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Studies on the Distribution and Integrated Management of Fruit Flies in Sudan

دراسات على التوزيع والإدارة المتكاملة لذباب الفاكهة في السودان

A Thesis Submitted in Partial Fulfillment of the Requirements for the PhD Degree in Plant Protection

By

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الآية

فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ ۗ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَىٰ إِلَيْكَ وَحْيُهُ ۖ وَقُلْ رَبِّ زِدْنِي عِلْمًا ﴿١١٤﴾

سورة طه

DEDICATION

To those who guided me in my first steps

Mother and Father

To my Brother and sister

To my dear Husband

With love

Maisoon

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Abstract

This Study was carried out at three States which are Khartoum,Sennar, and Kasala States during the period 2010 to 2018.

During the period 2010 – 2011 field and laboratory studies were undertaken through surveying and monitoring of fruit flies species, and the results revealed that, four species were found in association with mango and guava in the study sites. These species were, *Bacterocera invadens*, *Ceratitis cosyra*, *C. quinaria* and *C. capitata.* Among them, *B. invadens* was the most dominant in all study sites. Moreover, the sex ratios of the different fruit flies species were determined and showed that, females of *B. invadens* outnumber the males by three to four folds, while females of other *Ceratitis* species were two to five folds of the males.

During 2012 to 2014 studies were carried out in laboratory and field to evaluate the effectiveness of naturally extracted *B. invadens* host marking pheromone (HMP). Treating of mango and guava fruits with different concentrations of *B. invadens* faeces pheromonal extracts (2.5, 5, 10 and 15%) significantly reduced fruit infestation (number of larvae per fruit ($P< 0.0001$). The effectiveness of the faeces pheromonal extract was surprisingly uniform. As the results were promising, more studies were carried out for Structural Elucidation of the natural HMP using HPLC and GC-MS. The results of the experiments showed that, the natural pheromone extract according to the development of chromatographs of HPLC in the first analysis contain 17 peaks, 32 peaks for the second analysis and the samples analysis by GS-MS showed the presence of 42 compounds.

The last part of the study was carried out during 2015-2018, as we encountered problems in fruit flies control measures in study sites that lead to increase in fruit fly infestation and reduction in fruit production. Further experiments were carried out

to investigate the problem. It was found that fruit flies numbers trapped by locally manufactured Lynfield traps baited with Methyl Eugenol, mixed with malathion (57%) ranged from 4 to 440 flies per trap per week. The present results compared to former studies in same study sites showed a decrease in hatchability. To investigate the reasons of the decrease in traps catchability, we evaluate different types traps. The results showed that, the Sticky Traps catched the highest mean number of trapped fruit flies, followed by Lynfield trap baited with Methyl Eugenol mixed with malathion (57%) and Guava fruit juice. During checking of the traps and counting the numbers of flies, a new species of fruit flies was noticed, which was identified later as, Bactrocera zonata.

B. zonata was reported to be resistant to malation and this could be the reason catchability of traps decreased in comparison with previous studies. A newly designed two liter plastic bottle trap was compared with the locally manufactured Lynfield trap which is used for mass trapping in the study area (1st Trap)".The result showed that the range of catched flies per trap per week between (28 to 75) for the 1st trap and (863 to 1659) for the 2nd trap , The structure of the new designed two liter plastic bottle trap, with one upside down hole make it difficult for the flies to get out of the trap and hence be exposed to the pesticide for long time.

ملخص البحث

نفذت هذه الدراسة في ثلاث ولايات هي الخرطوم، سنار، كسلا في الفترة من 2010 الى 2018 خلال الفترة 2010–2011 اجريت دراسات معملية وحقلية لعمل مسوحات ومراقبة انواع ذبابة الفاكهة، اظهرت الدراسة وجود اربعة انواع مرتبطة بالمانجو والجوافة في مناطق الدراسة وهي ذبابة الفاكهة الأسيوية (الغازية) *Bacterocera invadens* ، ذبابة المانجو *Ceratitis cosyra* ، ذبابة الفاكهة الروديسية *C. quinaria*، وذبابة البحر الأبيض المتوسط *C. capitata*. اوضحت الدراسة أن ذبابة الفاكهة الأسيوية (الغازية) هي الأكثر انتشارا بين هذه الأنواع، بالإضافة إلى ذلك فقد تمت دراسة نسبة الذكور للإناث للأنواع الأربعة واظهرت الدراسة أن إناث نوع ذبابة الفاكهة الأسيوية*B. invadens* تتفوق على الذكور بثلاث الى اربعة اضعاف بينما الأنواع الأخرى *Ceratitis spp.* تفوق إناثها ذكورها بضعفين الى خمسة اضعاف.

خلال الفترة 2012–2014 اجريت دراسات في الحقل والمعمل لتقييم مفعول المستخلص الطبيعي لفرمون ذبابة الفاكهة الأسيوية المحدد للعائل (HMP) حيث ادت معاملة ثمار المانجو والجوافة بالتركيزات المختلفة للمستخلص الفرموني (2.5%،5%، 10%، 15%) الى خفض الإصابة معنويا. فعالية المستخلص الفرموني للفضلات اعطت نتائج متجانسة.

بما ان النتائج كانت واعدة اجريت مزيد من الدراسات على التركيب الكيميائي بواسطة جهازي الكروماتوجرافيا السائلة عالية الدقة HPLC وجهاز كروماتوجرافيا الغاز GC-MS. نتائج التجارب اوضحت احتواء الفرمون الطبيعي في التحليل الأول بجهاز HPLC على 17 مركب للعينة الأولى

واحتوت العينة الثانية على 32 مركب واضح التحليل الثاني بجهاز GC-MS احتواء العينة على 42 مركب.

الجزء الأخير نفذ خلال الفترة 2015–2018 حيث واجهتنا مشكلة في مكافحة ذبابة الفاكهة ادت الى زيادة الإصابة وتدني الإنتاج. اجريت المزيد من التجارب لبحث المشكلة وكانت اعداد ذباب الفاكهة الذي تم اصطياده بواسطة مصيدة لينفيلد Lynfield المصنعة محليا والمزودة بفرمون الميثايل يوجنول Methyl Eugenol المخلوط بمبيد الملاثيون Malathion 57% تتراوح بين 4–440 حشرة في المصيدة في الأسبوع. أوضحت النتائج انخفاض قدرة المصيدة على الإصطياد عند مقارنتها بالدراسات السابقة في نفس المنطقة. لبحث أسباب هذا الإخفاض تم تقييم انواع مختلفة من المصائد أوضحت النتائج ان المصائد اللاصقة اعطت اعلى متوسط لعدد الحشرات تليها مصيدة لينفيلد المزودة بالميثايل يوجنول المخلوط بمبيد الملاثيون 57% والمضاف اليها عصير الجوافة. أثناء فحص المصائد وحساب أعداد الحشرات تمت ملاحظة نوع جديد من ذباب الفاكهة، والذي تم تعريفه لاحقا بذبابة الخوخ *Bactrocera zonata* والتي ذكرت مقاومتها لمبيد الملاثيون في دراسات سابقة، وهذا قد يكون سبب انخفاض كفاءة مصيدة لينفيلد عند مقارنتها بالدراسات السابقة.

تم تقييم مصيدة جديدة مصنوعة محليا من قارورة بلاستيكية سعة 2 لتر (مصيدة 2) مع مصيدة لينفيلد (مصيدة 1) المستخدمة سابقا في المكافحة. اعطت التجربة (28–75) حشرة في المصيدة في الأسبوع للمصيدة الأولى بينما اعطت(836–1659) حشرة في المصيدة في الأسبوع للمصيدة الثانية. المصيدة الجديدة بفتحتها الواحدة المقلوبة ادت الى صعوبة خروج الحشرة وبالتالي تعرضها للمبيد لفترة أطول.

CHAPTER ONE

INTRODUCTION

1. INTRODUCTION

The rapid population growth of sub-Saharan Africa and progressive urbanization has resulted in increasing rates of malnutrition and vitamin deficiency in large sectors of rural and urban populations **(World Bank, 1996; World Resources, 1999; IFPRI, 2002 and WHO, 2002)**. This, along with the developing awareness of the nutritive value of fruits and the increased purchasing power of affluent segments of local populations translated into increased domestic demand for fresh fruits. In addition, the demand for quality tropical fruit in Europe, America and Japan is also growing. The above factors, combined with increasingly liberal global trade arrangements, have created new and lucrative production and trade opportunities.

Horticulture is the fastest growing agricultural sector in Africa, providing income and employment. However, profitable fruit production in Africa is greatly hampered by fruit flies. According to **Lux *et al*., (2003),** nearly 1.9 million tons of mangoes are produced annually in Africa. About 40% of the harvest is lost due to fruit flies. Fruit infestation rates vary among countries and seasons, ranging from 5-100%. Other factors such as the strict quarantine and the maximum residue levels set by the European Union (EU) are affecting the production and export of fresh mangoes from Africa. According to data presented during the meetings of the FAO Inter-Governmental Sub-Group on Tropical Fruits held in Australia in 1999 and Costa Rica in 2001, mango exports are estimated at 35,000 – 40,000 t annually and worth over 42 million US $.

During the last decade an increasing interest in the chemical communication system of insects has developed with the concern over the environmental safety of insecticides. Entomologists have turned their attention to pheromones as environmentally safe potential alternatives. A considerable amount of literature has

been built up in recent years on the chemical isolation and identification of pheromones and to a lesser extent on pheromone-controlled behavior.

After ovipositing in a fruit, some phytophagous insects leave behind chemical markers. These markers, known as host-marking pheromones (HMP) **(Nufio and Papaj, 2001)**, indicate to other females of the same species that the limited resource for larval nutrition has already been occupied. Females landing on such a resource usually reject it as a site not suitable for the development of their own offspring and avoid oviposition. HMPs are mainly produced by temperate species like the cherry fruit fly *Rhagoletis cerasi* and related species, which are mainly oligophagous, feeding on small fruits, and need to achieve economy in egg production **(Averill and Prokopy, 1989)**. Up to now, HMPs are known from 11 species of *Rhagoletis,* 2 species of *Anastrepha* and from *Ceratitis capitata* **(Fletcher and Kitching, 1995)**. Thus, a major concern regarding the existence of HMPs in tropical mango-infesting fruit flies like *Bactrocera invadens* and *Ceratitis cosyra* could be that in this case the resource for larval nutrition is not really limited, because one fruit can feed many individuals of fruit fly larvae. But a HMP is already known from the closely related polyphagous *C. capitata*, and the ancient endemic host plant of *C. cosyra* is the marula tree, which produces also small fruits in the size of cherries **(Lux *et al.*, 2003)**. These chemical signals may be secreted by females after egg laying, as seen in tephritid flies **(Averill and Prokopy, 1989)**, or it can be found among some larval secretions or frass, as seen in some lepidopterans and chrysomelids **(Williams *et al*., 1986; Hilker, 1989; Hilker and Klein, 1989; Gross and Hilker 1995)**.

The discovery of a host-marking pheromone that deters oviposition in the cosmopolitan and polyphagous medfly *C. capitata* has stimulated studies to develop a similar control method for these important pests **(Arredondo and Di´az-Fleischer 2006)**.

