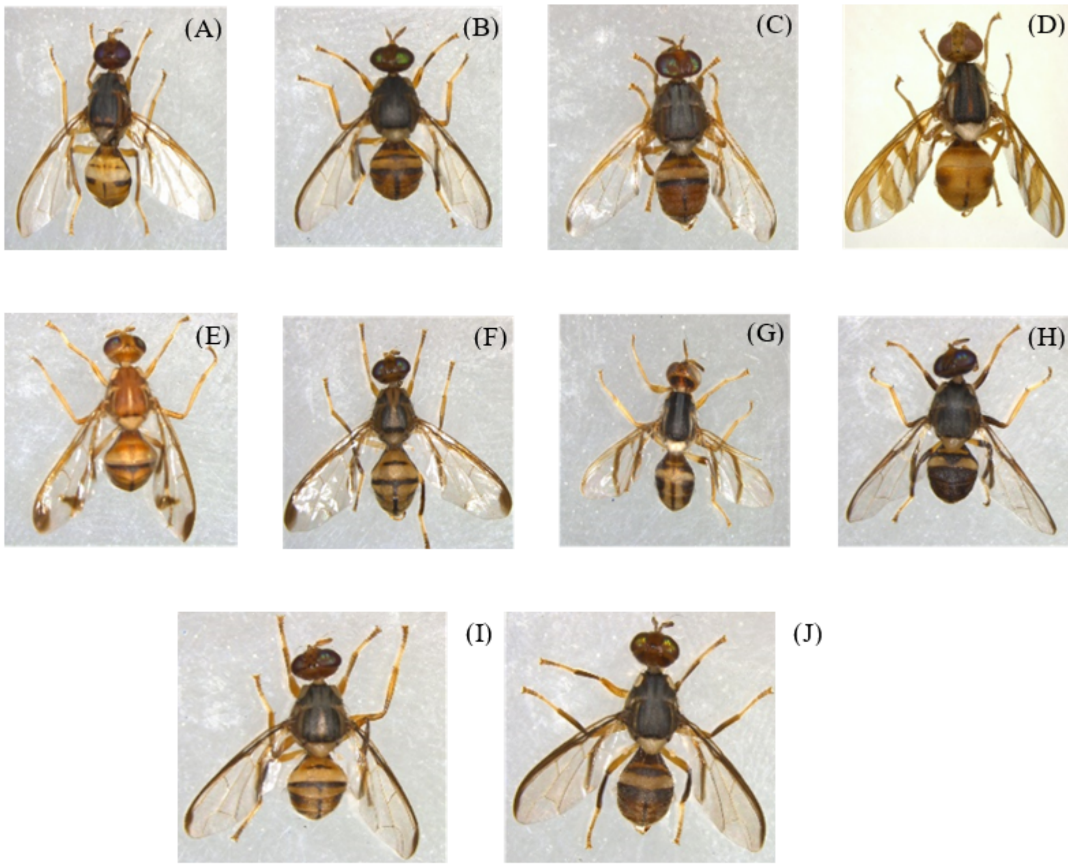



**Figure 20**. Species identified from the study under sterio-microcope *Bactrocera dorsalis* (A), *Bactrocera carambolae* (B), *Bactrocera correcta* (C), *Bactrocera umbrosa* (D), *Zeugodacus cucurbitae* (E), *Zeugodacus tau* (F), *Bactrocera albestrigata* (G), *Bactrocera nigrofemoralis* (H), *Bactrocera caudata* (I) *and unknown species* (J).

## 4.2 The toxicity test of four essential oils in foam to adult flies under different exposure periods

### 4.2.1 Mortality assay for *B. dorsalis* with plant essential oils

On day 0 of weathering, death of flies due to exposure of clove and eucalyptus oils started on the 1$^{st}$hour of exposure, citronella and basil oils became effective in 6 and 12 hours, respectively. Eucalyptus oil outperformed the other three oils, giving 100.0% mortality within 12 hours on 0 days of weathering ($F_{4,20}$ = 10.728; $P$<0.01) (Fig. 21A). After 72 hours of 0 days of weathering, 60.0 ± 16.3% and 60.0 ± 12.2%

mortalities were recorded with clove and citronella oils, respectively, while 40.0 ± 4.1% for basil oil ($F_{4,20}$ = 131.832; $P$<0.01).

On day 15 of weathering, the mortality effect of all the essential oils was decreased and flies needed more exposure time. After 72 hours of 15 days of weathering, basil oil caused 95.0± 2.9% mortality followed by clove oil with 87.5 ± 2.5% mortality ($F_{4,20}$ = 1737.687; $P$<0.01). The other two oils were less active, with below 40.0% mortalities (Fig. 21B).

On day 30 of weathering, after 72 hours of exposure of the flies, 72.5 ± 2.5% and 67.5 ±10.3% mortalities in flies were recorded for basil and clove oils, respectively. Citronella and eucalyptus oil performed mortality poorly ($F_{4,20}$ = 42.390; $P$<0.01) (Fig. 21C). On day 45 of weathering, the effectiveness of all essential oils ranged within 12.5% of mortality and higher than control ($F_{4,20}$ = 3.441; $P$<0.05) (Fig. 21D).

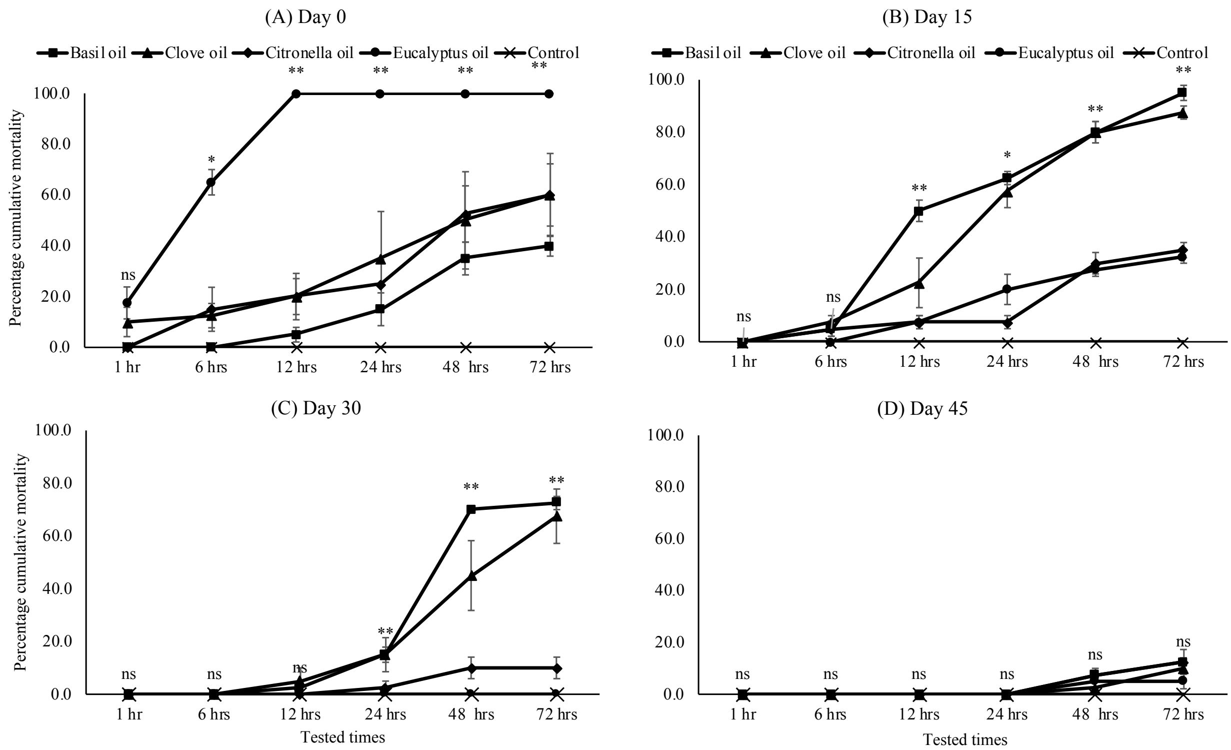



**Figure 21.** Cumulative mortalities of adult *Bactrocera dorsalis* from rubber foam injected with a plant essential oil (basil oil, clove oil, citronella oil and eucalyptus oil) and weathered for (A) 0, (B) 15, (C) 30 and (D) 45 days before application. The control was rubber foam without plant essential oil.

### 4.2.2 Mortality assay for *Z. cucurbitae*

On day 0 of weathering, the eucalyptus oil showed the quickest effect (100.0% mortality) in *Z. cucurbitae* adult within 12 hours of the exposure, while other EOs were very slow in becoming active (Fig. 22A). On day 15 of weathering, after 72 hours the basil and clove oils caused 76.7 ± 6.7% and 40.0 ± 11.5% mortalities in flies, respectively. Whereas, citronella and eucalyptus oils caused 16.7 ± 8.8%and 16.7 ± 3.3% mortalities in flies, respectively ($F_{4,15} = 76.565$; $P<0.01$) (Fig. 22B). On day 30 of weathering, all the treatments had lost effectiveness and took a long time to show activity. Basil oil caused only26.7 ± 16.7% mortality in flies. At the same time, other EOs was less than 20.0% mortalities in tested flies ($F_{4,15} = 18.630$; $P<0.01$) (Fig. 22C). The weathered rubber foams on day 45, all EOs tested caused <10.0% mortalities in *Z. cucurbitae* adults with no significant difference ($F_{4,15} = 3.392$; $P>0.05$) (Fig. 22D).

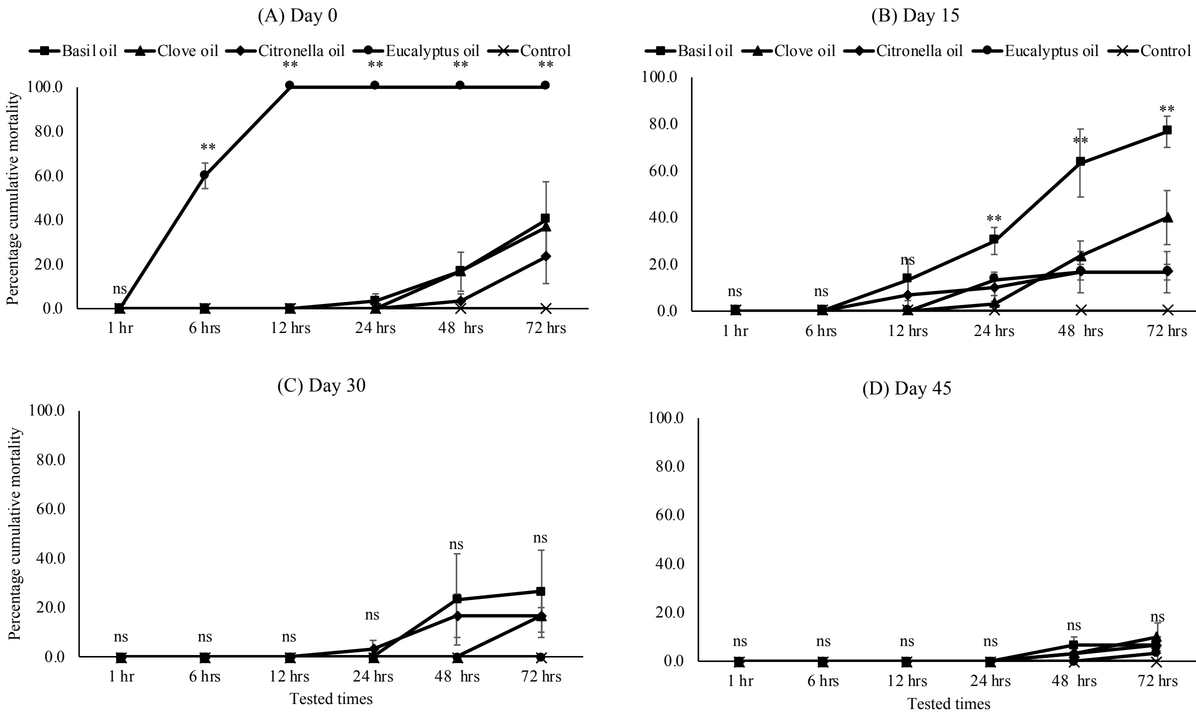



**Figure 22.** Cumulative mortalities of adult *Zeugodacus cucurbitae* from rubber foam injected with plant essential oils (basil oil, clove oil, citronella oil or eucalyptus oil) and weathered for (A) 0, (B) 15, (C) 30 or (D) 45 days before application. The control was rubber foam without plant essential oil.

The EOs of basil, clove, citronella and eucalyptus were tested and displayed the insecticidal activity to *B. dorsalis* and *Z. cucurbitae*. In the present study, eucalyptus oil was the most toxic, with 100% mortality within 12 hours, followed by clove, basil, and citronella oils for both *B. dorsalis* and *Z. cucurbitae*. This finding is supported by other studies with similar results. The most toxic oil was eucalyptus against peach fruit fly, *B. zonata* (Sunders) pupa ($LC_{50}$ value 38.88ml/L) followed by basil and other EOs (Ali, 2018). Also, on the first and second instar of Queensland fruit fly, *B. tryoni* (Froggatt), eucalyptus oil had a strong fumigant effect (Hidayat &Yusup, 2014).

Besides, *Eucalyptus globules* oil is very toxic against *Aedes aegypti* (L.) larvae and had a $LC_{50}$ of 32.4 ppm (Lucia et al., 2007). The present study, our experiments with various exposure times as well as different weathering time of EOs in rubber foam showed that the basil oil was highly effective against *B. dorsalis,* and *Z. cucurbitae,* after weathering for 15 days in natural conditions, compared to clove, citronella and eucalyptus essential oils. Similar results have been reported by Ling et al. (2009) with basil oil and its three major active constituents (trans-anethole, estragole, and linalool) obtained from basil (*Ocimum basilicum* L.) on *Ceratitis capitata* (Wiedemann), *B. dorsalis* and *Z. cucurbitae*. The toxic action of basil oil in *C. capitata* was significantly faster than in *Z. cucurbitae,* and slightly faster than in *B. dorsalis* (Chiou et al., 2009).

In another study, tea tree oil was tested on Mediterranean fruit fly (*C. capitata)* and its parasitoid *Psyttalia concolor* by contact and fumigation assay. The tea tree oil was more toxic on *C. capitata* to that of parasitoid *P. concolor* (Benelli et al., 2013), suggested that essential oils was safe for the parasitoids and other natural enemies. EOs from fresh leaves of rosemary, lavender, chan, poiteau and arborvitae were tested against *C. capitata,* where lavender oil was found the most toxic at 24 hours of exposure with LC50 of 9.09 µg/L (Benelli et al., 2012).

Some plant essential oils are very effective with quick action on some insect pests, by affecting the neuromodulator octopaminergic receptor (Kostyukovsky et al., 2002). This receptor is absent in mammals, making such actions less hazardous to other non-target organisms and the environment. For *B. dorsalis,* the death rates were 60% and 87.5% after 72 hours on day 0 and day 15 of weathering, whereas 36.7- 40% mortality has been found in *Z. cucurbitae* after 72 hours on day 0 and at 15 days of weathering with clove essential oil.

Toxicity of plant EOs was also tested against Mexican fruit fly *Anastrepha ludens* (Loew). EOs from *Eugenia caryophyllus*, *Ocimum basilicum,* and *Thymus vulgaris* were used to determine the ingestion in against adult Mexican fruit fly. Among the three EOs, *E. caryophyllus* was the most toxic with $LC_{50}$ of 3,529ppm, followed by *T. vulgaris* (5,347 ppm) and *O. basilicum* (8,050 ppm) (Buentello-Wong et al., 2016). In the present study, essential oil of basil was the most effective oil up to 45 days of weathering time against *B. dorsalis* and *Z. cucurbitae* under laboratory conditions. However, further research is needed to assess the effectiveness and chemical firmness of the essential oils in the field conditions to know the impact of air, moisture, light, temperature and humidity on effectiveness of basil essential oil against *B. dorsalis* and *Z. cucurbitae*. Finally, we conclude that among the four plant essential oils basil oil was the most toxic during 15 days for *Z. cucurbitae* & 30 days for *B. dorsalis* of after application.

### 4.3 Attractancy test of *B. dorsalis* and *Z. cucurbitae* in combination of lures with best essential oil from experiment 2 at same/ different point in foam under laboratory condition

#### 4.3.1 Attractancy of adult male of *B. dorsalis* and *Z. cucurbitae* to rubber foam with methyl eugenol (ME) and cue lure (CL) singly or mixed

On day 0 of weathering, the highest attractancy of *B. dorsalis* was with ME and for *Z. cucurbitae* by ME + CL at 32.5 ± 4.8% and 27.5 ± 4.8%, respectively. For both the species, ME + CL showed the highest attractancy of 50.0 ± 6.1% ($F_{4,20}$ = 83.227; $P$<0.01). On day 15 of weathering, the attractancy was slightly less than day zero in all of the treatments. It was 30.0 ± 2.0%, 22.5 ± 3.2%, and 48.8 ± 1.3% for *B. dorsalis* ($F_{4,20}$ = 171.395; $P$<0.01), *Z. cucurbitae* ($F_{4,20}$ = 27.101; $P$<0.01), and both the species ($F_{4,20}$ = 229.450; $P$<0.01) with ME, ME + CL and ME/ CL, respectively. On day 30 of weathering the rubber foam showed reduced attractancy of *B. dorsalis* ($F_{4,20}$ = 121.471; $P$<0.01) with all of the treatments. Though, there was a slight rise in the attractancy of *Z. cucurbitae* ($F_{4,20}$ = 37.935; $P$<0.01) by all the treatments. On day 45 of weathering, the attractancies were 17.5 ± 4.3% and 18.8 ± 5.2% by ME and ME + CL for *B. dorsalis* ($F_{4,20}$ = 23.924; $P$<0.01) but *Z. cucurbitae* ($F_{4,20}$ = 84.347; $P$<0.01) had a lower attractancy than on day 15 (Table 5).

**Table 5.** Percentage attraction of adult male *Bactrocera dosalis* and *Zeugodacus cucurbitae* to rubber foam loaded with single and mixed of methyl eugenol (ME) and cue lure (CL) under laboratory condition.

| Tested day | Fruit fly's species | Mean percentage of attraction (mean ± S.E.) | | | | | F value |
|---|---|---|---|---|---|---|---|
| | | ME | CL | ME+CL | ME/CL | Control | |
| 0 | *B. dorsalis* | 32.5 ± 4.8a | 0.0 ± 0.0c | 22.5 ± 1.4ab | 21.3 ± 2.4b | 0.0 ± 0.0c | ** |
| | *Z. cucurbitae* | 0.0 ± 0.0c | 17.5 ± 1.4ab | 27.5 ± 4.8a | 13.8 ± 2.4b | 0.0 ± 0.0c | ** |
| | Total | 32.5 ± 4.8b | 17.5 ± 1.4c | 50.0 ± 6.1a | 35.0 ± 2.0ab | 0.0 ± 0.0c | ** |
| 15 | *B. dorsalis* | 30.0 ± 2.0a | 0.0 ± 0.0b | 26.3 ± 2.4a | 25.0 ± 3.5a | 0.0 ± 0.0b | ** |
| | *Z. cucurbitae* | 0.0 ± 0.0c | 18.8 ± 2.4ab | 22.5 ± 3.2a | 8.8 ± 3.1b | 0.0 ± 0.0c | ** |
| | Total | 30.0 ± 2.0b | 18.8 ± 2.4c | 48.8 ± 1.3a | 33.8 ± 2.4b | 0.0 ± 0.0d | ** |
| 30 | *B. dorsalis* | 15.0 ± 2.0a | 0.0 ± 0.0b | 16.3 ± 2.4a | 17.5 ± 1.4a | 0.0 ± 0.0b | ** |
| | *Z. cucurbitae* | 0.0 ± 0.0b | 28.8 ± 4.3a | 31.3 ± 4.7a | 18.8 ± 6.9a | 0.0 ± 0.0b | ** |
| | Total | 15.0 ± 2.0c | 28.8 ± 4.3b | 47.5 ± 3.2a | 36.3 ± 5.5ab | 0.0 ± 0.0d | ** |
| 45 | *B. dorsalis* | 17.5 ± 4.3a | 0.0 ± 0.0b | 18.8 ± 5.2a | 8.8 ± 3.8a | 0.0 ± 0.0b | ** |
| | *Z. cucurbitae* | 0.0 ± 0.0c | 20.0 ± 2.0a | 15.0 ± 2.0ab | 11.3 ± 2.4b | 0.0 ± 0.0c | ** |
| | Total | 17.5 ± 4.3ab | 20.0 ± 2.0a | 33.8 ± 5.6a | 20.0 ± 4.1a | 0.0 ± 0.0c | ** |

[*]Different letters in the same row indicate statistical significances by Tukey' HSD test ($P$<0.01).

**4.3.2 Attractancy of adult male *B. dorsalis* and *Z. cucurbitae* to rubber foam injected with basil oil (BO) in the same spot with methyl eugenol (ME) and cue lure (CL) singly or mixed**

After injection of basil oil in the same spot either a single or mixed lure of ME and CL, the attractancy was very low on day 0 of weathering. The ME + BO and ME + CL+BO showed the highest attractancies of *B. dorsalis* and *Z. cucurbitae* at 5.0 ± 2.9% ($F_{4,15}$ = 1.731; $P$>0.05) and 8.3 ± 4.4% ($F_{4,15}$ = 3.157; $P$>0.05), respectively, without significant difference from other treatments tested. For both species, the ME+CL+BO and ME/CL+BO showed the same attractancies at 10.0 ± 2.9% and 10.0 ± 5.0% without significant difference from the other treatments ($F_{4,15}$ = 2.557; $P$>0.05).Then, attractancy increased for flies in all the treatments on day 15 of weathering.

The ME + CL + BO and CL + BO showed the highest attractancies and significantly differed from the other treatments for *B. dorsalis* and *Z. cucurbitae* with 28.3 ± 7.3% ($F_{4,15}$ = 16.403; $P$<0.01) and 13.3 ± 1.7% ($F_{4,15}$ = 58.334; $P$<0.01), respectively. For both species, ME + CL + BO showed the highest attractancy of 35 ± 8.7%, that was significantly different from the control only ($F_{4,15}$ = 12.362; $P$<0.01) (Table 6). On day 30 of weathering, the attractancy for *B. dorsalis* rose slightly (16.7-23.3%) and was highly significantly different ($F_{4,15}$ = 14.422; $P$<0.01), whereas there was a sharp fall in the attractancy for *Z. cucurbitae* (3.3-5.0%) but without significant difference ($F_{4,15}$ = 1.107; $P$>0.05).

The attractancy of both species across all treatments ranged within 5.0-26.7% and was significantly different ($F_{4,15}$ = 7.156; $P$<0.01). On day 45 of weathering, the attractancy of all treatments for *B. dorsalis* had decreased with highly significant difference ($F_{4,15}$ = 32.515; $P$<0.01), while it for *Z. cucurbitae* increased with significant difference ($F_{4,15}$ = 5.634; $P$<0.05). Both species showed similar attractancies (18.3-21.7%) and all treatments were not significantly different except in the control ($F_{4,15}$ = 13.841; $P$<0.01) (Table 6).

**Table 6.** Percentage attraction of adult male *Bactrocera dosalis* and *Zeugodacus cucurbitae* to rubber foam injected basil oil (BO) in the same spot with single and mixed of methyl eugenol (ME) and cue lure (CL) under laboratory condition.

| Tested day | Fruit fly's species | Mean percentage of attraction (mean ± S.E.) | | | | | F value |
|---|---|---|---|---|---|---|---|
| | | ME+BO | CL+ BO | ME+CL+ BO | ME/CL + BO | Control | |
| 0 | *B. dorsalis* | 5.0 ± 2.9a | 0.0 ± 0.0a | 1.7 ± 1.7a | 3.3 ± 1.7a | 0.0 ± 0.0a | ns |
| | *Z. cucurbitae* | 0.0 ± 0.0a | 6.7 ± 1.7a | 8.3 ± 4.4a | 6.7 ± 3.3a | 0.0 ± 0.0a | ns |
| | Total | 5.0 ± 2.9a | 6.7 ± 1.7a | 10.0 ± 2.9a | 10.0 ± 5.0a | 0.0 ± 0.0a | ns |
| 15 | *B. dorsalis* | 18.3 ± 7.3a | 0.0 ± 0.0b | 28.3 ± 7.3a | 18.3 ± 6.0a | 0.0 ± 0.0b | * |
| | *Z. cucurbitae* | 0.0 ± 0.0c | 13.3 ± 1.7a | 6.7 ± 1.7b | 8.3 ± 1.7ab | 0.0 ± 0.0c | ** |
| | Total | 18.3 ± 7.3a | 13.3 ± 1.7a | 35.0 ± 8.7a | 26.7 ± 7.3a | 0.0 ± 0.0b | * |
| 30 | *B. dorsalis* | 20.0 ± 7.6a | 0.0 ± 0.0b | 16.7 ± 6.0a | 23.3 ± 4.4a | 0.0 ± 0.0b | * |
| | *Z. cucurbitae* | 0.0 ± 0.0a | 5.0 ± 5.0a | 3.3 ± 1.7a | 3.3 ± 1.7a | 0.0 ± 0.0a | ns |
| | Total | 20.0 ± 7.6a | 5.0 ± 5.0ab | 20.0 ± 5.0a | 26.7 ± 6.0a | 0.0 ± 0.0b | * |
| 45 | *B. dorsalis* | 13.3 ± 1.7a | 0.0 ± 0.0b | 13.3 ± 3.3a | 15.0 ± 5.0a | 0.0 ± 0.0b | * |
| | *Z. cucurbitae* | 0.0 ± 0.0b | 15.0 ± 7.6a | 6.7 ± 4.4ab | 6.7 ± 1.7ab | 0.0 ± 0.0b | * |
| | Total | 13.3 ± 1.7a | 15.0 ± 7.6a | 20.0 ± 2.9a | 21.7 ± 4.4a | 0.0 ± 0.0b | ns |

*Different letters in the same row indicate statistical significances by Tukey' HSD test (*P*<0.01).

### 4.3.3 Attractancy of adult male *Bactrocera dorsalis* and *Zeugodacus cucurbitae* to rubber foam injected with basil oil (BO) in a different spot than with methyl eugenol (ME) and cue lure (CL) singly or mixed

With injection of basil oil in different spot by either single or a mixed lures of ME and CL in a rubber foam dispenser, all the treatments showed low attractancy for *B. dorsalis* (3.3-8.3%), for *Z. cucurbitae* (3.3-18.3%) and for both species (3.3-18.3%) on day 0 of weathering (Table 7). On day 15 of weathering, the attractancy increased for all the treatments. The highest attraction for *B. dorsalis, Z. cucurbitae* and for both species by ME + CL/BO, CL/BO and ME + CL/BO was 23.3 ± 3.3% ($F_{4,15}$ = 6.202; *P*<0.01), 15.0 ± 2.9% ($F_{4,15}$ = 6.801; *P*<0.01) and 30.0 ± 2.9% ($F_{4,15}$ = 4.980; *P*<0.05), respectively. After that, on day 30, the attractancy again increased slightly for *B. dorsalis*. Here, the ME + CL/BO showed high attractancy for *B. dorsalis*, *Z. cucurbitae* and both species at 28.3 ± 1.7%, 6.7 ± 3.3% and 35 ± 5.0%, respectively. Further, on day 45, the treatments were significantly differed for *B. dorsalis* ($F_{4,15}$ = 26.258; *P*<0.01) and *Z. cucurbitae* ($F_{4,15}$ = 42.625; *P*<0.01). For both species, all treatments were not significantly different except with the control ($F_{4,15}$ = 20.148; *P*<0.01) (Table 7).