However, the use of this type of infochemicals for control has been questioned, especially because some females reuse oviposition sites and use HMP marks as a cue to localize the oviposition puncture **(Papaj *et al*., 1989; Papaj, 1994; Dı´az-Fleischer *et al.*, 2000)**. Further, the main components are mainly related to hydroxy fatty acids which are substituted by taurine and glucose **(Fletcher and Kitching, 1995)**, and thus they can easily removed by rain. This is one of the reasons that HMP of *R. cerasi* is not yet used in Integrated Pest Management in Europe. In contrast, in Sudan the peak period of most fruits production actually out of the rainy season.

While studies on several fruit fly species conducted in Mexico showed that mango infesting fruit flies of the genus *Anastrepha* are using HMPs **(Aluja *et al.*, 2003)**, it is unknown if dominant species of fruit fly species in Sudan will also produce such type of infochemicals.

Trapping surveys are applied to determine species presence, or monitor established fruit fly populations. Also it can be applied to reach a fruit fly low prevalence area or to reach a fruit fly free area and to minimize the risk of introduction or re-introduction of a pest in a free area.

Traps used for fruit flies are dependent on the nature of the attractant. The most widely used traps contain para-pheromone or pheromone lures that are male specific. The para-pheromone trimedlure (TML) captures medfly and Natal fruit fly. The para-pheromone methyl eugenol (ME) captures a large number of *Bactrocera* species including: Oriental fruit fly (*B. dorsalis)*, peach fruit fly (*B. zonata)*, carambola fruit fly (*B. carambolae*), Philippine fruit fly (*B. philippinensis),* and banana fruit fly (*B. musae*). The para-pheromone cuelure (CUE) also captures a large number of *Bactrocera* including: melon fly (*B. cucurbitae*) and Queensland fruit fly (*B. tryoni*). The pheromone Spiroketal (SK) captures *B. oleae* **IAEA (2003)**.

Lures for capturing female fruit flies are based on food or host odours. Historically, liquid protein baits have been used to catch a wide range of different fruit fly species Liquid protein baits capture both females and males, with a higher percent of females captured.

These liquid baits are generally not as sensitive as the para-pheromone traps in low populations. The usage of liquid baits results in capturing large numbers of non-target insects **IAEA (2003)**.

Objectives:

1- Identification of the most damaging fruit fly species in Khartoum State and some other mango and guava producing States (Kasala and Sennar).
2- Identification of invasive species and their competitive displacement.
3- Investigating effects of chemical compounds extracts from faeces of major mango-infesting fruit fly species.
4- Evaluation of different types of traps catchability using Para pheromone methyl eugenol (ME) and food lures.
5- Evaluation of two types of locally manufactured traps.

CHAPTER TWO
LITERATURE REVIEW

2. LITERATURE REVIEW

2.1. Taxonomic status of fruit flies:

The identification of fruit flies is very difficult even for the professional taxonomist **(Dallwitz, 2000)**. For this reason a fruit fly identification system was developed by many authors as part of a long project in order to make taxonomic information on Tephritidae available. Other components of the project include comprehensive data on fruit flies names **(Norrbom *et al*., 1999)**.

True fruit flies belong to Dipter: Typhritidae includes more than 4000 species assigned to 500 genera. Approximately 250 of them are of economic importance and are associated with fruits and vegetables. **(Mohamed and Taha, 2008; PHA, 2011)**

Most of the Tephritidae species which attack fruits belong to the genera: *Ceratitis, Bactrocera, Dacus, Anastrepha* and *Rhagoletis*.

Bactrocera is the most economically significant genus, with about 40 important pests' species **(White and Elson–Harris, 1992 and Norrbom *et al*., 1999)**.

2.2 Origin and distribution:

The genus *Ceratitis* is native to Tropical Africa, while the genus *Bactrocera* is of Asian origin **(White and Elson–Harris, 1992)**. West Africa had been considered by the dipterologist Bezzi as the probable home of *Ceratitis capitata* **(Silvestri, 1914)**. Although, Widemann who first described it in 1824 under the name *Trypeta capitata*, considered the East Indies as the country of origin **(Silvestri, 1914)**.

Bactrocera invadens was first found in Kenya in 2003 **(lux *et al*., 2003)** and it was reported from Tanzania shortly afterwards **(Mwatawala *et al*., 2004)**. Taxonomic studies by **Drew *et al*., (2005)** showed that, it was an unknown exotic species which was described as *Bactrocera invadens*. Within two years of its

detection in East Africa, it was reported from several other countries throughout the African continent **(Drew *et al*., 2005)**.

In Sudan, *B. invadens* was first recorded in 2005 and is spreading fast replacing the already existing species **(Mohammed and Ali, 2008)**.

The family Tephritidae is represented all over the world, except in Antarctica, and has limited natural distribution, as follows:

2.2.1 *Anastrepha spp.*

According to **White and Elson-Harris (1992)**, it was found to attack a wide range of fruits in South and Central America and the West Indies, with a few species occurring in the extreme south of the U.S.A, but no species has become established outside those areas.

2.2.2 *Bactrocera spp.*

These are natives to tropical Asia, Australia and South Pacific region, with a few species found in Africa and warm temperate areas of Europe. Some species have become established in Hawaii, French Guiana and Suriname as a result of recent fruit trade movements **(White and Elson–Harris, 1992).**

2.2.3 *Ceratitis spp.*

They attack a wide range of fruits and are native to tropical Africa. *Creatitis capitata* (Wiedmann) has been established in all regions except Asia, whilst several out breaks in North America have been eradicated **(White and Elson–Harris, 1992).**

2.2.4 *Rhagoletis spp.*

These are found in South and Central America, mostly on Solanaceae, and in the temperate areas of Europe and North America, where most species are associated with fruits of a single family of plants, and often a single genus. The most important pest species are associated with Rosaceae family and some of these have the potential to become established in new areas **(White and Elson–Harris, 1992).**

2.2.5 *Dacus spp*.

These are almost associated with flowers and fruits of Cucurbitaceae, or with the pods of Asclepiadaceae and most species are found in Africa. *Dacus ciliatus* (Loew) has become established in the Indian Ocean Islands **(White and Elson–Harris, 1992).**

2.3. Relative abundance of fruit flies species in Sudan:

In Sudan, fruit flies were reported at Khartoum State by **Venkatraman and Elkhidir, (1965)**. **Ali (1967)** found fruit flies in the Northern region (Shendi, Hudieba), Khartoum, Kassala and the Southern Region (Yambio, Meridi, Yei, and Juba). Now they are wide spread in Sudan, occurring in all regions of fruits and vegetables.

Deng (1990) stated that, *Ceratitis cosyra* has been recorded in Khartoum, while **Beji (1996)** recorded it from Kassala. Recently, it has been recorded in Eastern, Western and Central Sudan **(Ahmed, 2001; Elhewaris, 2003, and Bashir, 2007).** An earlier study in the Gezira State showed that, *C. cosyra* was the predominant species of fruit flies on mango **(Ahmed, 2001).**

In North and South Kordofan States, *C. cosyra* was the most commonly occurring species on fruit trees followed by *B. invadens* **(Bashir, 2007 and Ali, 2007).** Also in the River Nile State, *C. cosyra* was the dominant species on mango and second to it was *B. invadens* **(Abdalla, 2007).** Recently, it was found that *B. invadens* is the most prevalent species followed by *C. capitata* and *C. cosyra* in the second degree on mango and guava in the Gezira area **(Mohammed and Ali, 2008)**.

2.4 Host plants:

Most of fruit-infesting Tephritids are polyphagous. **Liquido *et al*. (1991)** reported 353 plant species as hosts or potential hosts for fruit flies. Its close relatives, *C. cosyra* and *C. rosa* have fairly wide host range in Africa. Although *C. cosyra* is

primarily considered to be a pest of mango, the host range of *B. cucurbitae* is primarily cucurbits, but has been recorded from a few non–cucurbit hosts **(White and Elson–Harris, 1992)**.

Bactrocera zonata infests most of the known fruits, such as mango (*Mangifera indica*), guava (*Psidium guajava*), peach (*Prunus persica*), papaya (*Carica papaya*), pear (*Pyrus armeniaca*), plum (*Spondia scytherea*), apple (*Malus domestica*), citrus (*Citrus spp.*), dates (*Phoenix dactylifera*), in addition to secondary vegetable hosts such as cucurbits and tomatoes **(FAO, 2004).**

In Sudan, **Venkatraman and Elkhidir (1965)** found *C. capitata*, along with *Dacus spp.* attacking eggplant (*Solanum melongena*), guava (*Psidium guajava*), some Cucurbit and citrus fruits.

Schmutterer (1969) stated that, the med fly attacked ripen fruits of guava, citrus (orange, grape fruit, and tangerine), red pepper, eggplant and coffee berries (*Coffea arabica*). **Prokpy (1978)** suggested that the primary host fruit for particular fruit fly species could be a secondary host for the same fly species in other places.

2.5 Economic importance:

The damage caused by this pest is due to the oviposition punctures in the fruits.

The infested fruits develop watery soaked appearance. Young fruits become distorted and usually drop. The oviposition punctures and the larval tunnels provide entry passage for bacteria and fungi that cause rotting and lead to complete destruction of the fruit **(Schmutterer, 1969)**. The symptoms vary from one type of fruit to another e.g. infestation appears as dark spots in citrus and as black sunken areas in the lower half of the guava fruits. In mango the symptoms appear as fluids which exude from ovipunctures in the form of droplets that later dry up and turn brown. The genus *Bactrocera* is the most economically important, with about 40

pest species **(White and Elson–Harris 1992)**. About 70 species of fruit flies are considered important agricultural pests and many others are minor potential pests **(White and Elson-Harris, 1992)**. The economic effects of any pest species include not only direct yield losses and increased control cost, but also the loss of export markets and/or the cost of constructing and maintaining fruit treatment and eradication facilities. In many countries, the exportation of most commercial fruits is severely restricted by quarantine laws to prevent the spread of fruit flies.

The cost of managing an established infestation of *C. capitata* and several major fruit flies in California was estimated at hundred million dollars annually **(Jackson and Lee, 1985)**.

2.6 Life cycle and behavior:

Singh (1960), stated that all species of fruit flies were similar in their mode of reproduction, infestation and damage. The life cycles of various stages were reviewed by many authors. **Hill (1983)**, stated that the biology of the mango fruit fly *C. cosyra* was simillar to that of *C. capitata*.

Females pierce the ripening fruit and insert the eggs into the punctures. The maggots feed on the pulp, making the fruit unacceptable. Pupation occurs either inside the fruit or in the ground.

The adult is a small fly, which holds its wings partly extended when at rest; it is about 4-5mm long, and the wing span is 10mm. There are probably 2-10 generations per year in Africa according to the species and climate. **Deng (1990)**, reported that the life cycle of *C. cosyra* was closely related to that of *C. capitata* and *C. quinaria*.

2.6.1 The egg stage:

The adult female punctures the skin of the fruit with its sharp pointed long ovipositor and lays the eggs in the pulp **(Singh, 1960)**.

The eggs are laid singly or in clusters. They are tiny (0.8mm long, 0.2mm wide) and white in colour. **Back and Pemberton (1918)** and **Hill (1983)** reported that the incubation period for med fly eggs ranged between 2-4 days, in summer in the tropics and might be prolonged to 16-18 days in winter. **Hanna (1947)** in Egypt found that, the incubation period ranged between 2-4 days in summer. **Hill (1983)** reported that the mango fruit fly, *C. cosyra* needed 2-4 days from ovipostion to hatching in summer.