On the other study, we incorporate the basil oil with male lures ME and CL, at the same point and on the different positions on rubber foam to observe the attractancy. But, in case of single lures the result was not satisfactory. For both species, mixed lures ME + CL gave the highest attractancy of 50%. Basil oil with ME decreases the effectiveness of ME in attracting *B. dorsalis*. With CL, it mostly reduces the action of CL in attracting *Z. cucurbitae*. This result was similar to Chiou et al. (2009) when methyl eugenol (*B. dorsalis* male attractant) was mixed with basil oil to increase the toxicity of basil oil to *B. dorsalis*. Methyl eugenol additionally might also play a physiological role in toxicity reduction. Cue lure at excessive concentrations (50 and 90%) in combination with basil oil decreased the mortality/knockdown in *Z. cucurbitae* males and females.

**Table 7.** Percentage attraction of adult male *Bactrocera dosalis* and *Zeugodacus cucurbitae* to rubber foam injected basil oil (BO) in different spot with single and mixed of methyl eugenol (ME) and cue lure (CL) under laboratory condition.

| Tested day | Fruit fly's species | Mean percentage of attraction (mean ± S.E.) | | | | | F value |
|---|---|---|---|---|---|---|---|
| | | ME/BO | CL/BO | ME+CL/BO | ME/CL/BO | Control | |
| 0 | *B. dorsalis* | 3.3 ± 3.3a | 0.0 ± 0.0a | 3.3 ± 1.7a | 8.3 ± 4.4a | 0.0 ± 0.0a | ns |
| | *Z. cucurbitae* | 0.0 ± 0.0b | 18.3 ± 1.7a | 3.3 ± 3.3ab | 6.7 ± 4.4ab | 0.0 ± 0.0b | * |
| | Total | 3.3 ± 3.3a | 18.3 ± 1.7a | 6.7 ± 4.4a | 15.0 ± 7.6a | 0.0 ± 0.0a | ns |
| 15 | *B. dorsalis* | 18.3 ± 10.1ab | 0.0 ± 0.0b | 23.3 ± 3.3a | 13.3 ± 6.0ab | 0.0 ± 0.0b | * |
| | *Z. cucurbitae* | 0.0 ± 0.0b | 15.0 ± 2.9a | 6.7 ± 4.4ab | 1.7 ± 1.7b | 0.0 ± 0.0b | * |
| | Total | 18.3 ± 10.1ab | 15.0 ± 2.9ab | 30.0 ± 2.9a | 15.0 ± 5.8ab | 0.0 ± 0.0b | ** |
| 30 | *B. dorsalis* | 16.7 ± 1.7b | 0.0 ± 0.0c | 28.3 ± 1.7a | 26.7 ± 1.7a | 0.0 ± 0.0c | ** |
| | *Z. cucurbitae* | 0.0 ± 0.0a | 6.7 ± 4.4a | 6.7 ± 3.3a | 5.0 ± 2.9a | 0.0 ± 0.0a | ns |
| | Total | 16.7 ± 1.7ab | 6.7 ± 4.4bc | 35.0 ± 5.0a | 31.7 ± 3.3a | 0.0 ± 0.0c | ** |
| 45 | *B. dorsalis* | 15.0 ± 5.0a | 0.0 ± 0.0b | 15.0 ± 2.9a | 10.0 ± 2.9a | 0.0 ± 0.0b | * |
| | *Z. cucurbitae* | 0.0 ± 0.0b | 15.0 ± 2.9a | 13.3 ± 3.3a | 8.3 ± 1.7a | 0.0 ± 0.0b | * |
| | Total | 15.0 ± 5.0a | 15.0 ± 2.9a | 28.3 ± 4.4a | 18.3 ± 4.4a | 0.0 ± 0.0b | ns |

*Different letters in the same row indicate statistical significances by Tukey' HSD test ($P$<0.01).

A boxplot analysis of attractancy from day-0 to day-45 was performed. For *B. dorsalis*, the ME singly, ME + CL and ME/CL with and without BO were not significantly different and ranged from 15.5-20.3%, while the CL alone and CL with and without BO were not attracted this fly species (Fig. 23A). In contrast, *Z. cucurbitae* showed higher attraction towards ME + CL (21.5 ± 2.3%) and CL alone (20.5 ± 1.5%) than when mixed with BO (6.7-13.3%) (Fig. 23B). For both species, ME + CL (39.5 ± 3.8%), ME/CL (27.5 ± 2.6%), and ME +CL/BO (27.6 ± 3.6%), showed higher attraction than the other treatments (Fig. 23C).

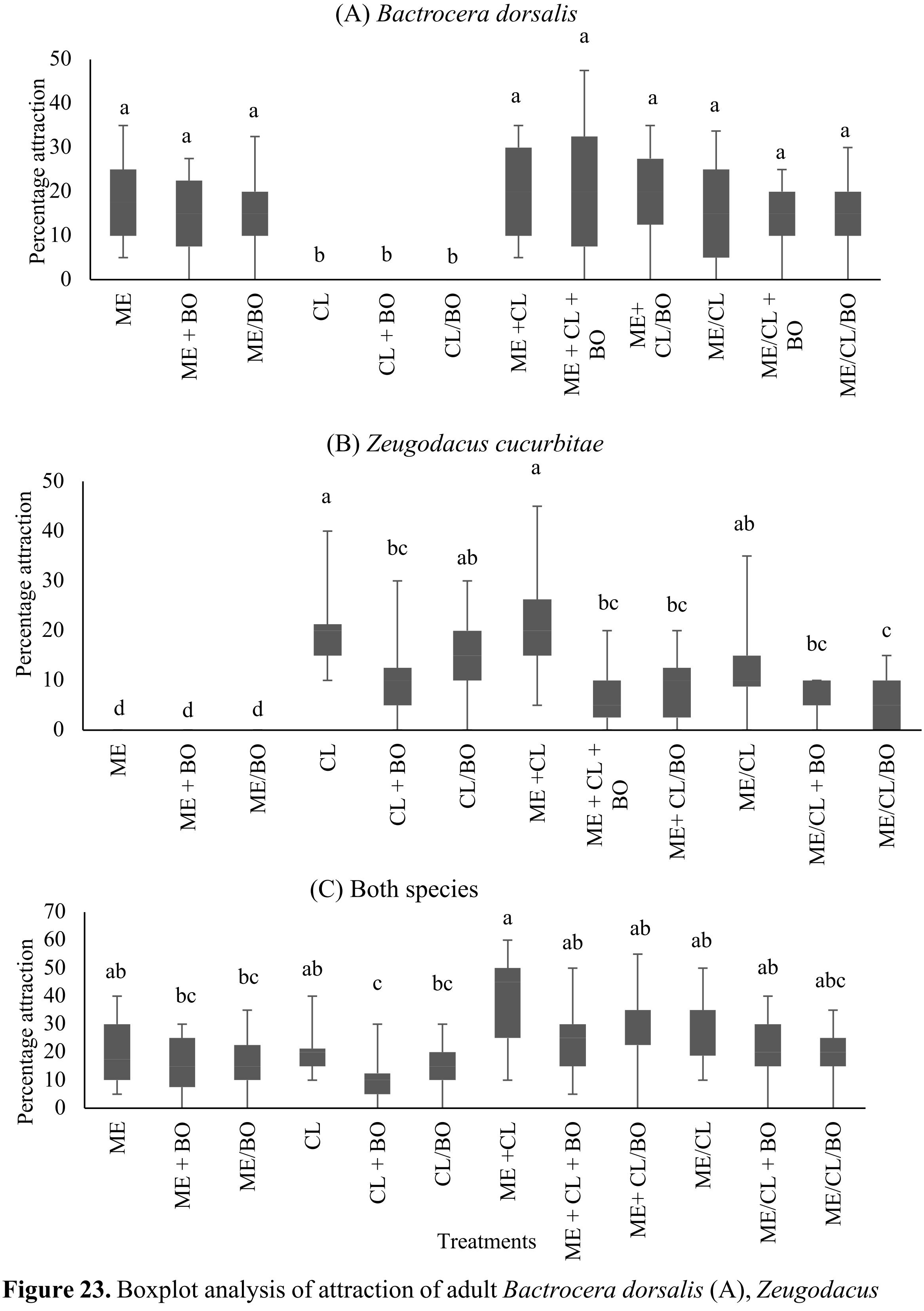



**Figure 23.** Boxplot analysis of attraction of adult *Bactrocera dorsalis* (A), *Zeugodacus cucurbitae* (B) and both species (C) to rubber foam with methyl eugenol (ME) and cue lure (CL) singly or mixed, with and without basil oil (BO) injected at the same or a different spot. Mean with different letter were significantly different by Tukey's HSD test ($P$<0.05).

Effect of mixed lure system was tested for the mass trapping of *B. dorsalis* and *Z. cucurbitae.* In the present investigation, mix lure of ME+CL attracts similar number of *B. dorsalis* adults as ME alone. Whereas, slightly increased attraction of *Z. cucurbitae* has been observed in treatment ME+CL. However, Shelly et al. (2004) observed significantly decreased attraction of *B. dorsalis* in ME + CL than ME alone and significantly increased attraction of *Z. cucurbitae* in ME + CL than CL alone. Slight lower and higher attraction has been observed in ME+CL for *B. dorsalis* and *Z. cucurbitae* than ME and CL alone, respectively but these lower and higher attractions in both species were not significantly different than ME and CL alone. This suggests that the trap with ME + CL would be a better option for the mixed cropping system where fruits and cucurbitaceous vegetables grown in an area for mass trapping, which reduce the cost of trapping system and labour charges for installation of only one trap instead of two traps for both the species. However, field testing needs to be conducted before its inclusion into the IPM program.

Further investigations on basil oil with male fruit fly attractant *i.e.* ME (methyl eugenol) and CL (cue lure) at the same point and on different positions on the rubber foam, and the attractancy was observed against *B. dorsalis* and *Z. cucurbitae* under laboratory conditions. Attraction of male *B. dorsalis* as well as *Z. cucurbiate* towards the ME and CL alone as well as in combination reduced when use the basil oil as toxicant in rubber foam dispenser for first 15 days of weathering. However, no significantly different effects were observed when used the basil oil at same point and different point with lures in rubber foam dispenser. This suggests that the effect of basil oil has not been different when use alone and in combination of lure.

However, the effectiveness of lures (ME and CL alone and in combination) towards attraction of *B. dorsalis* and *Z. cucurbitae* reduces when used with basil oil. This suggests that the basil oil also affect the physiology of attraction in *B. dorsalis* and *Z. cucurbitae.* This result was similar to Ling Chang et al., (2009), when methyl eugenol (*B. dorsalis* male attractant) was mixed with basil oil, it did not affect the toxicity of basil oil to *B. dorsalis*. Methyl eugenol additionally might also play a physiological role in toxicity reduction. Cue lure at excessive concentrations (50 and 90%) in combination with basil oil decreased the mortality or knockdown in *Z. cucurbitae* males and females.

Ling Chang et al., (2009) reported that the linalool with cue lure reduces the potency of linalool to kill the species with increased concentration of cue lure.

Dharmadasa et al. (2015) used two morphotypes of *Ocimum tenuiflorum* (L.) for attracting *B. dorsalis* and compared with methyl eugenol. They also showed results on the mean number of attracted flies in the first few weeks (53-111 flies) but did not mix plant essential oil with lures. In the present studies, *B. dorsalis* attraction towards single ME and ME mixed with basil oil showed decreased attractancy up to fifteen days of weathering after that the attractancy of *B. dorsalis* was at par in both conditions. For *Z. cucurbitae,* the CL and the CL mixed with basil oil showed a relative decrease in attractancy. The mix of CL and basil oil would have an antagonistic effect. Whereas, mixed lure of ME+CL with basil oil provide similar attraction like ME + basil oil for *B. dorsalis*.

Traps baited with ME+ CL would be the better option for mass trapping of *B. dorsalis* and *Z. cucurbitae* with single trap which benefit the farmers with lower labour charges and trapping system in the countries of South Esat-Asia as well as South-Asia region where small holding farmers grow fruits and vegetables crops in nearby field some times in the same field. The single ME and mix of ME with basil oil were similar in attraction of *B. dorsalis,* however CL with basil oil showed decreased attraction of *Z. cucurbitae* for entire duration of experiment. Further, field trails needed to evaluate the compatibility of basil oil with ME and CL in potential implementations of the lure and kill approach against fruit flies.

### 4.4 The proportion efficiency of methyl eugenol-cue lure mixed and alone in rubber foam device for controlling Tephritidae fruit fly

#### 4.4.1 Effect of different proportions of mixed ME and CL in rubber foam

For all species of caught flies, mixed ME-CL proportion traps significantly varied among them in catching total species of flies ($F_{4,40}$ =10.961; $P$<0.01). The proportion of methyl eugenol: cue lure (ME: CL) at 100: 0 traps (100% ME) caught the highest number of flies with 35.89 ± 6.23 FTD and significantly different from other traps. While all other traps were found catching statistically similar value of mean flies. The mean number of flies by 75: 25 traps (21.69 ± 3.43 FTD), 50: 50 traps (12.40 ±

1.50 FTD), 25: 75 traps (10.35 ± 1.26 FTD) and 0: 100 traps (9.58 ± 1.36 FTD) were not significantly different (Fig. 24A).