2.6.2 The larval stage:

The larvae moult twice; the second instar larva measures 7.5-10 mm in length and 1.5-2 mm in width **(White and Elson –Harris, 1992)**. Fully grown larvae measure about 1.3cm in length **(Singh, 1960)**.

The maggots are white, broader at the posterior end and pointed at the anterior end. They feed on the internal tissues of infested fruits causing rot, and the fruits fall from the tree **(FAO, 2004)**. Larvae pass through three instars within 9-25 days after which they drop into the soil for pupation to a depth of about 5cm, depending on soil type. The development of maggots depends on temperature, moisture, ripeness, hardness, decay, dryness and acidity of fruits **(Severin, 1913)**.

2.6.3 The pupal stage:

Deng (1990) in Sudan reported that the fully grown med fly maggots turned to creamy colour at the time of pupation; they leave the fruit and drop to the soil to pupate. The pupae change their colour gradually from creamy to light brown and, finally to brown. **Severin (1913)** found that the pupal period was about 15-17 days, while **Back and Pemberton (1918)** found it to be 17 days.

2.6.4 The adult stage:

Back (1915) observed that the adults of *C. capitata* emerged in large numbers early in the morning during warm weather and were more scattered during cool weather. Adult flies feed on various kinds of food, such as glandular secretions of

plants, flower nectar, plant sap, rotten fruits, bird droppings, and honey-dew secreted by homopterous insects **(Hagen, 1953)**. Adult longevity is influenced by availability of food and climatic conditions. **Hagen (1953)** found the longevity of *C. capitata*, ranging between 20-65 days. Adult longevity can become longer when vitamins and hydrolyzed proteins are added to the food. In Egypt, **Hanna (1947)** found that the longevity of the med fly ranged from 32–96 days. The longevity of *Bactrocera* females ranged between 50-70 days while that of the males ranged between 30–45 days **(FAO, 2004)**.

2.6.5 Number of annual generations:

Bactrocera spp. was reported to have 6-10 annual field generations **(FAO, 2004)**.The duration of the different stages varies with species, host plant, and climatic conditions.

2.7 Behavior of the fruit flies:

Tephritids exhibit a wide array of interesting and sometimes spectacular behaviors in many aspects of their life especially during their dispersal, feeding and ovipostion.

2.7.1 Oviposition behavior:

Females often discharge a marking pheromone on the fruit (or other part of the plant in which eggs are deposited). This pheromone deters ovipostion by other females **(Headrichs and Goeden, 1994)**.

The ovipostion behavior appears to be more uniform than epigamic behavior. The female lays several eggs singly or in cluster beneath the skin of mature ripe fruits during an extended period of many hours **(Aluja and Norrbom, 1999)**.

2.7.2 Feeding behavior:

Feeding behavior of Tephritids in nature is poorly understood **(Headrichs and Goeden, 1994)**. The larvae feed on the internal tissues of infested fruits causing rot and the fruit drop **(FAO, 2004)**. Adult nutritional requirements vary and largely

depend on the quality of the larval food and usually include at least carbohydrates and water, **(Aluja and Norrbom,1999)**. Adults may feed on plant exudates, including those from the ovipostion punctures or rotting fruits, bird feces,nectar, honey dew, pollen grains and rain drops **(Headricks and Goeden, 1994)**.

2.7.3 Courtship behavior:

Courtship can be elaborate in some species, or simple and brief in others. **Headrick and Goeden (1994)** defined 14 movements or behaviors that commonly occur in courtship, which may include various types of body, leg, and wing movements, and/or transfer of a nupitual gift (trophallaxis). The letter behavior has been observed in diverse taxa, including species of Dirioxa (Acanthonevrini), Anastrepha (Toxotrypanini), and various genera of Tephritinae **(Headrick and Goeden, 1994)**. The gift may be passed before or after copulation, and it may consist of liquid transferred by direct contact of the mouth parts or may be a solidified froth deposited on the substrate. Copulation is determined by female choice **(Headrick and Goeden1994)** and may last from several minutes to several hours.

2.8 Copulation:

Many fruit flies mate on their host plants, but mating tactics vary, even within same species. Lek formation by males, usually on non-hosts, has been observed in *Ceratitis capitata* and species of *Dacina*, *Anastrepha*, *Rhagoletis*, and *Procecidochares* **(Aluja, 1994)**.

Males of most species of Tephritidae that have been studied secrete some type of sex-attractant chemical, either by inflating the lateral abdominal membranes or by extruding an anal pouch **(Headrick and Goeden, 1994)**. Visual stimuli, as well as chemical and auditory stimuli, play an important role in communication between and among the sexes and with other insects. Mate-guarding and male defense of food resources attractive to females also have been reported **(Headrick and Goeden, 1994)**.

2.9 Ecological factors affecting biology and behavior:

2.9.1 Temperature:

The development of the immature stages of Tephritids is possible under temperature range of 10–30°C. A temperature of 45°C is the upper limit for a few hours of survival of all stages of flies **(Bess and Harmamoto, 1969)**. The role of temperature as determinant of abundance in Tephritids is mediated either directly or indirectly through its effect on rates of developments, mortality and fecundity **(Clark, 1957). Prokopy (1978)** stated that egg lying is usually restricted to a few weeks in summer in tropical regions.

2.9.2 Moisture:

According to **Bateman (1972)** moisture is an important factor for the determination of abundance of Tephritids and there is a high correlation between rainfall and the peak number of fruit flies recorded each year. However, **Vergas (1983)** found a negative correlation between total monthly rainfall and the number of *C. capitata*. **Bateman (1972)** stated that Tephritids were rarely found in extreme dry parts of the world. This might be due to a limitation on the distribution of their host plants, rather than on the capacity for physiological adaptation. According to **Nelson (1964)**, the survival rate of pupae at a relative humidity of 60% and below was virtually zero. However, **Shoukry and Hafiz (1969)** reported that the effect of relative humidity on the pupal duration had no significance, though the percentage of adult emergence was found to be high at 60% and low at 30% relative humidity.

2.10 Control:

In many cases, the study of the biology of an insect pest has offered some clue for its effective control. Some weak links in the life history of a pest are discovered

which the economic entomologists exploit. However, the study of the biology of the pests belonging to this family offers no clue because the larvae live in the fruits, vegetables, nuts, or in the buds of the growing plants and therefore, insecticides that may be applied in the form of dusts or spray cannot reach them and cannot be recommended because of the risk of the residues and environmental contamination. The economic entomologist is left with only non-chemical methods such as trapping the adult flies, especially before they start laying eggs in order to reduce the incidence of the pest population **(Narayanan and Batra, 1960)**.The methods of control that can be used for the management of fruit flies are:

2.10.1 Cultural control:

The following cultural control measures were recommended:

1- Phytosanitary measure: such as destruction of infested fruits which is effective when carried out in the fields **(Agarwal *et al*., 1989).**
2- Flooding of infested fields kills the pupae in the soil **(Liu and Lee, 1987)**.
3- Light ploughing after harvest can destroy the pupae and /or expose them to the adverse weather conditions, parasites and predators.
4- Bagging of fruits with muslin bags which is time consuming but it can be effective in small plots **(Schmutterer, 1969 and Barsome, 1975)**.

2.10.2 Biological control:

Biological control is a potential useful approach in suppressing fruit fly densities **(Wharton, 1989; Knipling, 1992; Waterhouse, 1993; and Sivinski *et al.*,1996)**. Recently, natural enemies were used to reduce the population of medfly, *C. capitata* **(Wong and Ramadan, 1992 and Headricks and Godden, 1996)**.

2.10.2.1 Predators:

Some predators from different families such as Staphlinidae, Chrysopidae, Pentatomidae, Eulophidae and few mites were reported to prey on tephritids **(Bateman, 1972)**. Some earwigs were predators on *Bactrocera dorsalis*(Handel) in

Hawaii **(Marucci, 1955)**; the Argentine ant, *Lridomyrme xhumilis* (Mayer) attacks *C. capitata* under laboratary conditions and causes 50% mortality of medfly pupae **(Wong *et al*., 1984)**. The lizard, *Anolis graham* (Gray) was introduced from Jamaica to Bermuda to control some fruit flies, but their role in controlling the pest has not been indicated (Clasuesn, 1978). Birds and rodents were reported to consume infested fruits resulting in a high level of larval mortality **(Drew, 1987)**.

2.10.2.2 Parasitoids:

Many parasitoid species especially in the family Braconidae are used for Biological control of fruit flies **(Wharton, 1989)**.*Tetrastichus giffardii* Silvestri (Hymenoptera: Eulophidae) is a gregarious, larval–pupal endo parasitoid of many fruit fly species **(Lasalle and Wharton, 2002)**.

2.10.2.3 Microbial agents:

2.10.2.3.1 Fungi:

The genera, Penicillium, Serrata and Mucorae were reported to cause considerable mortality to the larvae and pupae of *B. dorsalis* **(Newel and Haramoto,1968)**. Studies carried out by **Ekesi *et al*. (2002)** and **Dimbi (2003)** proved that *Metarhizium anisopliae* had very high potential in suppressing fruit fly population.

2.10.2.3.2 Bacteria:

Bacillus thuringiensis (Beliner) sub species darmadiensis, when mixed with a protein diet and sugar and introduced as bait was found to kill *Anastrepha ludens* (Loewin) **(Robarker *et al*., 1996; and Martinez *et al*., 1997)**.

2.10.2.3.3 Nematodes:

The medfly, *C. capitata* was found susceptible to the entomopathogenic nematode, *Steinernemafelitiae* (Filipjevi). Fieldexposure of final instars of *C. capitata* larvae to a dose of 500 nematodes /cm caused high mortality **(Lindegren *et al*., 1990)**.

2.10.3 Sterile insect technique (SIT):

The sterile insect technique has been successfully used to eradicate fruit flies in several parts of the world. Early examples were the eradication of the melon fly from the Mariana Island **(Steiner *et al*., 1965)** and Kume Island in Japan **(Iwahasi, 1977)** as well as the oriental fruit fly from Guan **(Stein *et al*., 1970)** as a result of which Japan was declared free of the oriental and melon flies **(Kawasaki, 1991)**.

SIT has been extensively investigated in 13 species of Tephritidae **(Hooper, 1989)**. The use of SIT is not a simple procedure and involves a high degree of technical expertise, time and funds.

There are several discrete components of SIT that must be investigated to ensure success of the project and these were reviewed by **Hooper (1991)**. The important components include: appropriate diets and mass rearing techniques to produce 500–1000 million individuals per week; suitable techniques to sterilize flies; transport and release methods; and the methods to evaluate the progress of the control or eradication programme.

SIT techniques were used successfully to eradicate *B. dorsails* from Okinawa and neighboring Islands in the Ryukyu, Archipelago and Japan **(FFEPO, 1987)**.

2.10.4 Eradication:

Most countries that are successful in horticultural industries do not have fruit flies or have embarked on procedures to totally eliminate them. A very good example is the State of California that has a huge fruit industry which is supported by an extensive fruit fly survey, detection and eradication services. Adventives populations of oriental fruit fly, med fly; melon fly and several other species of tropical fruit flies are regularly intercepted and eradicated by the state authorities. The med fly was eradicated from Mexico and the government has expanded this to

a large national campaign for the elimination of 4 species of native Anastrepha **(Hendrichs *et al*., 1982)**.