For *B. dorsalis* mean capture of flies were highly significantly different (F4,40 = 124.375; *P*<0.01) with all the traps. Mean capture of *B. dorsalis* consistently increased with the amount of ME in proportion traps. The highest capture was found in 100 % ME traps 100 : 0 (33.09 ± 5.84 FTD) followed by 75 : 25 traps (16.94 ± 3.56 FTD), 50 : 50 traps (7.04 ± 1.11 FTD), 25 : 75 traps (1.69 ± 0.27 FTD) and 100 : 0 traps (0.00 ± 0.0 FTD) traps, respectively (Fig. 24B).

For *Z. cucurbitae* the figure showed quite a different result. The total number of caught flies in all traps showed a significantly different results ($F_{4,40}$ = 28.236: *P*<0.01). The proportion of ME: CL at 25: 75 traps gave the highest mean capture of *Z. cucurbitae* with 2.63 ± 0.22 FTD. The mean captured flies from 0: 100 traps (100% CL) was 1.73 ± 0.27 FTD which statistically same with 25: 75 traps and also statistically same with the captured flies with 75: 25 traps (1.25 ± 0.35 FTD) and 50: 50 traps (0.98 ± 0.21). No flies of *Z. cucurbitae* was got in 100: 0 traps (100% ME) (Fig. 24C).

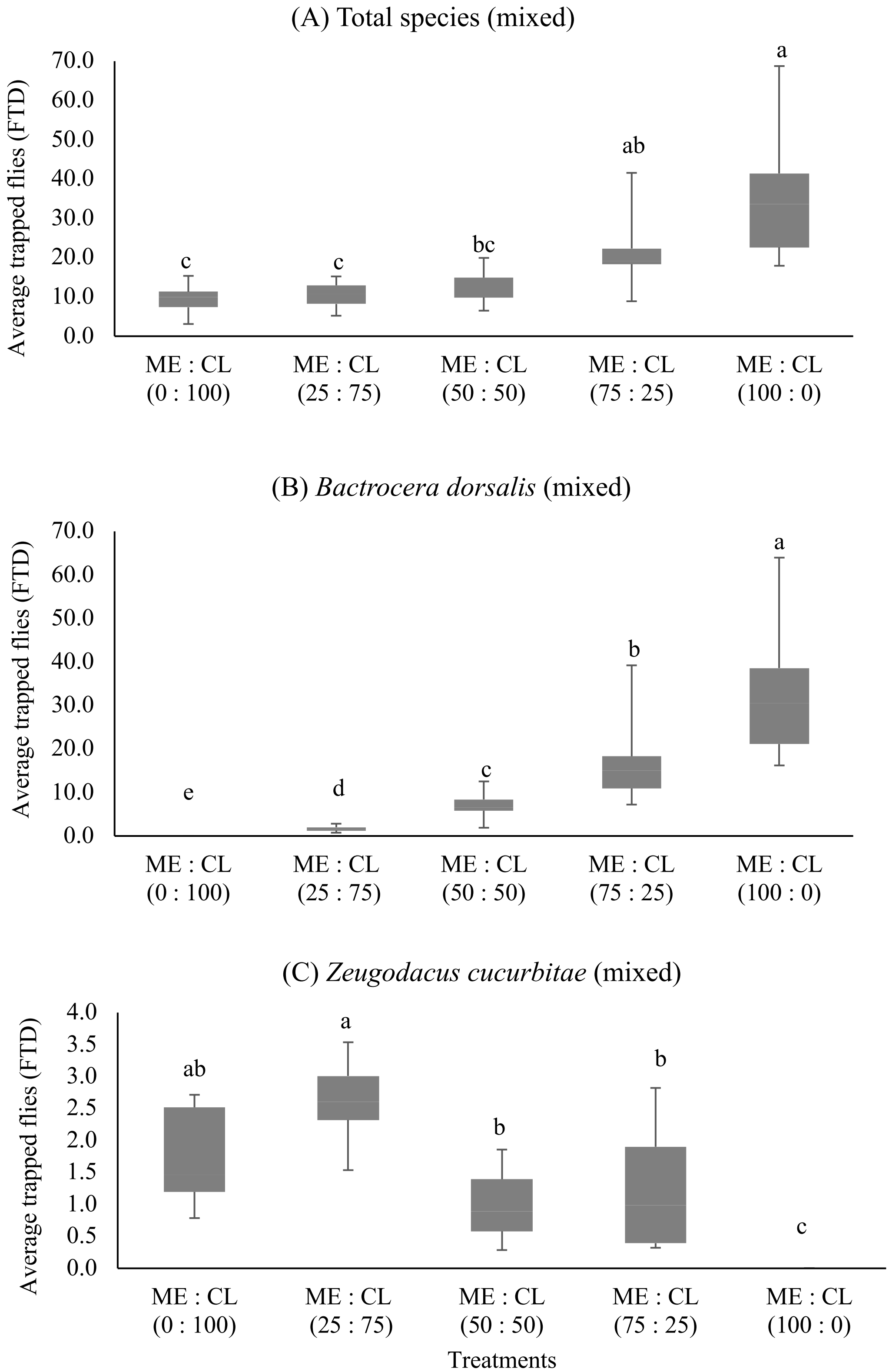



**Figure 24.** Boxplot analysis of average trapped flies of adult total species (A) *Bactrocera dorsalis* (B) and *Zeugodacus cucurbitae* (C) to rubber foam with methyl eugenol (ME) and cue lure (CL) mixed proportions of (0: 100), (25: 75), (50: 50), (75: 25), (100: 0). Mean with different letter were significantly different by Tukey's HSD test ($P$<0.05).

**4.4.2 Effect of different proportions of ME and CL placed separately in rubber foam**

For all species of caught flies, separate ME-CL proportion traps, there was significant variation in all traps ($F_{4,40}$ = 4.935; $P$<0.01). The maximum number of caught flies were found in 100: 0 traps (100% ME) with 26.20 ± 4.39 FTD. The mean caught flies by 100: 0 traps were not significantly different from the 25: 75 and 75: 25 traps with 18.98 ± 2.95 FTD and 16.05 ± 2.66 FTD, respectively. These two traps statistically followed by 50: 50 and 0: 100 traps with 14.66 ± 1.41 FTD and 9.45 ± 1.19 FTD, respectively (Fig. 25A).

There was a significant difference in all traps for catching *B. dorsalis* ($F_{4,40}$ = 55.039; $P$<0.01). For *B. dorsalis* 100: 0 traps (23.62 ± 4.13 FTD) gave the highest mean capture. The proportion 50: 50 traps (4.69 ± 0.91 FTD) caught more flies than 25: 75 traps (3.01 ± 0.84 FTD) but not statistically different. Again, the proportion 50: 50 traps caught less flies than 75: 25 traps (7.33 ± 1.79) but not significantly different. *B. dorsalis* were not caught in 0: 100 traps (Fig. 25B).

Significant difference had found in all traps regarding *Z. cucurbitae* capture ($F_{4,40}$ = 38.558; $P$<0.01). The maximum number of mean flies were caught in 25: 75 traps with 3.68 ± 0.61 FTD and it was statistically different from other traps. Mean number of flies caught by the proportion at 50: 50 and 75: 25 traps were 1.67 ± 0.23 and 1.76 ± 0.34 FTD, respectively, and were not significantly different. *Z. cucurbitae* were not caught in 100: 0 traps (Fig. 25C).

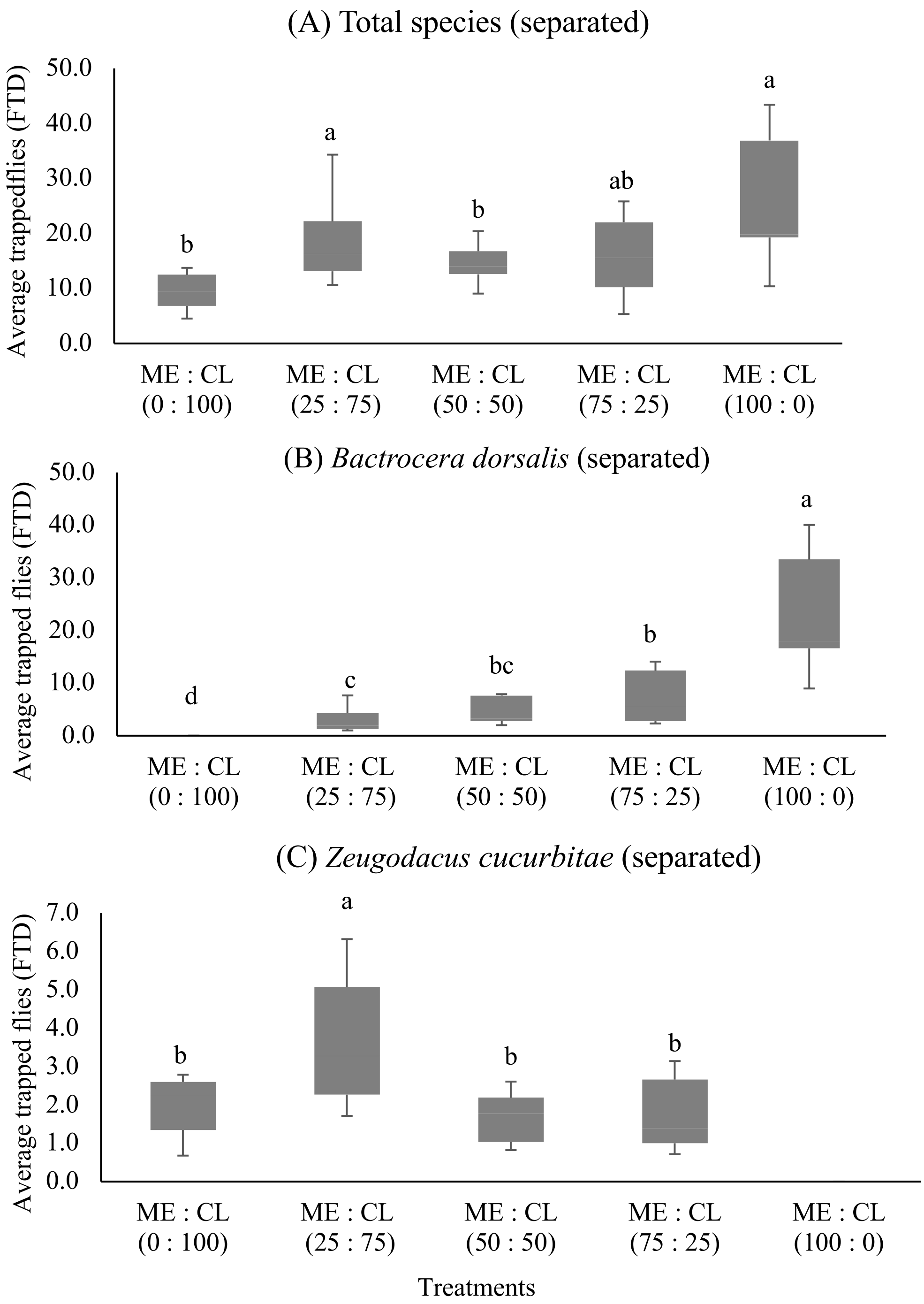



**Figure 25**. Boxplot analysis of average trapped flies of adult total species (A) *Bactrocera dorsalis* (B) and *Zeugodacus cucurbitae* (C) to rubber foam with separate methyl eugenol (ME) and cue lure (CL) proportions of (0: 100), (25: 75), (50: 50), (75: 25), (100: 0). Mean with different letter were significantly different by Tukey's HSD test ($P$<0.05).

**4.4.3 Effect of different proportions of cumulative mixed ME and CL in rubber foam for all the species caught**

After eight weeks of trapping period, 10 species were identified from the five proportional traps captured flies. The total number of flies in all proportion traps varied significantly among them ($F_{4,20}$=52.544; P<0.01). The highest mean capture of flies by 100: 0 traps was 35.89 ± 1.45 FTD, while the lowest number of trap capture were found in 0: 100 traps with 9.58 ± 0.64 FTD.

The number of total captured flies of ME responsive species increased with the amount of ME in rubber foam. For *B. dorsalis* the same trend observed and there was a highly significant difference in the treatments ($F_{4,20}$ = 653.98; P<0.01). The maximum number of means captured flies by 100: 0 traps was 33.09 ± 1.36 FTD whereas the least number of means captured flies by 0: 100 traps. In the case of *B. carambolae* also noticed a highly significant difference among all traps (F4,20 =496.707; P<0.01). Here also the highest and the lowest number of means captured flies found by 100: 0 and 0: 100 traps with 2.03 ± 0.13 and 0.00 ± 0.00 FTD, respectively.

Regarding *B. umbrosa*, all the traps varied significantly ($F_{4,20}$ = 31.672; P<0.01). The maximum and the minimum number of trapped flies by 100: 0 traps and 0: 100 traps were 0.21 ± 0.03 and 0.00 ± 0.00 FTD, respectively. The least common ME responsive species was the *B. correcta*. They also showed significant difference ($F_{4,20}$ = 92.453; P<0.01). They captured only by 100: 0 and 75: 25 traps with 0.56 ± 0.06 and 0.06 ± 0.03 FTD, respectively.