The med fly eradication programme in Guatemala enabled 57% (62.000km) of the country to be free of the pest and is continuing toachieve total elimination **(Linares, 1991)**. Eradication can be achieved by a number of means. Target population can be first reduced by insecticide cover spray or male inhalation technique or combination of all **(Bateman, 1982)**.

2.10.5 Legislative control:

1. Avoid transferring infested fruits from a highly infested area to slightly infested area or pest-free areas without, post-harvest treatment such as quarantine disinfection.
2. Avoid planting different types of hosts at one place in order to break the food cycle of the fly throughout the year **(FAO, 2004)**.

2.10.6 Chemical methods:

The chemical or the insecticidal methods of control of fruit flies fall under three main categories: these are spraying the adult flies with suitable insecticides, trapping of the adult flies by means of chemical attractants, and bait spray that in essence consists of an insecticide mixed with bait **(Narayanan and Batra, 1960)**.

2.10.6.1 Trapping and bait spray of fruit flies:

Different traps and lures have been developed and used over decades to survey fruit flies population. The para-pheromones attract male flies and are highly species-specific while the food baits attract both females and males and are not species-specific **(Billah, 2006)**.

The first attractant for male fruit flies was Methyl Eugenol (ME), for

Bactrocera zonata followed by kerosene for the Mediterranean fruit fly, *C. capitata* **(Severin, 1913)**.

Beroza *et al*. (1961) discovered Trimedlure (TML) to be effective against *Ceratitis capitata*. However, the male lures are highly species- specific e.g. methyl eugenol attracts *B. invadens*, *B. zonata*, *B. carambolae* and *B. dorsalis*. Trimedlure attracats *C. capitata*. Terpinyl acetate attracts *C. cosyra* and culure attracts *B. cucurbitae* and *Dacus spp*. **(IAEA, 2003; FAO, 2008; Billah, 2006 and Mohamed and Ali, 2008)**.

Baited food traps based on protein, sugar, fruit juice and vinegar had been used since 1918 to capture females of several fruit fly species. The Mcphail trap was the first device to be used with protein baits **(Mcphail, 1939)**. Jackson traps in 1971 for TML **(Harris *et al*., 1971)**. These traps are currently being used in various countries for fruit fly survey, control activities and eradication campaigns.

Global trends in increasing food quality, revenue sources, fruit and vegetable trade, have resulted in an increased worldwide movement of fruit fly species which requires refinement of survey systems. The Joint Division FAO/IAEA proposes the use of proven technologies in improving trap sensitivity in area–wide fruit fly control programs **(IAEA, 1996 and 1998)**. These proven technologies include the use of synthetic food lures such as female attractants that can be used for several species.

It is well known that bait–spray offers one of the most effective methods of control especially in the pre-oviposition stage when the flies require protein for egg maturation. This habit of the flies has been utilized, to poison and kill them. A few branches and foliage in each tree in an orchard are swiftly sprayed so that the spray does not drizzle down, but is retained on the leaves as droplets. The insecticides employed included lead arsentate, Paris green, potassium arsentae, sodium arsenate, copper carbonate, sodium flu silicate

and tartar emetic; the insecticides are dissolved in syrup of cane sugar **(Narayanan and Batra, 1960)**.

The fruit fly is attracted to the deposits of the protein materials, which contain nutrients essential for their sexual development, and quickly ingest enough to kill it. The contact action of the deposits and contamination of natural food sources also contribute to fly mortality **(Narayanan and Batra, 1960)**.

2.11. Integrated pest management (IPM):

The use of some or all the previously mentioned control measures were adopted to minimize the pest population of fruit flies **(FAO, 2004)**. **Verghese *et al*. (2005)** found that in India, during 2004 and 2005, a pre-harvest IPM combination of Male Annihilation Technique (MAT) using methyl eugenol as a lure and cultural methods like orchard sanitation (collection and destruction of all fallen fruits) in mango, brought down *B. dorsalis* infestation to 5% from an infestation ranging from 17-60% in no-treatment plots in both years.

2.12. Phermones:

Pheromones were originally defined as 'substances secreted to the outside by an individual and received by a second individual of the same species in which they release a specific reaction, for instance, a definite behavior [releaser pheromone] or developmental process [primer pheromone]. The word pheromone comes from the Greek pherein, meaning to carry or transfer, and hormon, meaning to excite or stimulate. The action of pheromones between individuals is contrasted with the action of hormones as internal signals within an individual organism.

Pheromones are often divided by function, such as sex pheromones, aggregation pheromones and trail pheromones.

The main methods for utilizing an understanding of pheromones to control pests are monitoring, mating disruption, 'lure and kill' or mass trapping, and other manipulations of pest behavior. Some of these techniques have been applied to control other animal pests, including vertebrate herbivores, such as deer. A major strength of pheromones is their effectiveness as part of integrated pest management (IPM) schemes because of their compatibility with biological control agents and other beneficial invertebrates, such as bees and spiders. Pheromones fit neatly into the *virtuous* spiral, for example, in greenhouse IPM, where the use of one biological control agent, such as a predatory spider mite, encourages (or requires) moving away from conventional pesticides for other pests **(Lenteren&Woets 1988)**.

2.12.1. Sex pheromones:

Sex pheromones have been identified for a large number of insect pests, particularly Lepidoptera. These chemicals have a number of useful attributes for the attract-annihilate method, including specificity, eliciting long-distance responses and longevity in the field.

However, because most sex pheromones are produced by females and elicit responses from males, they have been used primarily in the mating disruption method, or for monitoring, rather than for the attract-annihilate method. The removal of adult males, unless at a very high proportion of the population, is unlikely to have a large impact on the size of subsequent generations compared with the removal of females **(Lanier, 1990)**. Sex pheromones have also been used as attractants to facilitate contact with and the dispersal of pathogens in pest populations **(Pell *et al*., 1993)**. Pheromones have been identified for many insect pests. The website 'Pherolist', for example, cites more than 670 genera from nearly 50 families of Lepidoptera in which female sex pheromones have been identified **(Arn *et al*., 1995)**.

2.12.2. Aggregation pheromones:

Aggregation pheromones lead to the formation of animal groups near the pheromone source, either by attracting animals from a distance or by stopping (‘arresting’) passing conspecifics **(Wyatt, 2003)**. In contrast to sex pheromones (which attract only the opposite sex), aggregation pheromones, by definition, attract both sexes (and/or, possibly, larvae).

The pheromones’ ability to attract females makes them well suited for the attract annihilate method **(Lanier, 1990)**. Aggregation pheromones have been used successfully for controlling various Coleoptera, including the cotton boll weevil *Anthonomus grandis* in the United States **(Hardee, 1982)** and bark beetles in North America and Europe **(Lanier,1990)**. **Innocenzi *et al*. (2001)** characterized a male-produced aggregation pheromone of *An. rubi*as a 1:4:1 blend of grandlure I, grandlure II and lavandulol (note: ‘grandlure’ is the name given to four components in the aggregation pheromone lure of the cotton boll weevil, *An. Grandis* Boh.). A blend of the synthetic compounds was shown to attract both male and female beetles.

2.12.3. Alarm pheromones:

Alarm pheromones have been identified most frequently from social insects (Hymenoptera and termites) and aphids, which usually occur in aggregations. In many cases, these pheromones consist of several components. The function of this type of pheromone is to raise an alert in conspecifics, to raise a defense response, and/or to initiate avoidance **(Rechcigl & Rechcigl, 1998). Weston *et al*. (1997)** showed a dose response of attraction and repellence for several pure volatiles from the venom of the common wasp *Vespula vulgaris* and the German wasp *V. germanica*.

The compounds are usually highly volatile (low molecular weight) compounds, such as hexanal, 1-hexanol, sesquiterpenes (e.g., (E)-β - farnesene for aphids), spiroacetals, or ketones **(Francke *et al*., 1979)**. The alarm pheromones of

aphids have been used commercially to increase the effectiveness of conventional pesticides or biological control agents, such as the fungal pathogen *Verticillium lecanii* **(Howse et al., 1998)**. Synthetic alarm pheromones and the increased activity of the aphids in response to their alarm pheromones increases mortality because they come in contact more often with insecticide or fungal spores **(Pickett *et al*., 1992)**.

2.12.4. Host marking pheromones:

Spacing or host marking (epidietic) pheromones are used to reduce competition between individuals and are known from a number of insect orders. One of the best studied is from the apple maggot *Rhagoletis pomonella* (Tephritidae), where females ovipositing in fruit mark the surface to deter other females. This behavior has also been studied in the related cherry fruit fly (*R. cerasi*). Egg laying is a key stage determining subsequent population density; therefore, it is perhaps unsurprising that there is considerable evidence of such pheromones affecting gravid females of herbivores. There is also exploitation of prey host marking and sex pheromones by parasitoids, which use the signal persistence of these intraspecific cues to find their hosts. Mating-deterrent pheromones are also known from a number of insects, including tsetse flies, houseflies, and other Diptera. These pheromones are released by unreceptive females to deter males from continuing mating attempts **(Rechcigl & Rechcigl, 1998)**.

2.12.5. Trail pheromones:

Chemical trail communication allows group foragers to exploit conspicuous food sources efficiently, and it is the most prevalent form of recruitment behavior. Trail communication is commonly based on a multicomponent system, in which the secretions of different glands (or a blend of pheromones produced by the same gland) may contribute to the structure of the trail and regulate different behaviors in the process of recruitment **(Hölldobler& Wilson 1990; Jackson *et al*. 2006)**.

Trail pheromones are used by animals as navigational aids in directing other members of the colony to a distant location, varying in length from hundreds of meters in bees to meters in terrestrial insects. The reasons for orienting members of the colony to a distant point may vary. In most cases, trails are laid by foraging workers as they return from a food source.

These trails are then used by other foragers **(Wilson &Pavan, 1959)**. In other cases, however, trails may be laid to recruit workers for slave raids, colony emigration, or the repair of a breach in the nest wall **(Wilson, 1963)**. Different types of trail marking are found in terrestrial insects and flying insects. The terrestrial insects appear to lay a continuous or nearly continuous trail between points. **Wilson (1962)** showed that the fire ant *(Solenopsis saevissima)* drags its stinger and lays a trail in a manner similar to a pen inking a line. If the food source is of good quality, other workers choose to reinforce this trail, and a highway several centimeters wide may be formed.

2.13. Tephritid Host Marking Pheromones:

In the case of true fruit flies (Diptera: Tephritidae), host marking behavior has been described in the frugivorous genera *Anastrepha*, *Bactrocera*, *Ceratitis*, *Paraceratitella*, and *Rhagoletis* (work reviewed by **Aluja *et al*., 2000; Dı´az-Fleischer and Aluja, 2000; Dı´az-Fleischer *et al*., 2000; Nufio and Papaj, 2001**). In non frugivorous tephritids, the phenomenon is less well studied but has been reported for *Tephritis bardanae* (Schrank) **(Straw, 1989)**, *Chaetorellia australis* Hering **(Pittara and Katsoyannos, 1990)**, and *Terellia ruficauda* (Fabricius) **(Lalonde and Roitberg, 1992)**. Flies in the genera *Anastrepha*, *Ceratitis*, and *Rhagoletis* mark hosts by dragging of the aculeus tip on the fruit surface **(Roitberg and Prokopy, 1987; Aluja *et al*., 2000)**, while in the case of the olive fruit fly, *Bactrocera oleae* Gmelin, marking is achieved through labellar spreading of fruit juices oozing from an oviposition puncture **(Cirio, 1971; Girolami *et al*., 1981)**.