Contrastingly, CL responsive species captured more in 25: 75 traps mostly than any other traps and no flies were found in 100: 0 traps. For *Z. cucurbitae* significant difference among the traps ($F_{4,20}$ = 9.172; P<0.01). The highest number of mean flies found in 25: 75 traps with 2.63 ± 0.69 FTD and it was statistically similar to the other three traps. Mean captured of flies found 1.73 ± 0.51 FTD, 1.25 ± 0.62 FTD and 0.98 ± 0.13 FTD by 0: 100, 75: 25 and 50: 50 traps, respectively. In the case of *Z. tau*, the mean capture of flies was significantly different (F4,20 = 8.121; P<0.01). The maximum number of means captured flies was 1.80 ± 0.36 FTD by 25: 75 traps and marginally different from the other three traps. At the same time 1.38 ± 0.38 FTD, 1.13 ± 0.46 FTD and 0.77 ± 0.23 FTD mean captured flies got in 0: 100, 75: 25 and 50: 50 traps, respectively. (Table 8).

Regarding *B. nigrofemoralis*, significant differences among treatments found ($F_{4,20}$ = 108.557; P<0.01). The 0: 100 traps (100% CL) captured the highest number of flies with 2.56 ± 0.36 FTD. For *B. albistrigata*, also found significant difference (F4,20 = 15.274; P<0.01). The maximum number of flies captured by 25: 75 traps was 0.30 ± 0.02 FTD.

For *B. caudata* all the treatments varied significantly ($F_{4,20}$ = 20.683; P<0.01). The 0: 100 traps (100% CL) caught the greatest number of flies with 1.24 ± 0.15 FTD. In case of *B. bryonae* significant difference found among the traps ($F_{4,20}$ = 32.969; P<0.01). The highest mean number of flies captured in 0: 100 traps was 2.38 ± 0.36 FTD. Mean capture of flies by 25: 75 and 50: 50 traps were 0.82 ± 0.22 and 0.62 ± 0.18 FTD, respectively, and statistically different from 0: 100 traps. The least number of mean flies caught by 75: 25 traps were 0.05 ± 0.02 FTD (Table 8).

**Table 8.** The mean number of trapped (mean ± SE) of different species (FTD) trapped in the proportions of mixed ME : CL

| **Fruit flies species** | **Mean number (± S.E.) of trapped flies (FTD) in the proportion of ME : CL*** | | | | | **F value** |
|---|---|---|---|---|---|---|
| | **(0 : 100)** | **(25 : 75)** | **(50 : 50)** | **(75 : 25)** | **(100 : 0)** | |
| ***B. albestrigata*** | 0.29 ± 0.07a | 0.30 ± 0.02a | 0.23 ± 0.03ab | 0.12 ± 0.04bc | 0.00 ± 0.00c | ** |
| ***B. bryoniae*** | 2.38 ± 0.36a | 0.82 ± 0.22b | 0.62 ± 0.18b | 0.05 ± 0.02c | 0.00 ± 0.00c | ** |
| ***B. carambolae*** | 0.00 ± 0.00e | 0.08 ± 0.01d | 0.37 ± 0.03c | 1.02 ± 0.04b | 2.03 ± 0.13a | ** |
| ***B. caudata*** | 1.24 ± 0.15a | 0.93 ± 0.12a | 0.73 ± 0.12ab | 0.34 ± 0.18bc | 0.00 ± 0.00d | ** |
| ***B. correcta*** | 0.00± 0.00b | 0.00 ± 0.00b | 0.00 ± 0.00b | 0.06 ± 0.03b | 0.56 ± 0.06a | ** |
| ***B. dorsalis*** | 0.00 ± 0.00e | 1.69 ± 0.26d | 7.04 ± 0.33c | 16.94 ± 1.15b | 33.09 ± 1.36a | ** |
| ***B. nigro*** | 2.57 ± 0.36a | 2.10 ± 0.09ab | 1.60 ± 0.10b | 0.66 ± 0.06c | 0.00 ± 0.00d | ** |
| ***B. umbrosa*** | 0.00 ± 0.00c | 0.01 ± 0.01c | 0.06 ± 0.02bc | 0.12 ± 0.00b | 0.21 ± 0.03a | ** |
| ***Z. cucurbitae*** | 1.73 ± 0.51a | 2.63 ± 0.69a | 0.98± 0.13a | 1.25 ± 0.62a | 0.00 ± 0.00b | ** |
| ***Z. tau*** | 1.38 ± 0.38a | 1.80 ± 0.36a | 0.77 ± 0.23a | 1.13 ± 0.46a | 0.00 ± 0.00b | ** |

*Different letters in the same row indicate statistical significances by Tukey' HSD test (*P*<0.01).

### 4.4.4 Effect of different proportions of cumulative separate ME and CL in Rubber foam for all the species caught

Over the whole period of trapping, 10 species also identified from all the traps. Total number of captured flies showed significant difference ($F_{4,20}$ = 38.534; *P*<0.01).

The highest number of means captured flies were found in 100: 0 traps (100% ME) with 26.20 ± 2.12 FTD. Here also the number of ME sensitive species captured increased with the more amount of ME in rubber foam and no flies were detect in 0: 100 traps. For *B. dorsalis*, a highly significant difference observed in all traps ($F_{4,20}$ = 314.587; *P*<0.01). The greatest number of flies were found in 100 : 0 traps with 23.62 ± 1.99 FTD. Mean captured flies by all the traps were statistically different with increasing amount of ME.

In case of *B. carambolae*, all trap captures varied significantly ($F_{4,20}$ = 260.458; *P*<0.01). The 100: 0 traps gave the highest number of mean trap capture with 1.60 ± 0.11 FTD than the other three traps. Regarding to *B. umbrosa* significant difference among all the traps noticed ($F_{4,20}$ = 41.005; *P*<0.01). The maximum number of means captured flies gave by 100 : 0 traps with 0.77 ± 0.13 FTD from rest of the traps.

For the *B. correcta* the maximum number of means captured flies found in 100: 0 traps with 0.21 ± 0.02 FTD and there was a significant difference for all the species ($F_{4,20}$ = 53.828; *P*<0.01). No fly was found in 50 : 50 traps. Mean captured flies by 25 : 75 and 75 : 25 traps were 0.02 ± 0.01 FTD and 0.02 ± 0.02 FTD, respectively, and significantly different from 100: 0 traps. Oppositely, trapping of CL responsive species found the highest in 25 : 75 traps except for *B. bryoniae*.

For *Z. cucurbitae*, a significant difference was present in the treatments ($F_{4,20}$ = 18.742; *P*<0.01). The highest number of these species was caught in 25 : 75 traps with 3.68 ± 0.79 FTD. Regarding to *Z. tau*, there also found a significant difference ($F_{4,20}$ = 13.217; *P*<0.01). Also, the highest number of this species caught in 25 : 75 traps with 2.08 ± 0.33 FTD. For *B. nigrofemoralis*, all the treatments differed significantly ($F_{4,20}$ = 195.511; *P*<0.01). The 25 : 75 traps caught the greatest number of means captured flies with 7.45 ± 0.67 FTD.

For *B. albistrigata*, also found significant difference ($F_{4,20}$ = 5.476; *P*<0.01). The maximum number of flies by 25 : 75 traps were 0.10 ± 0.03 FTD. Regarding *B. caudata* all the treatments varied significantly ($F_{4,20}$ = 141.005; *P*<0.01). The 25: 75 traps caught the greatest number of flies with 2.14 ± 0.08 FTD. In case of *B. bryonae* significant difference found among traps ($F_{4,20}$ = 8.860; *P*<0.01). The highest mean number of flies captured in 0: 100 traps (100% CL) with 0.24 ± 0.01 FTD (Table 9).

**Table 9.** The mean number of trapped (mean ± SE) of different species (FTD) trapped in the proportions of separate ME : CL

| Fruit flies species | Mean number (± S.E.) of trapped flies (FTD) in the proportion of ME : CL* | | | | | F value |
|---|---|---|---|---|---|---|
| | (0: 100) | (25: 75) | (50: 50) | (75: 25) | (100: 0) | |
| ***B. albestrigata*** | 0.06 ± 0.00ab | 0.10 ± 0.03a | 0.06 ± 0.02ab | 0.05 ± 0.02ab | 0.00 ± 0.00b | ** |
| ***B. bryoniae*** | 0.24 ± 0.01a | 0.23 ± 0.07a | 0.15 ± 0.05ab | 0.03 ± 0.01b | 0.00 ± 0.00b | ** |
| ***B. carambolae*** | 0.00 ± 0.00d | 0.18 ± 0.02c | 0.27 ± 0.03c | 0.48 ± 0.03b | 1.60 ± 0.11a | ** |
| ***B. caudata*** | 1.01 ± 0.08b | 2.14 ± 0.08a | 1.25 ± 0.03b | 1.10 ± 0.16b | 0.00 ± 0.00c | ** |
| ***B. correcta*** | 0.00 ± 0.00b | 0.02 ± 0.01b | 0.00 ± 0.00b | 0.02 ± 0.02b | 0.21 ± 0.02a | ** |
| ***B. dorsalis*** | 0.00 ± 0.00e | 3.01 ± 0.34d | 4.69 ± 0.51c | 7.33 ± 0.60b | 23.62 ± 1.99a | ** |
| ***B. nigro*** | 4.47 ± 0.11b | 7.45 ± 0.67a | 5.41 ± 0.53ab | 3.85 ± 0.53b | 0.00 ± 0.00c | ** |
| ***B. umbrosa*** | 0.00 ± 0.00c | 0.09 ± 0.02bc | 0.12 ± 0.01bc | 0.18 ± 0.04b | 0.77 ± 0.13a | ** |
| ***Z. cucurbitae*** | 1.97 ± 0.34a | 3.68 ± 0.79a | 1.67 ± 0.60a | 1.76 ± 0.35a | 0.00 ± 0.00b | ** |
| ***Z. tau*** | 1.70 ± 0.38a | 2.08 ± 0.33a | 1.04 ± 0.34a | 1.25 ± 0.38a | 0.00 ± 0.00b | ** |

*Different letters in the same row indicate statistical significances by Tukey' HSD test ($P$<0.01).

In the current study, we studied different proportions of ME and CL to find the best ratio for catching targeted fruit flies' species. Overall, the total trapped specimens collected in mixed proportions of ME and CL were 20,141 flies, of which *B. dorsalis* made up 65.3%, *Z. cucurbitae* 7.3% and other species 27.4%, respectively. Prevalence of *B. dorsalis* is related to the presence of abundant host plants like mango (Vayssières et al., 2005; Mwatawala et al., 2006). It is also a highly competitive species that can replace other indigenous species significantly (Aketarawong et al., 2007; Rattanapun et. al., 2009; Ambele et al., 2012). However, it has been reported that the populations of *Z. cucurbitae* growing with the increasing temperatures especially at the warmer months of the year, vice-versa population fall with less heat simultaneously with colder months (Laskar and Chatterjee, 2010).

In our study, we got that the number of *B. dorsalis* caught more in 100: 0 traps than other proportions of ME and CL. The high number of *B. dorsalis* capture associated with higher amount of ME. The highest number got in only ME traps than mixed with CL and these results corroborate other studies (Hooper, 1978; Vargas et al., 2000; LeBlanc et al., 2011; Shelly et al., 2004; Wee and Shelly 2013; Royer and Meyer et al., 2018). It appears when CL is added to ME or placed near ME it decreases the capture of male *B. dorsalis* and this decrease can be prevented by increasing the quantity of ME exit when loaded together with CL (Vargas et al., 2015). However, a combination of ME and CL also have been reported to increase trap capture of *B. dorsalis* (Leblanc et al., 2011; Vargas et al., 2012). Shelly and Kurashima (2016) got the best proportion ME: CL (90: 10) over 85: 15 and 90: 5 for *B. dorsalis*. As we got a low percentage of *Z. cucurbitae* than *B. dorsalis*, because of occupying less cucurbitaceous crops. As its more number associated with host plants like bitter melon, watermelon, cucumber and zucchini, respectively (Vargas et al., 1989; Gnanvossou et al., 2008). This species trapped markedly by 25: 75 traps (ME: CL). Though it is a CL sensitive species, it caught more in 25: 75 traps than only 0: 100 traps (100% CL) but not statistically. It means the ratio of 25: 75 is just equally attractive as only CL. This finding has seen in some other studies (Leblanc et al., 2011; Wee and Shelly 2013).

The total fruit flies obtained from separate proportions of ME and CL were 19,115 flies among which 45.2% *B. dorsalis*, 10.6% *Z. cucurbitae* and other species 44.2%, respectively. In this case, *B. dorsalis* was trapped the highest number by 100: 0 traps than combined with CL ratios and same result was found by Shelly et al. (2004). The traps with ME and CL mixed or separate caught significantly less *B. dorsalis* males than only ME traps (Shelly et al., 2004). Mean capture of flies of *Z. cucurbitae* also noticed significantly highly by 25: 75 traps than other proportional traps. There are many factors behind these results differ.

The longevity period of ME and CL is different that might somehow be associated with these results. Variations in male captured of *B. dorsalis* and *Z. cucurbitae* with different proportions are also correlated with abiotic factors like season, temperature, relative humidity, rainfall, etc. (Gnanvossou et al., 2017). However, Shelly and Kurashima (2016) did not found any significant difference for *B. dorsalis* and *Z. cucurbitae* with single and three different mixed proportions of ME-RK blend. The performance of a trapping program is determined by trap and lure combination, their spatial arrangements (Berec et al., 2015). Some other reasons might be the state of lure (liquid or solid), different dispensers (cotton, wafer, rubber foam, fiberboard, sticky paper, rubber foam), different toxicant (DDVP, naled, borax, malathion, dichlorovos) different trap type (Jackson, Steiner, McPhail, bucket, tephritrap, PEET), and amount of lure (0.1 ml to 10 ml), etc.

**4.5 Efficiency of best mixed lure and best essential oil controlling fruit flies in field condition**

**4.5.1 The efficacy of ME, CL, ME + CL, ME/BO, CL/BO, ME + CL//BO and control combinedly**

In this study, we evaluate the efficacy of single lure of ME and CL, the mixed lure of ME + CL and basil oil with those single ME/BO, CL/BO and mixed lure ME+ CL/BO. There was also a comparison of their efficacy. There were three different orchards from where data were collected. For an easy and clear understanding of this result, we put the data on boxplot and analyze it. As our major concern was related to *Bactocera dorsalis* and *Zeugodacus cucurbitae* species, their mean comparisons computed with total species captured in the traps. There was a highly significant difference in capturing the total number of flies among the treatments ($F_{6,84}$ =33.723; $P$<0.01).The highest mean capture of flies found in ME traps (6.0 ± 1.1 FTD) which was statistically same with ME + CL, ME/BO, ME + CL/BO and CL with number of mean captured flies (5.2 ± 0.6 FTD), (4.1 ± 0.8 FTD), (2.9 ± 0.5 FTD) and (1.9 ± 0.2 FTD), respectively. The mean capture of flies by CL/BO (0.5 ± 0.1 FTD) and is statistically different from the former treatments. While no flies found in control traps (Fig. 26A).

The mean captured flies for *B. dorsalis*, showed a highly significant difference in all traps ($F_{6,84}$=54.622; $P$<0.01). The maximum number of means captured flies were also got from ME traps (5.5 ± 1.0 FTD). The mean captured flies by ME/BO and ME + CL were (3.8 ± 0.8 FTD). (3.4 ± 0.5 FTD) which were statistically the same with ME and ME + CL/BO traps (2.0 ± 0.4 FTD). CL, CL/BO, and control traps had no flies (Fig. 26B).

In the case of *Z cucurbitae*, there was a highly significant difference in mean captured flies ($F_{6,84}$ =41.014; P<0.01). ME + CL and CL traps caught same mean captured flies (0.4 ± 0.1 FTD) (0.4 ± 0.0 FTD). Whereas, ME + CL/BO and CL/BO traps caught statistically same mean captured flies (0.2 ± 0.0 FTD) and (0.1 ± 0.0 FTD). ME, ME/BO, and control traps did not catch any *Z. cucurbitae* fly (Fig. 26C).

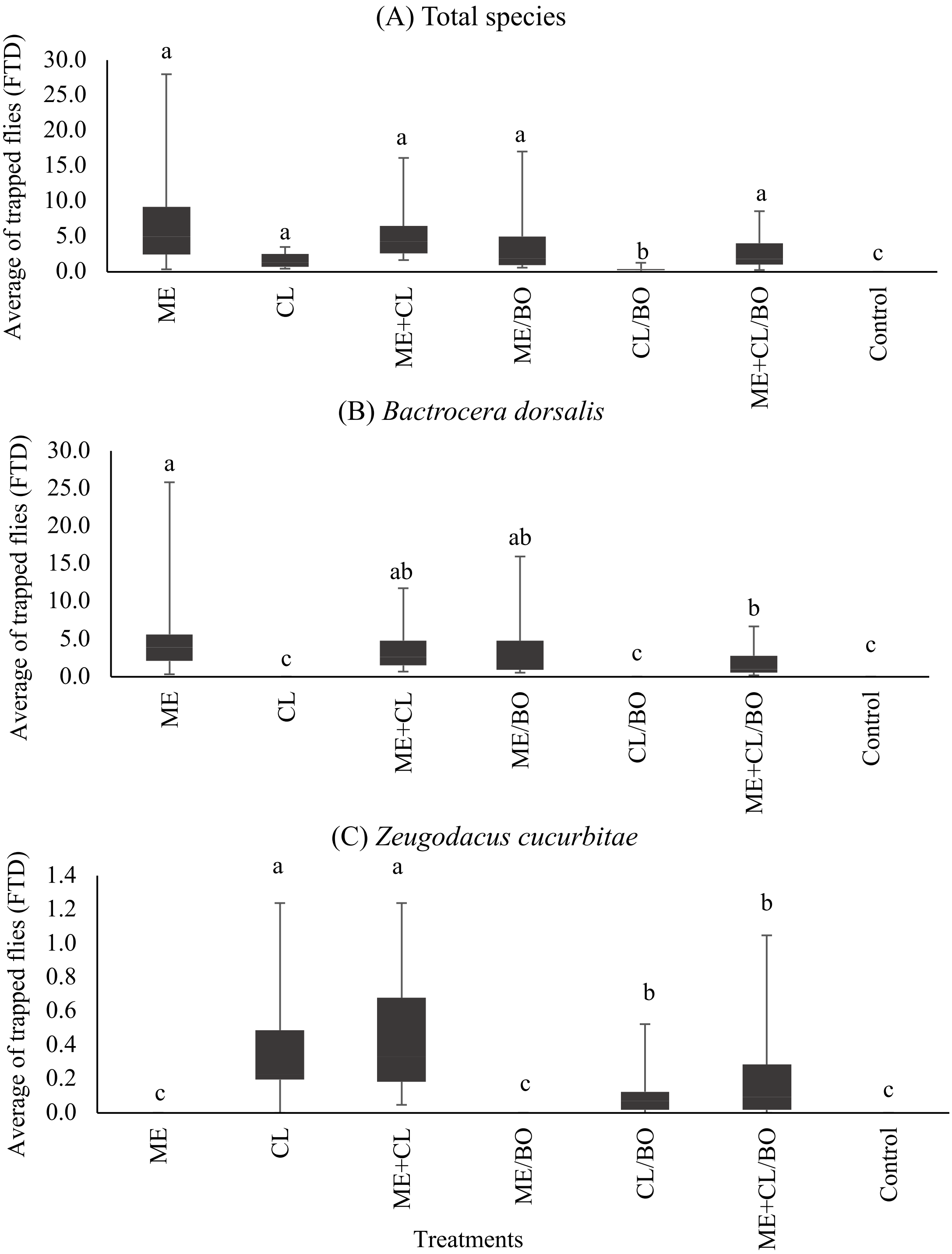



**Figure 26**. Boxplot analysis of mean number of trapped total flies (A) *Bactrocera dorsalis* (B) and *Zeugodacus cucurbitae* (C) to rubber foam with methyl eugenol (ME) and cue lure (CL) ME + CL, ME/BO, CL/BO, ME + CL/BO and control.

**4.5.2 The efficacy of ME, CL, ME + CL, ME/BO, CL/BO, ME + CL/BO and control per weekly (14 days)**

The present study also determined the mean captured flies for six months in twelve weeks at fifteen days interval, the data put in line graphs for total species, *B. dorsalis* and *Z. cucurbitae*. Immediately after hung the traps in orchards in the first week, there was a highly significant difference in mean captured flies from all the treatments ($F_{6,21}$ =15.309; $P$<0.01). ME/BO (9.6± 2.2 FTD) trap showed highly effective in trapping total species followed by ME (9.5 ± 2.1 FTD). During the whole trapping period, the second week produced the greatest number of trapped flies in all treatments and showed a highly significant difference ($F_{6,21}$ =62.674; $P$<0.01). Here the maximum mean trapped flies found by ME traps (22.2 ± 5.0 FTD) followed by ME/BO traps (14.4 ± 1.4 FTD). After 30 days, we changed the traps with new prepared foam. In the third week, there also a highly significant variation in all the traps ($F_{6,21}$ =22.558; $P$<0.01). The highest number of means trapped flies got in ME traps (10.5 ± 2.6 FTD) and ME + CL traps (10.3 ± 3.3 FTD). After that, the way trapped flies decreased gradually until the fifth week. Where the maximum mean trapped flies was (2.3 ± 0.3 FTD) by ME + CL traps. Again, the mean trapped flies increased slightly up the seventh week and backdropped in the ninth week. Eventually, the least number of trapped flies found in the twelfth week with a maximum number of means trapped flies by ME + CL traps (1.8 ± 2.2 FTD) (Fig. 27A)

For *B. dorsalis* combined three orchards in the line graph. There was a highly significant difference in all treatments found in the first week ($F_{6,21}$ =74.124; P<0.01). The highest number of means trapped flies found by ME/BO traps (9.0 ± 2.1 FTD) followed by ME traps (8.8 ± 1.9 FTD). The maximum number of flies caught in the Second week and also showed a significant difference ($F_{6,21}$ =192.516; P<0.01). Here the maximum number of means trapped flies got in ME traps (20.4 ± 4.5 FTD) followed by ME/BO traps (13.5 ± 1.4 FTD). In the third week mean trapped flies caught maximum by ME (9.5 ± 2.4 FTD and ME/BO traps (9.5 ± 3.1 FTD). Then the number of trapped flies decreased slowly until the fifth week. After that, a fluctuating trend of trapped flies found until the last week. From the seventh week, ME + CL gradually increased its efficiency and again decreased, and in the last week, it surpassed the mean trapped flies of ME traps. The maximum mean trapped flies were at last week by ME

+ CL traps (1.7 ± 0.3 FTD) and by ME traps (1.0 ± 0.0 FTD). CL and CL/BO did not catch any flies during the trapping period (Fig. 27B).

In the case of *Z. cucurbitae* the mean trapped flies from three orchards assembled in line graph. A significant variation among all treatments appeared there in the first week ($F_{6,21}$ =5.297; P<0.01). CL and ME + CL traps got the same mean trapped flies (0.3 ± 0.2 FTD) and (0.3 ± 0.1 FTD), respectively. The trend of trapping the most flies found in the second week and prevailed a highly significant variation ($F_{6,21}$ =5.297; P<0.01). ME + CL traps outperformed than CL in mean trapped flies' number (0.7 ± 0.3 FTD) and (0.3 ± 0.1 FTD), respectively. There was a fluctuating trend of mean trapped flies over the trapping period. Sometimes ME + CL and often CL traps caught the maximum trapped flies. Though the week ninth and week eleventh showed no significant difference in the treatments. Eventually, CL (0.1 ± 0.2 FTD) again caught more mean trapped flies than ME + CL traps (0.3 ± 0.2 FTD). There were no flies in ME, and ME/BO traps (Fig. 27C).

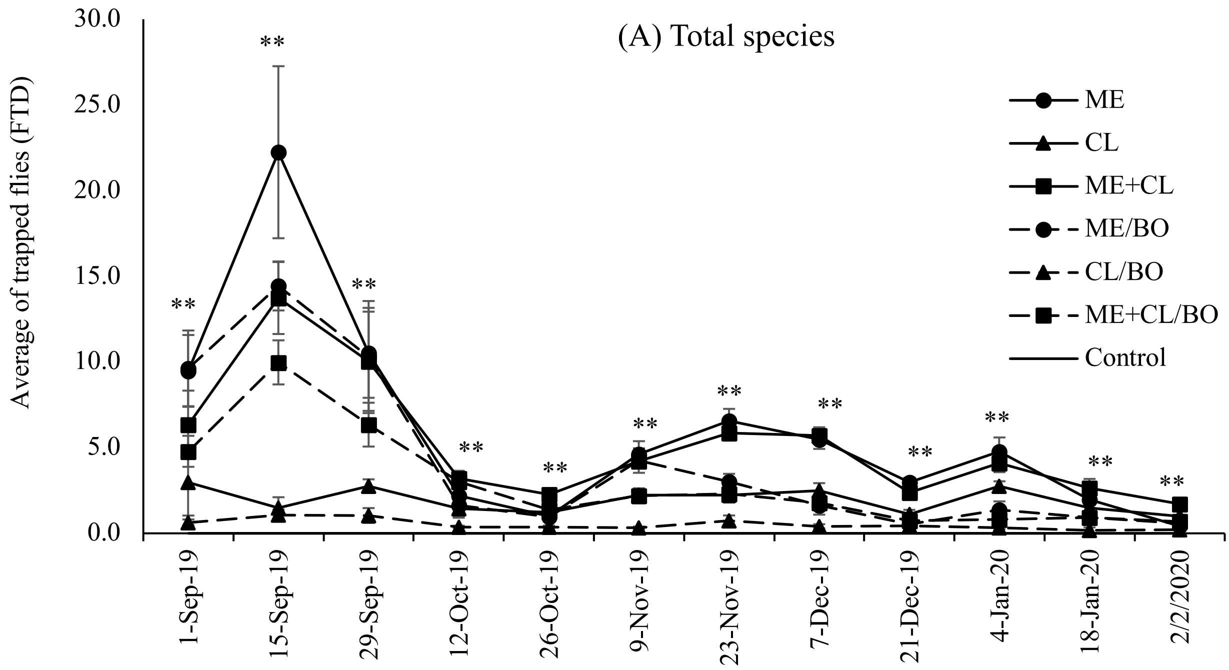
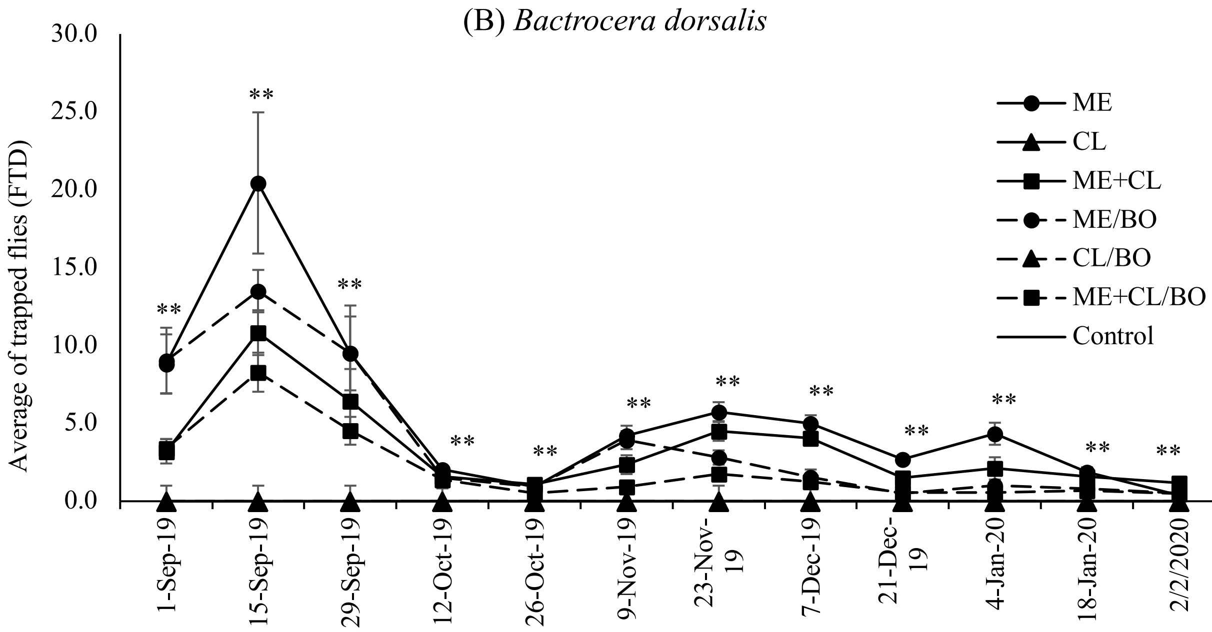



**Figure 27.** Number of trapped flies bi-weekly (FTD) total flies (A) *Bactrocera dorsalis* (B) and *Zeugodacus cucurbitae* (C) to rubber foam with methyl eugenol (ME) and cue lure (CL) ME + CL, ME/BO, CL/BO, ME + CL/BO and control.