The most striking behavioral responses exhibited by foraging female fruit flies upon encountering fruit covered with HMP are as follows: an increase in overall movement (e.g., number of leaf landings per minute, distance of between-tree displacements) and fruit visitation rates, reduction in tree residency time **(Roitberg and Prokopy, 1984; Aluja and Boller, 1992)**, reduction or increase in clutch size depending on HMP concentration and fruit size **(Papaj *et al*., 1990, 1996; Papaj and Aluja, 1993)**, and a reduction in the propensity of a female to initiate oviposition **(Nufio and Papaj, 2001)**. HMP recognition is contingent upon previous experience **(Roitberg and Prokopy, 1981)** but sensitivity to the pheromone is reduced if exposure is continual, apparently due to central habituation or peripheral adaptation of certain sensillain the tarsi **(Aluja and Boller, 1992)**. In the case of males of species exhibiting a resource–defense mating system (e.g., R. pomonella, R. cerasi), an encounter with an HMP-marked fruit causes arrestment **(Prokopy and Bush, 1972; Katsoyannos, 1975)**.

CHAPTER THREE

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Survey, Collection, Rearing and Identification of Fruit flies:

3.1.1. The Study Sites:

This part of the study was carried out from February 2010 to March 2011. Mango and guava fruits, at different stages of maturation, were collected randomly from different sites at the three states, directly from the trees or immediately after they fall to the ground. The collection sites in the different States include:

1. Khartoum State: Khartoum Central Market, Bahri Central Market, and from Al-kadroo and Elfaki-Hashim fruit orchards. **(Figure No. 1)**
2. Sennar State: Singa
3. Sennar State: Sennar
4. Kassala State: Kassala **(Figure No. 2)**

Collected fruits were placed in paper bags, labeled and then transported for further study at the Entomology laboratory, College of Agricultural Studies - Shambat, Sudan University of Science and Technology.

On arrival to the laboratory, the collected fruits were transferred to a rearing room. Fruits from separate collections were placed in pupae rearing cages (plastic boxes, each measuring: 25×18×18 cm) with moistened sterile sand at the bottom which serves as a substratum for pupation, while the upper cover of the container was cut and replaced by a fine mesh for ventilation as seen in **Plate No. 1**. When fruits partially rottened, dissection took place to allow for the movement of the larvae that may be stuck in the pulp.

3.1.2. Identification and Sex Ratio's of Fruit fly Species in the Study Sites:

For specific identification of the different species of fruit flies, and to determine the predominant species that invade different fruits at all sites, 100 pupae were collected randomly from each rearing cage of each type of fruits (mango and guava) according to their sites. Pupae were collected from the sand by sieving and held in Petri-dishes, 9 cm diameter and 1.5 cm depth, lined with moistened filter papers **(Plate No. 2)**.

After emergence, adult flies were provided with diet, consisting of: one part yeast and four parts sugar and water, for 3-4 days till they attained their full body coloration to facilitate easy identification. For Identification, keys of: **White and Harries (1992)** and **Billah (2004**) were used.

This treatment was replicated three times. The percentage of adults emerged was calculated according to species, and then separated into males and females to determine their sex ratio.

3.2 Rearing of Fruit Flies and Extraction of the Pheromone(S):

This part of the study was carried out through collection of infested fruits from Mango and Guava Orchards at the Study sites of Al-Kadaro and Alfaki- Hashim **(Figure No. 3)**. Rearing of fruit flies was made at the Entomology Laboratories, College of Agricultural Studies - Shambat, Sudan University of Science and Technology.