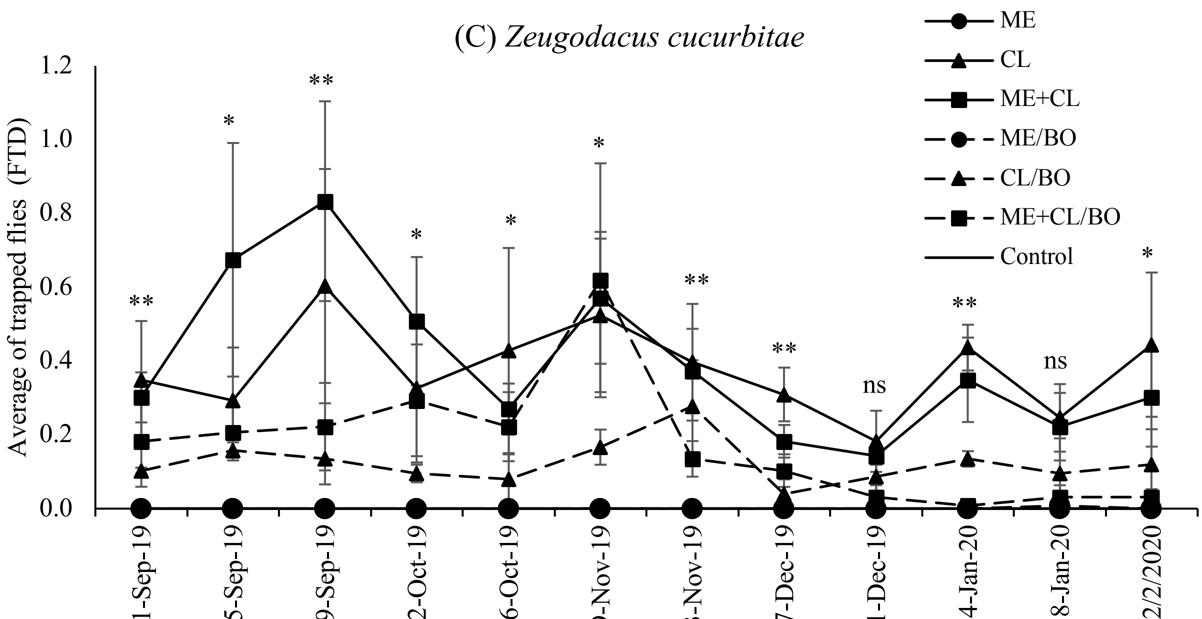




**Figure 27 (cont.).** Number of trapped flies bi-weekly (FTD) total flies (A) *Bactrocera dorsalis* (B) and *Zeugodacus cucurbitae* (C) to rubber foam with methyl eugenol (ME) and cue lure (CL) ME + CL, ME/BO, CL/BO, ME + CL/BO and control.

In this study we used lures singly (ME, CL) and mixed (ME + CL), again basil oil with single (ME/BO, CL/BO) and mixed lure (ME + CL/BO) with an assumption that basil oil contains methyl eugenol. It might increase the efficiency of ME in enhancing the trapped caught of ME responsive fruit flies. But our result indicated that in general trapping was maximum with only ME traps. Although ME + CL traps caught the flies statistically same with Only ME traps. The mean number of flies got much more in ME trpas than ME + CL than ME/BO traps. It could be because of ME in basil oil somehow decreased the attractancy of ME. Contrastingly, ME + CL did well than ME/BO traps. Mixing of two lures showed more attractive to CL responsive species than ME responsive flies.

Again, for capturing *B. dorsalis* ME traps trapped the best. In this case ME/BO trapped showed a bit better than ME + CL traps in terms of mean trapped flies. Contrastingly for *Z. cucurbitae* ME + CL trap was just as effective as CL traps while basil oil with CL and ME + CL was less effective than CL and ME + CL traps. Basil oil might inhibit the CL activity.

In terms of each time data collection, first time and second time ME/BO traps were best for the total number of flies and *B. dorsalis* mean trapped flies but after that, it was no longer active. It suggests that. After 30 days, basil oil started to decrease its activity. At this point, we serviced the traps to see what happen next. But after servicing the traps it gradually decreased the trapped flies. Because the fruit fly population already decreased than initial fly population. From then there was a fluctuation of mean trapped flies by ME, ME + CL and ME/BO in some weeks. Weather condition also might play role in fluctuating trend.

Singh et al., (2020) conducted an experiment where basil oil from it different parts () applied in mango, guava and vegetable orchards to trap *Z. cucurbitae*, control was methyl eugenol. Outcome got, *Z. cucurbitae* attracted to basil oil from its all part in maximum number from vegetables garden (103.25) fruit flies/week followed by guava (41.77) fruit flies/week So, there would have had a chance of using basil oil in managing fruit flies. Although in our work we used the basil oil with lures it not gave satisfactory result compare with only lures.

A study conducted in Srilanka by Dharmadasa et al. (2015) where attractancy capability of two morphotypes of *Ocimum tenuiflorum* morphotypes (MT1 purple and MT2 purple-green) along with commercial methyl Eugenol for *B. dorsalis* had evaluated. The result reveal that there was no significantly difference in the attractancy by essential oil of MT1 (106±8.1), MT2 (104±2) and commercial ME (111±8.5). This indicates basil oil could be used as an alternative para-pheromone in suppressing *B. dorsalis* in field level. But it contrasts with our works where basil oil combined with commercial ME and CL and the result was poor.

# Chapter V

## Conclusions and Recommendations

Results from experiment one showed that mixed lure combination (ME + CL) caught a greater number of flies of *Z. cucurbitae* and also more species. So, it is highly recommended in mixed cropping field.

Results from experiment 2 and 3 revealed that among five plant essential oils tested; basil oil was the most toxic one during 15-30 days. The single ME and mix of ME with basil oil were similar in the attraction of *B. dorsalis*, but for *Z. cucurbitae* mixing CL with basil oil decreased effectiveness and attractancy. In this case, the addition of basil oil would be more expensive. Thus, the use of basil oil with lures in the field is also discouraged.

For the different proportion of ME: CL placed mixed or separately on rubber foam experiment in both cases 25: 75 ratios explored gave the highest performance in trapping *Z. cucurbitae*. This ratio could be highly recommended in a mixed cropped field to suppress fruit flies.

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# Appendix

**Appendix I.** Data for boxplot analysis of average caught flies of total species, *Bactrocera dorsalis*, and *Zeugodacus cucurbitae* to rubber foam with methyl eugenol (ME) and cue lure (CL) mixed (ME + CL), and separated (ME/CL).

| Treatment | Average number of trapped flies FTD (Mean ± SE)* | | |
|---|---|---|---|
| | Total species | *Bactrocera dorsalis* | *Zeugodacus cucurbitae* |
| ME | 19.10 ± 3.51a | 15.17 ± 2.95a | 0.00 ± 0.00c |
| CL | 3.04 ± 0.60c | 0.06 ± 0.05c | 0.87 ± 0.14ab |
| ME+CL | 10.32 ± 1.77bc | 5.90 ± 0.94b | 1.61 ± 0.61abc |
| ME/CL | 12.95 ± 1.72ab | 10.36 ± 1.49ab | 0.44 ± 0.13b |

*Mean with different letter in the same row were significantly different by Tukey's HSD test ($P$<0.05).

**Appendix II.** Data for cumulative mortalities of adult *Bactrocera dorsalis* from rubber foam injected with a plant essential oil (basil oil, clove oil, citronella oil and eucalyptus oil) and weathered for 0, 15, 30 and 45 days before application. The control was rubber foam without plant essential oil.

| Days | Treatments | Percentage mortality of *Bactrocera dorsalis* (Mean ± SE) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 hr | 6 hrs | 12 hrs | 24 hrs | 48 hrs | 72 hrs |
| Day 0 | Basil oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 5.0 ± 2.9 | 15.0 ± 6.5 | 35. ± 6.5 | 40.0 ± 4.1 |
| | Clove oil | 10.0 ± 5.8 | 12.5 ± 4.8 | 20.0 ± 7.1 | 35.0 ± 18.5 | 50.0 ± 19.1 | 60.0 ± 16.3 |
| | Citronella oil | 0.0 ± 0.0 | 15.0 ± 8.7 | 20.0 ± 9.1 | 25.0 ± 9.6 | 52.5 ±11.1 | 60.0 ± 12.2 |
| | Eucalyptus oil | 17.5 ± 6.3 | 65.0 ± 5.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ±0.0 |
| | Control | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Level of Sig. | ns | * | ** | ** | ** | ** |
| Day 15 | Basil oil | 0.0 ± 0.0 | 5.0 ± 2.9 | 50.0 ± 4.1 | 62.5 ± 2.5 | 80.0 ± 4.1 | 95.0 ± 2.9 |
| | Clove oil | 0.0 ± 0.0 | 7.5 ± 2.5 | 22.5 ± 9.5 | 57.5 ± 6.3 | 80.0 ± 4.1 | 87.5 ± 2.5 |
| | Citronella oil | 0.0 ± 0.0 | 5.0 ± 2.9 | 7.5 ± 2.5 | 7.5 ± 2.5 | 30.0 ± 4.1 | 35.0 ± 2.9 |
| | Eucalyptus oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 7.5 ± 2.5 | 20.0 ± 5.8 | 27.5 ± 2.5 | 32.5 ± 2.5 |
| | Control | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Level of Sig. | ns | ns | ** | * | ** | ** |
| Day 30 | Basil oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 2.5 ± 2.5 | 15.0 ± 2.9 | 70.0 ± 0.0 | 72.5 ± 2.5 |
| | Clove oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 5.0 ± 5.0 | 15.0 ± 6.4 | 45.0 ± 13.2 | 67.5 ± 10.3 |
| | Citronella oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 2.5 ± 2.5 | 10.0 ± 4.1 | 10.0 ± 4.1 |
| | Eucalyptus oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Control | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Level of Sig. | ns | ns | ns | ** | ** | ** |
| Day 45 | Basil oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 7.5 ± 2.5 | 12.5 ± 2.5 |
| | Clove oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 2.5 ± 2.5 | 10.0 ± 4.1 |
| | Citronella oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 7.5 ± 2.5 | 12.5 ± 4.8 |
| | Eucalyptus oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 5.0 ± 2.9 | 5.0 ± 2.9 |
| | Control | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Level of Sig. | ns | ns | ns | ns | ns | ns |

**Appendix III.** Data for cumulative mortalities of adult *Zeugodacus cucurbitae* from rubber foam injected with a plant essential oil (basil oil, clove oil, citronella oil and eucalyptus oil) and weathered for 0, 15, 30 and 45 days before application. The control was rubber foam without plant essential oil.

| Days | Treatment | Percentage mortality of *Zeugodacus cucurbitae* (Mean ± SE) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 hr | 6 hrs | 12 hrs | 24 hrs | 48 hrs | 72 hrs |
| Day 0 | Basil oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 3.3 ± 3.3 | 16.7 ± 8.8 | 40.0 ± 17.3 |
| | Clove oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 16.7 ± 6.7 | 36.7 ± 3.3 |
| | Citronella oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 3.3 ± 3.3 | 23.3 ± 12.0 |
| | Eucalyptus oil | 0.0 ± 0.0 | 60.0 ± 5.8 | 100.0 ±0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 |
| | Control | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Level of Sig. | ns | ** | ** | ** | ** | ** |
| Day 15 | Basil oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 13.3 ± 8.8 | 30.0 ± 5.8 | 63.3 ± 14.5 | 76.7 ± 6.7 |
| | Clove oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 3.3 ± 3.3 | 23.3 ± 6.7 | 40.0 ± 11.5 |
| | Citronella oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 6.7 ± 6.7 | 10.0 ± 5.8 | 16.7 ± 8.8 | 16.7 ± 8.8 |
| | Eucalyptus oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 13.3 ± 3.3 | 16.7 ± 3.3 | 16.7 ± 3.3 |
| | Control | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Level of Sig. | ns | ns | ns | ** | ** | ** |
| Day 30 | Basil oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 23.3 ± 18.6 | 26.7 ± 16.7 |
| | Clove oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 16.7 ± 3.3 |
| | Citronella oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 3.3 ± 3.3 | 16.7 ± 8.8 | 16.7 ± 8.8 |
| | Eucalyptus oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Control | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Level of Sig. | ns | ns | ns | ns | ns | ns |
| Day 45 | Basil oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 6.7 ± 3.3 | 6.7 ± 3.3 |
| | Clove oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 3.3 ± 3.3 | 10.0 ± 5.8 |
| | Citronella oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 3.3 ± 3.3 | 6.7 ± 3.3 |
| | Eucalyptus oil | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 3.3 ± 3.3 |
| | Control | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Level of Sig. | ns | ns | ns | ns | ns | ns |