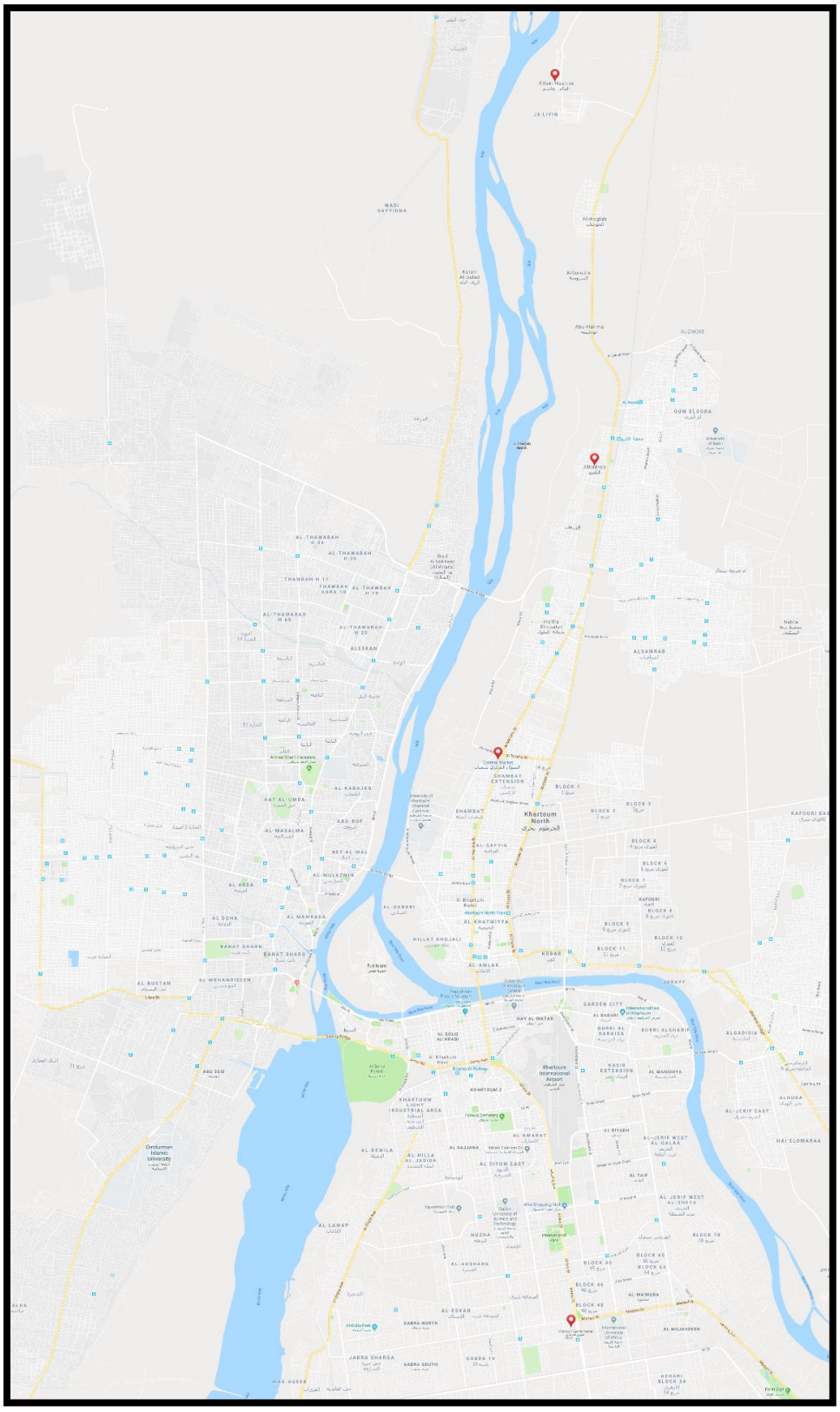


Fig (1) Khartoum State collection sites

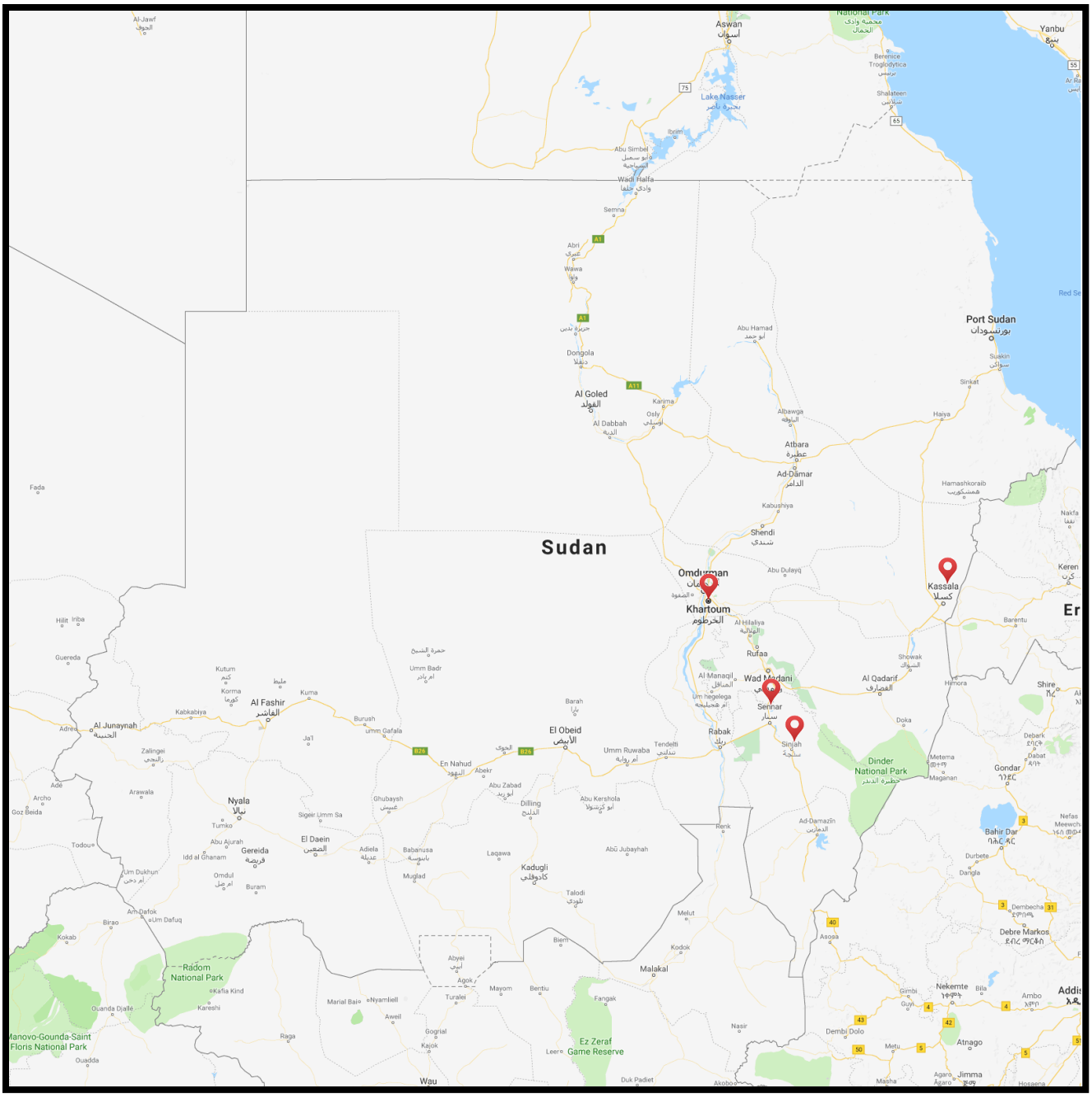


Fig (2) Different States collection sites

Plate No. 1 Pupae rearing cages

Plate No. 2 Randomly selected pupae

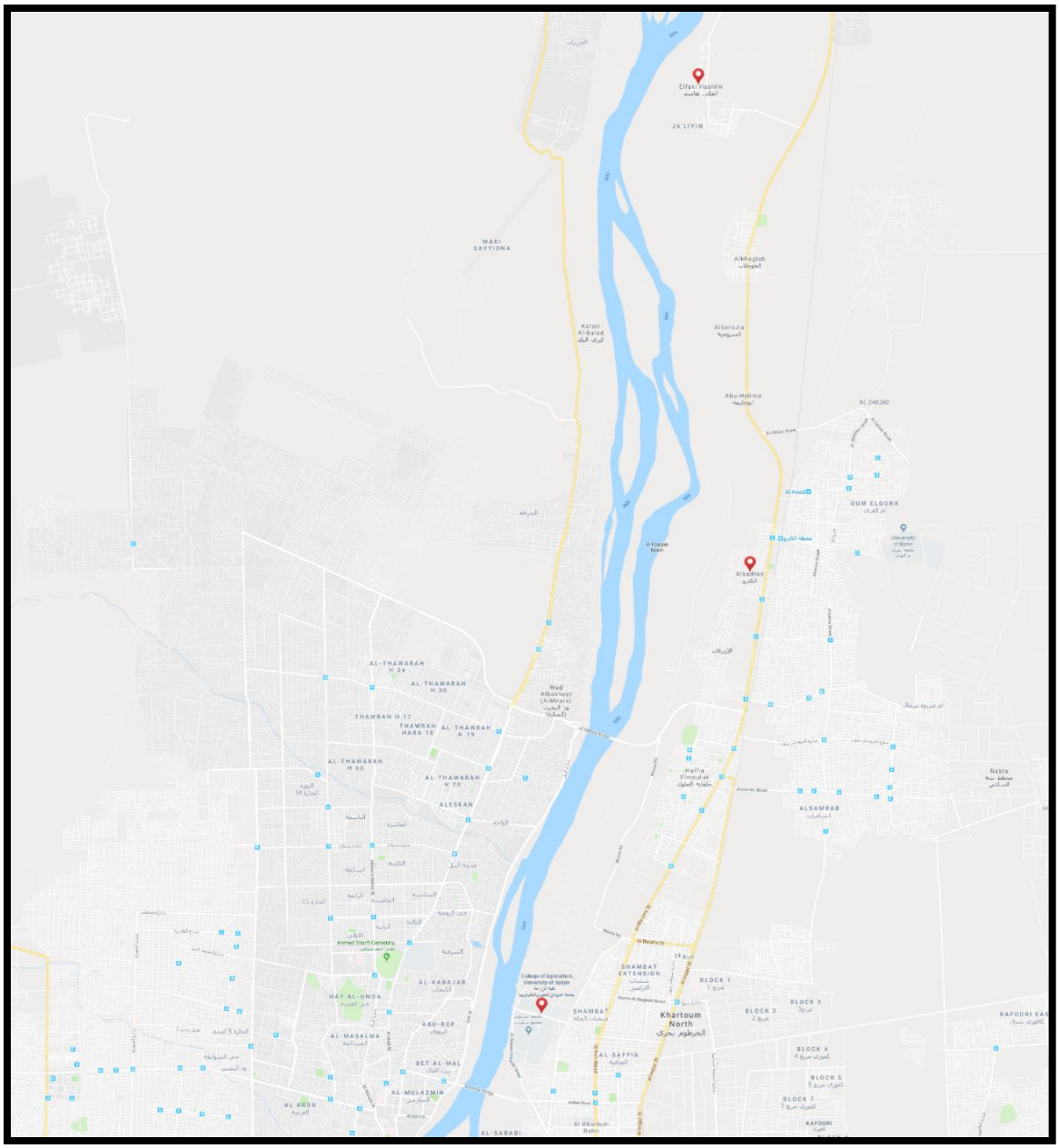


Figure (3) Collection, Rearing of Fruit Flies and Extraction of the Pheromone(S) sites

3.2.1 Mass Rearing of Fruit flies for Faeces Collection:

Methods of rearing and pheromone extraction were performed according to the methods described by **Aluja et *al.*, (2003 & 2009)** and Arrendo **and Dia'z-Fleischer (2006)**. Those authors successfully used fly faeces to obtain and isolate the "**HMPs**" of *Rhagoletis cerasi* , *Anastrepha ludens* and *Ceratitis capitata*.

Rearing started in March 2011 and continued up to May 2012, at a rearing room in the Entomology Laboratory at college of Agricultural Studies - Shambat . Large quantities of infested guava fruits were collected and kept under laboratory conditions in plastic boxes provided with moistened sterilized sand layer. Large numbers of emerging adults (up to 3000 individuals) were identified and transferred to separate culturing glass cages (30×30×30cm) , **Plate No. 3 and 4**, and provided with food (composed of : sucrose and hydrolyzed protein at a 3:1 ratio) and water, **Plate No. 5**.

Rearing conditions were maintained at 25±1ºC & 40± 5% relative humidity (RH), using a digital Thermo-Hygrometer **(Plate No. 6)**, and a photoperiod of L12:D12, controlled by a Timer **(Plate No. 7)** .

During each rearing period of (Three Months), when most of the flies were dead in all cages , the flies' faeces were scraped from the cages walls and kept in plastic bottles **(Plate No. 8)** at -15ºc in a refrigerator until further use.

These experiments of (Mass Rearing) of adult fruit flies were repeated 3-4 times during 2012 – 2013, in order to obtain large quantities of the fly faeces to be used for the extraction of the natural pheromone.

Plate No. (3) A Glass Cage for Rearing Adult Fruit Flies

Plate No. (4) Fruit Flies in A Rearing Cage

Plate No. (5) Food and Water for Adult Fruit Flies

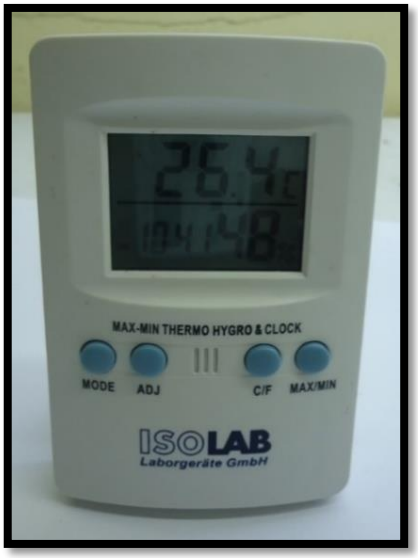


Plate No (6) Thermo-Hygrometer

Plate No (7) Timer

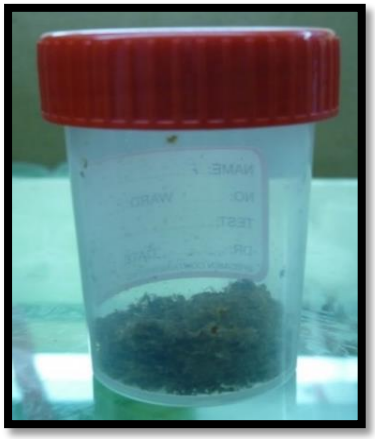


Plate No. (8) Collected Fruit Fly Faeces

3. 2. 2 Isolation of the Raw Material (Natural Pheromone):

Isolation of the pheromone from fly faeces to obtain crude pheromonal extracts for biological experimentation, was conducted and performed at JICA Laboratory , College of Agricultural Studies – Shambat, Sudan University of Science and Technology. The methods of **Aluja et al., (2009)** and **Arrendo and Dia'z-Flei scher (2006)** were adopted. One gm of fly Faeces was mixed with 10 ml of Methanol and shaken by an Automatic Shaker **(Plate No. 9)** for 15 min. Subsequently, the liquid subjected to centrifugation at 12,000 RPM during 20 min **(Plate No. 10)**. The supernatant **(Plate No. 11)** at a concentration of 100 mg/ml (Faeces / Methanol), was separated and kept as a stock solution **(Plate No. 12)** in the refrigerator at 2- 4^{o}c.

Plate No (9) An Automatic Shaker

Plate No (10) A Centrifuge

Plate No (11) Supernatant

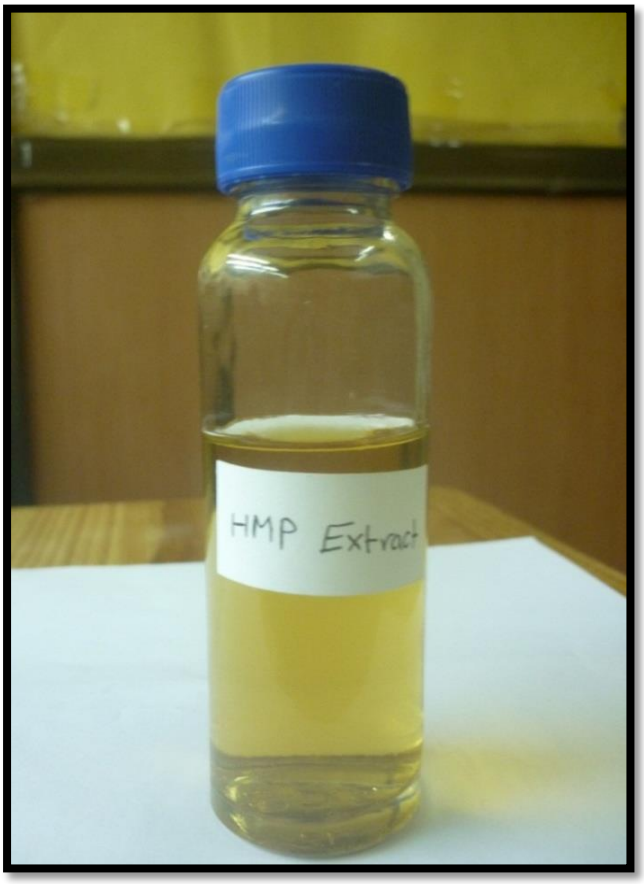


Plate No. (12) Natural (HMP Pheromone) Extract Stock Solution

3. 2. 3 Pheromones Tests:

For laboratory and semi-field experiments, the stock solution was diluted with water one day before application. The dilution was made to give four different concentrations of: 2.5, 5, 10, and 15% **(Plate No. 13)**. These experiments were carried out during Two Seasons: 2011 and 2012.

3.2. 3. 1 Laboratory Experiments:

These experiments were carried out at the College of Agricultural Studies – Shambat. Four Mango fruits were treated with the each concentrations of the natural pheromone extract (2.5, 5, 10, and 15%) and a fifth mango fruit was left untreated as control. Labeled treated fruits and the control fruit were introduced into glass cages and fixed with suction cups to the cages' roofs. Each cage was provided with ten pairs of *Bactrocera invadens* (males and females), food and water, the same procedures were applied for Guava. For each of the mango and guava treatments, Four glass cages were used as replicates as shown in **Plate No. 14** and **Plate No. 15**.

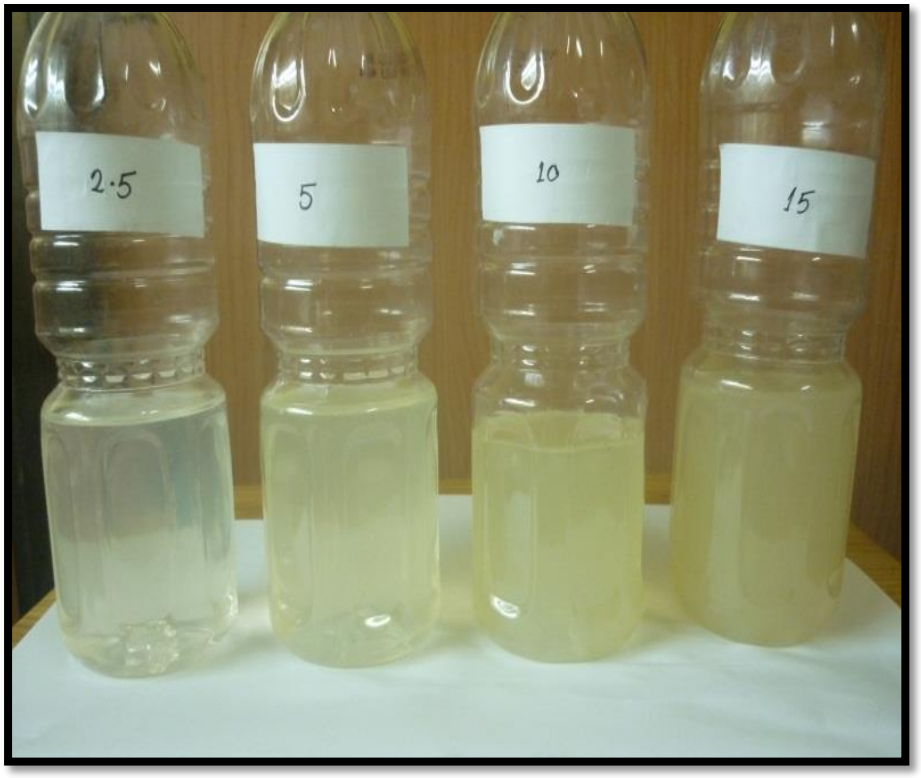


Plate No. (13) Concentrations of Natural HMP Extract

Plate No. (14) Laboratory Experiments

(Bioassay Tests of HMP Extract on Mango Fruits)

Plate No. (15) Laboratory Experiments

(Bioassay Tests of HMP Extract on Guava Fruits)

3.2. 3. 2 Semi-Field Experiments:

The pheromone containing materials (i.e., the Stock solution) extracted and tested in the laboratory were also tested in a semi-field conditions.

The tests were conducted under natural conditions of mango (*Mangifera indica*) and guava (*Psidium guajava*) orchards at Al-kadaroo and AlFaki-Hashim . Different concentrations of the natural pheromonal extracts were applied for treatment of mango and guava fruits using small Hand Sprayer (**Plate No. 16**).

For each Semi-Field Treatment, a wire frame, covered with fine net **(Plate No. 17)** was used to cover the treated and untreated mango and guava fruits and was provided with ten pairs of *Bactrocera invadens* (males and females), in addition to food and water. Each frame was considered as a replicate.

Mango frames were hanged on trees in a mango orchard , and guava frames were hanged on trees in a guava orchard (**Plates No. 18 & 19**).

All Fruits of mango and guava from laboratory and semi-field Experiments were checked every day for oviposition up to 7 days, and then, they were transferred into plastic cages for another 7 days before dissection **(plate 20 &21)** , to count the number of larvae in the pulp, so as to check the deterrent effect of the pheromone.

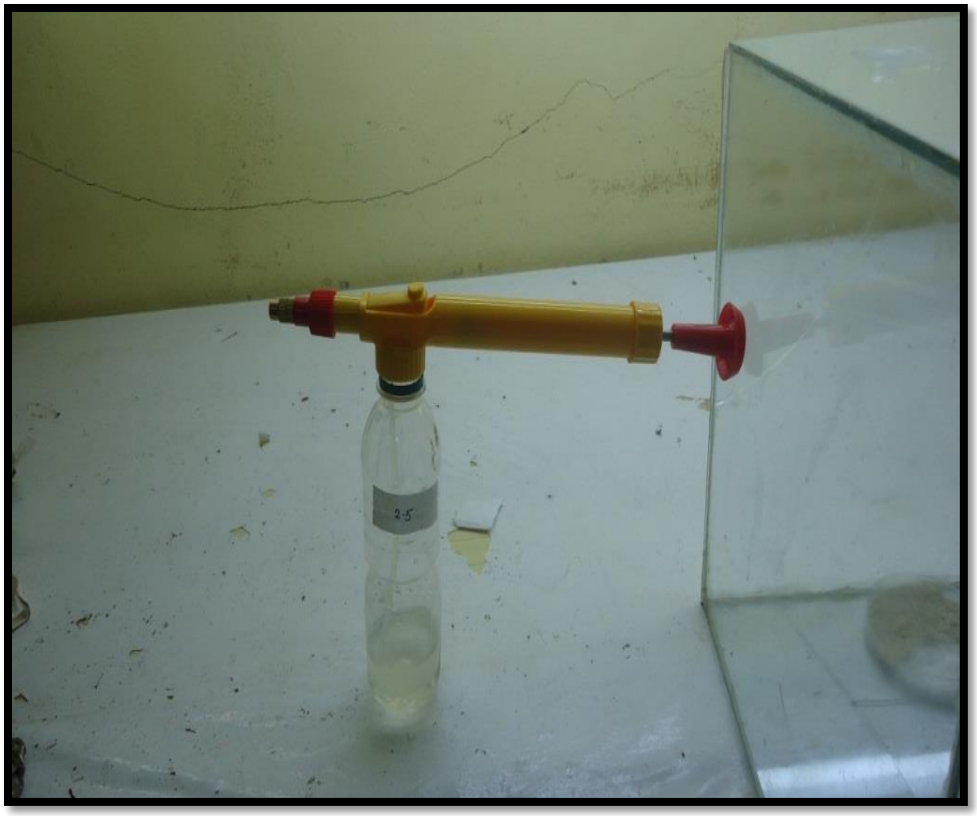


Plate No. (16) Small Hand sprayer

Plate No (17) Wire Frame and Fine Net

Plate No. (18) Semi - Field Experiments at a Mango Orchard
(Bioassay Tests of HMP Extract on Mango Fruits)

Plate No. (19) Semi - Field Experiments at a Guava Orchard
(Bioassay Tests of HMP Extract on Guava Fruits)

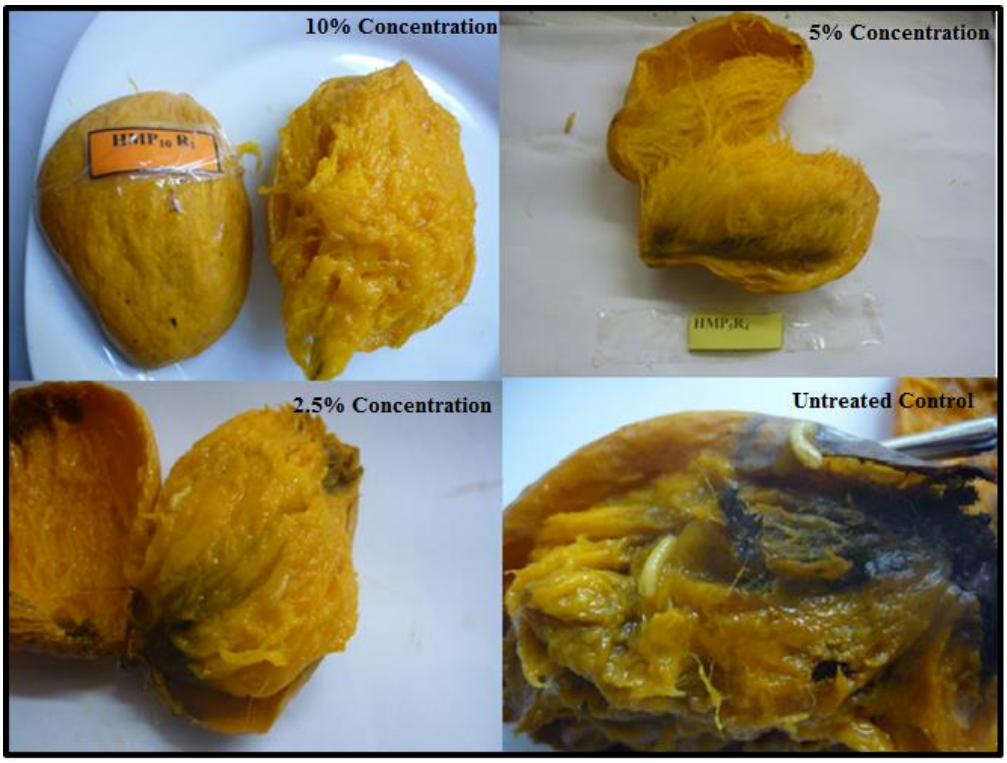


Plate No. (20) Dissected Mango Fruits

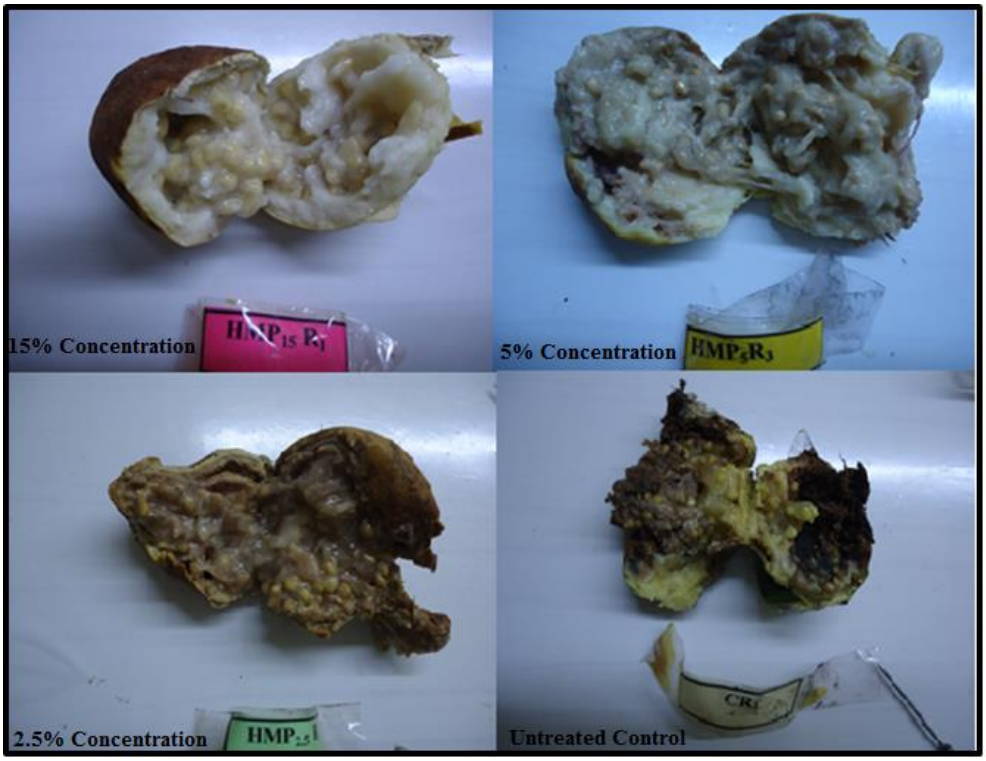


Plate No. (21) Dissected Guava Fruits

3. 2. 4 Extraction, Purification for Structural Elucidation of Host Marking Pheromone (HMP):

The main experiments of this section were made at " **JICA Laboratory** " at the College of Agricultural Studies – Shambat, Sudan University of Science and Technology. In addition, more advanced Chemical Analysis for Natural Host Marking Pheromone was carried out at **"The Chemical Laboratories"**, University of Medical Sciences and Technology, in Khartoum.