**Appendix IV.** Data for boxplot analysis of attraction of adult *Bactrocera dorsalis, Zeugodacus cucurbitae and* both species to rubber foam with methyl eugenol (ME) and cue lure (CL) singly or mixed, with and without basil oil (BO) injected at the same or a different spot.

| Treatment | Percentage attraction of fruit flies (Mean ± SE)* | | |
|---|---|---|---|
| | *Bactrocera dorsalis* | *Zeugodacus cucurbitae* | Total species |
| ME | 20.3 ± 2.6a | 0.0 ± 0.0d | 20.3 ± 2.6ab |
| ME + BO | 16.7 ± 2.7a | 0.0 ± 0.0d | 16.7 ± 2.7bc |
| ME/BO | 15.7 ± 2.8a | 0.0 ± 0.0d | 15.7 ± 2.8bc |
| CL | 0.0 ± 0.0b | 20.3 ± 1.5a | 20.3 ± 1.5ab |
| CL + BO | 0.0 ± 0.0b | 9.3 ± 2.0bc | 9.3 ± 2.0c |
| CL/BO | 0.0 ± 0.0b | 13.3 ± 1.7ab | 13.3 ± 1.7bc |
| ME +CL | 18.0 ± 1.9a | 21.5 ± 2.3a | 39.5 ± 3.4a |
| ME + CL + BO | 17.3 ± 3.1a | 6.7 ± 1.6bc | 24.0 ± 3.3ab |
| ME + CL/BO | 18.7 ± 2.7a | 9.0 ± 1.8bc | 27.7 ± 3.6ab |
| ME/CL | 15.5 ± 2.0a | 12.0 ± 1.8ab | 27.5 ± 2.6ab |
| ME/CL + BO | 16.7 ± 2.6a | 5.7 ± 1.0bc | 22.3 ± 2.7ab |
| ME/CL/BO | 15.7 ± 2.6a | 5.0 ± 1.3c | 20.7 ± 2.7abc |

*Mean with different letter in the same column were significantly different by Tukey's HSD test ($P<0.05$).

**Appendix V.** Data for boxplot analysis of average trapped flies of adult total species, *Bactrocera dorsalis* and *Zeugodacus cucurbitae* to rubber foam with methyl eugenol (ME) and cue lure (CL) mixed proportions of (0: 100), (25: 75), (50: 50), (75: 25), (100: 0).

| ME: CL ratio Mixed | Mean trapped flies FTD (Mean ± SE)* | | |
|---|---|---|---|
| | Total species | *Bactrocera dorsalis* | *Zeugodacus cucurbitae* |
| 0 : 100 | 9.6 ± 1.4b | 0.0 ± 0.0e | 1.7 ± 0.3ab |
| 25 : 75 | 10.4 ± 1.3b | 1.7 ± 0.3d | 2.6 ± 0.2a |
| 50 : 50 | 12.4 ± 1.5b | 7.0 ± 1.1c | 1.0 ±0.2b |
| 75 : 25 | 21.7 ± 3.4b | 16.9 ± 3.6b | 1.3 ± 0.3b |
| 100 : 0 | 35.9 ± 6.2a | 33.1 ± 5.8a | 0.0 ± 0.0c |

*Mean with different letter in the same column were significantly different by Tukey's HSD test (*P*<0.05).

**Appendix VI.** Data for boxplot analysis of average trapped flies of adult total species, *Bactrocera dorsalis* and *Zeugodacus cucurbitae* to rubber foam with separate methyl eugenol (ME) and cue lure (CL) proportions of (0: 100), (25: 75), (50: 50), (75: 25), (100: 0).

| ME : CL ratios separated | Mean trapped flies FTD (Mean ± SE)* | | |
|---|---|---|---|
| | Total species | *Bactrocera dorsalis* | *Zeugodacus cucurbitae* |
| 0 : 100 | 9.4 ± 1.2b | 0.0 ± 0.0d | 2.0 ±0.3b |
| 25 : 75 | 19.0 ± 2.9ab | 3.0 ± 0.8c | 3.7 ± 0.6a |
| 50 : 50 | 14.7 ± 1.4b | 4.7 ± 0.9bc | 1.7 ± 0.2b |
| 75 : 25 | 16.1 ± 2.7ab | 7.3 ± 1.8b | 1.8 ± 0.3b |
| 100 : 0 | 26.2 ± 4.4a | 23.6 ±4.1a | 0.0 ± 0.0c |

*Mean with different letter in the same column were significantly different by Tukey's HSD test (*P*<0.05).

**Appendix VII.** Data for boxplot analysis of mean number of trapped total flies, *Bactrocera dorsalis* and *Zeugodacus cucurbitae* to rubber foam with methyl eugenol (ME) and cue lure (CL) ME + CL, ME/BO, CL/BO, ME + CL/BO and control.

| Treatment | Mean trapped flies FTD of three orchards (Mean ± SE)* | | |
|---|---|---|---|
| | Total species | *Bactrocera dorsalis* | *Zeugodacus cucurbitae* |
| ME | 6.0 ± 1.1a | 5.5 ± 1.0a | 0.0 ± 0.0c |
| CL | 1.9 ± 0.2a | 0.0 ± 0.0c | 0.4 ± 0.0a |
| ME+CL | 5.2 ± 0.6a | 3.4 ± 0.5ab | 0.4 ± 0.1a |
| ME/BO | 4.1 ± 0.8a | 3.8 ± 0.8ab | 0.0 ± 0.0c |
| CL/BO | 0.5 ± 0.1b | 0.0 ± 0.0c | 0.1 ± 0.0b |
| ME+CL/BO | 2.9 ± 0.5a | 2.0 ± 0.4b | 0.2 ± 0.0b |
| Control | 0.0 ± 0.0c | 0.0 ± 0.0c | 0.0 ± 0.0c |

*Mean with different letter in the same column were significantly different by Tukey's HSD test ($P<0.05$).

**Appendix VIII.** Temperature (mean, maximum and minimum) and rainfall in Koh Yor, Songkhla, from September 2019 to January 2020.

| Month | Max temperature (°C) | Min temperature (°C) | Mean temperature (°C) | Rain fall (mm) |
|---|---|---|---|---|
| August | 33.9 | 25.5 | 29.7 | 52.4 |
| September | 32.6 | 25.3 | 29 | 103.2 |
| October | 31.4 | 24.7 | 27.7 | 210.3 |
| November | 31.4 | 24.8 | 27.8 | 256.1 |
| December | 30.1 | 25.0 | 27.4 | 107.9 |
| January | 31.1 | 25.4 | 28.3 | 1.3 |

**Appendix IX.** Number of trapped flies bi-weekly (FTD) total flies (A) *Bactrocera dorsalis* (B) and *Zeugodacus cucurbitae* (C) to rubber foam with methyl eugenol (ME) and cue lure (CL) ME + CL, ME/BO, CL/BO, ME + CL/BO and control

| Treatment | Average trapped flies FTD (Mean ± SE) for total species | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-Sep-19 | 15-Sep-19 | 29-Sep-19 | 12-Oct-19 | 26-Oct-19 | 9-Nov-19 | 23-Nov-19 | 7-Dec-19 | 21-Dec-19 | 4-Jan-20 | 18-Jan-20 | 2-Feb-20 |
| ME | 9.5 ± 2.1 | 22.2 ± 5.0 | 10.5 ± 2.6 | 2.2 ± 0.3 | 1.0 ± 0.2 | 4.6 ± 0.8 | 6.5 ± 0.7 | 5.5 ± 0.5 | 3.0 ± 0.2 | 4.8 ± 0.8 | 2.0 ± 0.3 | 0.4 ± 0.0 |
| CL | 3.0 ± 1.9 | 1.5 ± 0.6 | 2.8 ± 0.4 | 1.5 ± 0.5 | 1.2 ± 0.5 | 2.2 ±0.3 | 2.2 ± 0.4 | 2.5 ± 0.4 | 1.1 ± 0.3 | 2.8 ± 0.3 | 1.5 ± 0.4 | 1.0 ± 0.3 |
| ME+CL | 6.3 ± 2.0 | 13.7 ± 2.1 | 10.0 ± 2.9 | 3.2 ± 0.5 | 2.3 ± 0.3 | 4.2 ± 0.4 | 5.9 ± 0.4 | 5.7 ± 0.5 | 2.4 ± 0.1 | 4.1 ± 0.5 | 2.6 ± 0.5 | 1.7 ±0.3 |
| ME/BO | 9.6 ± 2.2 | 14.4 ± 1.4 | 10.3 ± 3.3 | 1.6 ± 0.7 | 1.0 ± 0.3 | 4.2 ± 0.7 | 3.0 ± 0.4 | 1.7 ± 0.6 | 0.5 ± 0.1 | 1.4 ± 0.5 | 0.9 ± 0.3 | 0.6 ± 0.1 |
| CL/BO | 0.6 ± 0.2 | 1.1 ± 0.3 | 1.0 ± 0.4 | 0.4 ± 0.1 | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.8 ± 0.3 | 0.4 ± 0.1 | 0.4 ± 0.3 | 0.3 ± 0.1 | 0.2 ± 0.2 | 0.2 ± 0.1 |
| ME+CL/BO | 4.8 ± 0.9 | 10.0 ± 1.3 | 6.3 ± 1.3 | 3.0 ± 0.2 | 1.4 ± 0.4 | 2.2 ± 0.4 | 2.3 ± 0.4 | 1.8 ± 0.2 | 0.7 ± 0.2 | 0.8 ± 0.4 | 0.9 ± 0.4 | 0.7 ± 0.2 |
| Control | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Level of Sig | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| Treatment | Average trapped flies FTD (Mean ± SE) for *Bactrocera dorsalis* | | | | | | | | | | | |
| ME | 8.8 ± 1.9 | 20.4 ± 4.5 | 9.5 ± 2.4 | 2.0 ± 0.2 | 0.9 ± 0.2 | 4.2 ± 0.6 | 5.8 ± 0.6 | 5.0 ± 0.5 | 2.7 ± 0.3 | 4.3 ± 0.7 | 1.9 ± 0.3 | 0.4 ± 0.0 |
| CL | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| ME+CL | 3.2 ± 0.8 | 10.8 ± 1.4 | 6.4 ± 2.1 | 1.6 ± 0.4 | 1.1 ±0.2 | 2.3 ± 0.6 | 4.5 ± 0.6 | 4.0 ± 0.3 | 1.5 ± 0.2 | 2.1 ± 0.7 | 1.6 ± 0.6 | 1.2 ± 0.3 |
| ME/BO | 9.0 ± 2.1 | 13.5 ± 1.4 | 9.5 ± 3.1 | 1.5 ± 0.7 | 0.9 ±0.2 | 3.9 ± 0.6 | 2.8 ± 0.5 | 1.5 ± 0.5 | 0.5 ± 0.1 | 1.0 ± 0.2 | 0.8 ± 0.2 | 0.5 ± 0.1 |
| CL/BO | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| ME+CL/BO | 3.4 ± 0.6 | 8.3 ± 1.3 | 4.5 ± 0.9 | 1.4 ± 0.5 | 0.5 ± 0.1 | 0.9 ± 0.1 | 1.7 ± 0.4 | 1.2 ± 0.2 | 0.6 ± 0.2 | 0.6 ± 0.3 | 0.7 ± 0.3 | 0.5 ±0.2 |
| Control | 0.0 ±0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Level of Sig | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| Treatment | Average trapped flies FTD (Mean ± SE) for *Zeugodacus cucurbitae* | | | | | | | | | | | |
| ME | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| CL | 0.3 ± 0.2 | 0.3 ± 0.1 | 0.6 ± 0.3 | 0.3 ±0.2 | 0.4 ± 0.3 | 0.5 ± 0.2 | 0.4 ± 0.2 | 0.3 ± 0.1 | 0.2 ± 0.1 | 0.4 ± 0.1 | 0.2 ± 0.1 | 0.4 ± 0.2 |
| ME+CL | 0.3 ± 0.1 | 0.7 ± 0.3 | 0.8 ± 0.3 | 0.5 ± 0.2 | 0.3 ± 0.1 | 0.6 ± 0.2 | 0.4 ± 0.1 | 0.2 ± 0.0 | 0.1 ± 0.0 | 0.3 ± 0.1 | 0.2 ± 0.1 | 0.3 ± 0.1 |
| ME/BO | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ±0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| CL/BO | 0.1 ± 0.0 | 0.2 ± 0.0 | 0.1 ± 0.1 | 0.1 ± 0.0 | 0.1 ± 0.1 | 0.2 ± 0.0 | 0.3 ± 0.1 | 0.0 ± 0.0 | 0.1 ± 0.1 | 0.1 ± 0.0 | 0.1 ± 0.1 | 0.1 ± 0.1 |
| ME+CL/BO | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.3 ± 0.2 | 0.2 ± 0.1 | 0.6 ± 0.3 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Control | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ±0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Level of Sig | ** | * | ** | * | * | * | ** | ** | ns | ** | ns | * |

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