For these experiments, the following equipments and materials were used:

1. Glass rearing cages (30×30×30 cm).
2. All facilities for the tested insect rearing, including food (sucrose and hydrolyzed protein).
3. Metal spatula for fruit fly faeces collection.
4. Plastic bottles for keeping collected faeces.
5. Glassware (Petri- dishes, Measuring Cylinders, Pipettes, Funnels, Flasks, Beakers, Glass stirring rods, and Laboratory Bottles) and filter papers to use in preparing faeces solution.
6. Absolute Methanol and Trifluoro-acetic acid as a solvent to prepare faeces extract.
7. Magnetic Stirrer and Hot Plate Stirrer for extract shaking.
8. Centrifuge to isolate active liquid from the solids.
9. Rotary Evaporator to concentrate the liquid so as to provide stock solution.
10. A Thermal Desorber for desorption of biological samples.
11. Gas Chromatography (GC) coupled with Mass Spectrometry
12. High Performance liquid chromatography (HPLC)
13. Electrophoresis (11-13 for structural determination).
16. Plastic cards, Rubber bands and Permanent ink Markers for experiment labeling.

17. Electronic Sensitive Balance for weighing.

18. Indoor/Outdoor Thermo -Hygrometer to measure temperature and relative humidity in the laboratory.

19. Timer to control the photoperiod.

3. 2. 4. 1 Experiments carried out at" JICA Laboratory "at the College of Agricultural Studies – Shambat:

3. 2. 4. 1. 1 Extraction and Purification:

Rearing to get " more faeces " for structural elucidation was done all through the year 2013, to provide enough amounts of faeces, using the same methods mentioned above.

Extraction took place in March 2014 at " **JICA Laboratory** ", **Aluja *et al*., (2003)** methods were adopted. 167 g of fly faeces were suspended in 5L of Ethanol and stirred for 17 hrs at room temperature. The solid material was filtered off, rinsed with 1L of Ethanol and the extraction procedure was then repeated once with 2L of Ethanol containing 3.5 ml Trifluoro - acetic acid. The combined Ethanol extracts were concentrated on a rotary evaporator at 50 °C and 20 mbar to almost dryness. After 6 hrs of lyophilization, the residue (33.8 g) was dissolved in 300 ml Methanol at 50 °C and cooled down to room temperature. After 2 hrs, the precipitated fat (10.5 g) was filtered off and rinsed with Methanol **(Plate No. 22)**. The solution then evaporated to dryness using Rotary evaporator (**Plate No. 23)** giving 23.3 g of a residue used for preparative of " High Performance Liquid Chromatography " (**HPLC).**

Plate No. (22) Filtering off and Rinsing

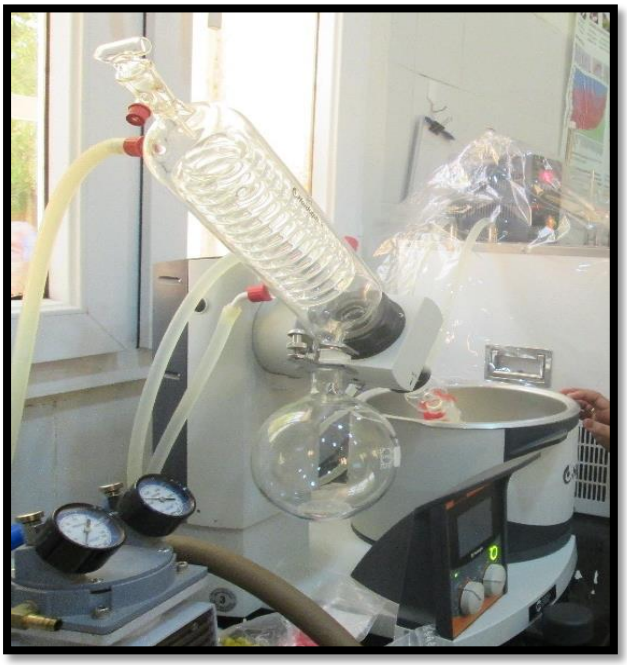


Plate No. (23) Rotary evaporator

3. 2. 4. 1. 2 Structural Elucidation:

After injection of the extracted and purified natural pheromone in **HPLC** column, The fractions were collected according to peak development of chromatograph. UV- Detection: 220nm. Electro – physiological activity 47-60 min. and UV – Detection: 200nm. Electro - physiological activity 47-60 min

3. 2. 4. 2 Experiments of Chemical Analysis at the Chemical Laboratories, University of Medical Sciences and Technology:

In these Laboratories, Other samples were injected in the " Gas Chromatography-Mass Spectrometry (GC.MS) ", using mass spectrometer, column: Rtx-5MS, Length (30 m), Diameter (0.25mm) and thickness (0.25µl), and carrier gas Helium.

3.3 Evaluation of fruit fly traps:

3.3.1 Study Sites:

This part of the study took place at Al-Halfaia, Al-kadroo, Al-khoglab and Elfaki-Hasim (**Figure No. 4**), in order to investigate the main reasons of the fruit fly problems at the study sites.

3.3. 2 Sex Pheromone Traps Catchability:

These experiments were carried out at:

1- Al-kadroo and Elfaki-Hasim, from June to July 2016.

2- Al-kadroo and Al-khoglab, from February to March 2017.

Lynfield Traps were manufactured locally, using a plastic bottle with four holes and a piece of gauze, cylindrical in shape, with a diameter of 1-2cm and length of 4 cm, attached to the cover of the trap (**Plate No. 24 A & B**) and (**Appendix No. 43**). Methyl Eugenol was mixed with the pesticide malathion (57%) at a ratio of 4: 1, respectively, and then each trap was baited with 3 ml of that solution, and hung by a wire on a shady tree branch, approximately 2 meters above the ground. The position of each trap was fixed throughout the study period and checked every week for about a month which is the period of bait activity. Five traps were used at every site.

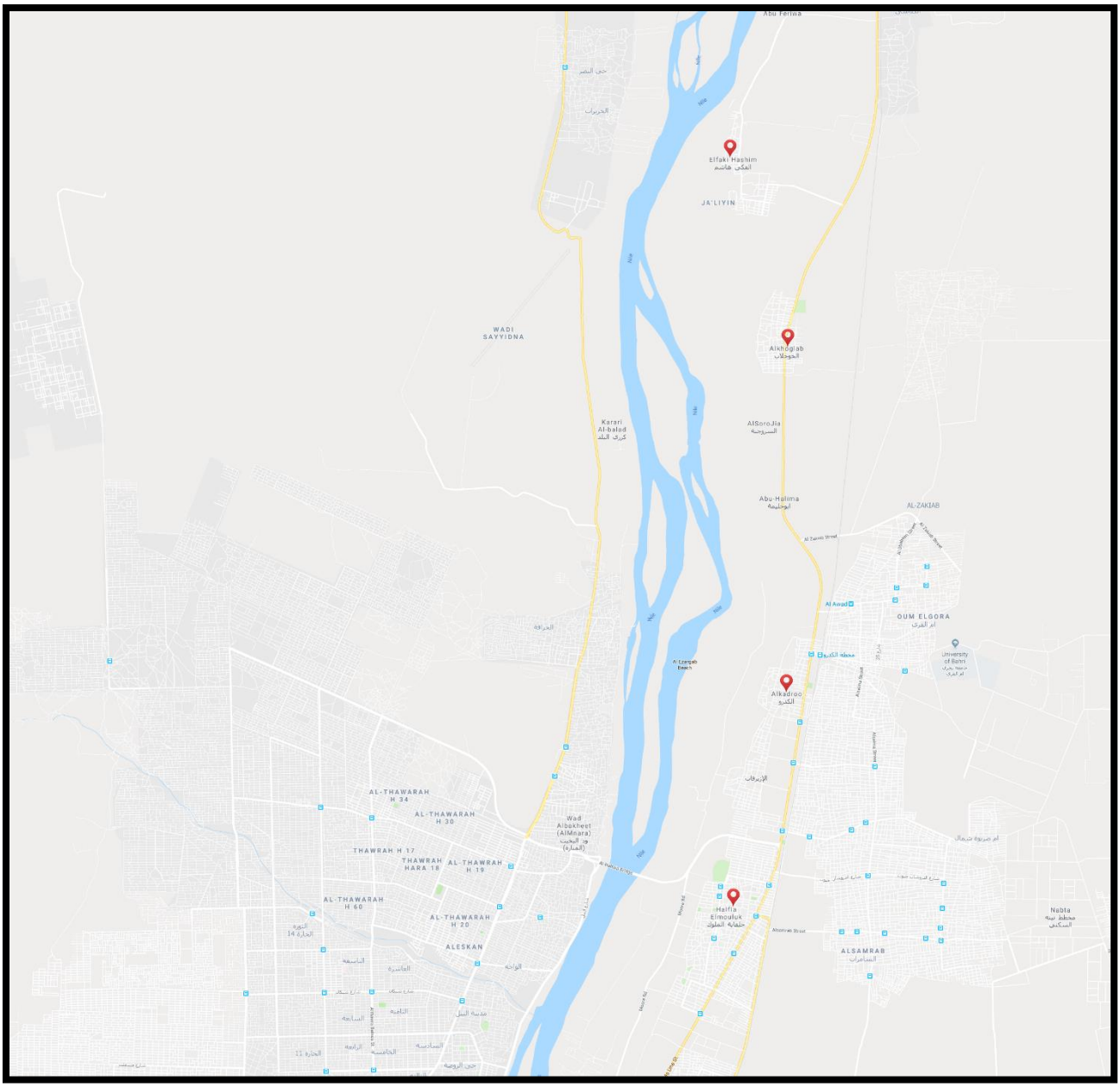


Figure (4) Evauation of fruit fly traps study sites

A

B

Plate No. 24 (A & B) locally Manufactured Lynfield Trap

3 .3. 3 Evaluation of Different Traps Efficacy:

The same Lynfield locally manufactured traps were used for these experiments in Al-halfaia , from May to June 2017, 4 traps for each treatment. Treatments were as follows:

1. Locally manufactured Lynfield trap, baited with 3 ml of Methyl Eugenol mixed with pesticide Malathion (57%) at ratio of 4: 1, respectively.
2. Locally manufactured Lynfield trap, baited with 3 ml of Methyl Eugenol mixed with pesticide Cypermethrin at ratio of 4: 1, respectively.
3. Locally manufactured sticky trap (**Plate No. 25**), baited with 3ml of pure Methyl Eugenol.
4. Locally manufactured Lynfield trap, baited with 3 ml of Methyl Eugenol mixed with pesticide Malathion (57%) at ratio of 4: 1, respectively, with added Guava fruit juice to the bottom of the trap.
5. Locally manufactured Lynfield trap, baited with 3 ml of Methyl Eugenol mixed with pesticide Cypermethrin at ratio of 4: 1, respectively, with added Guava fruit juice to the bottom of the trap.

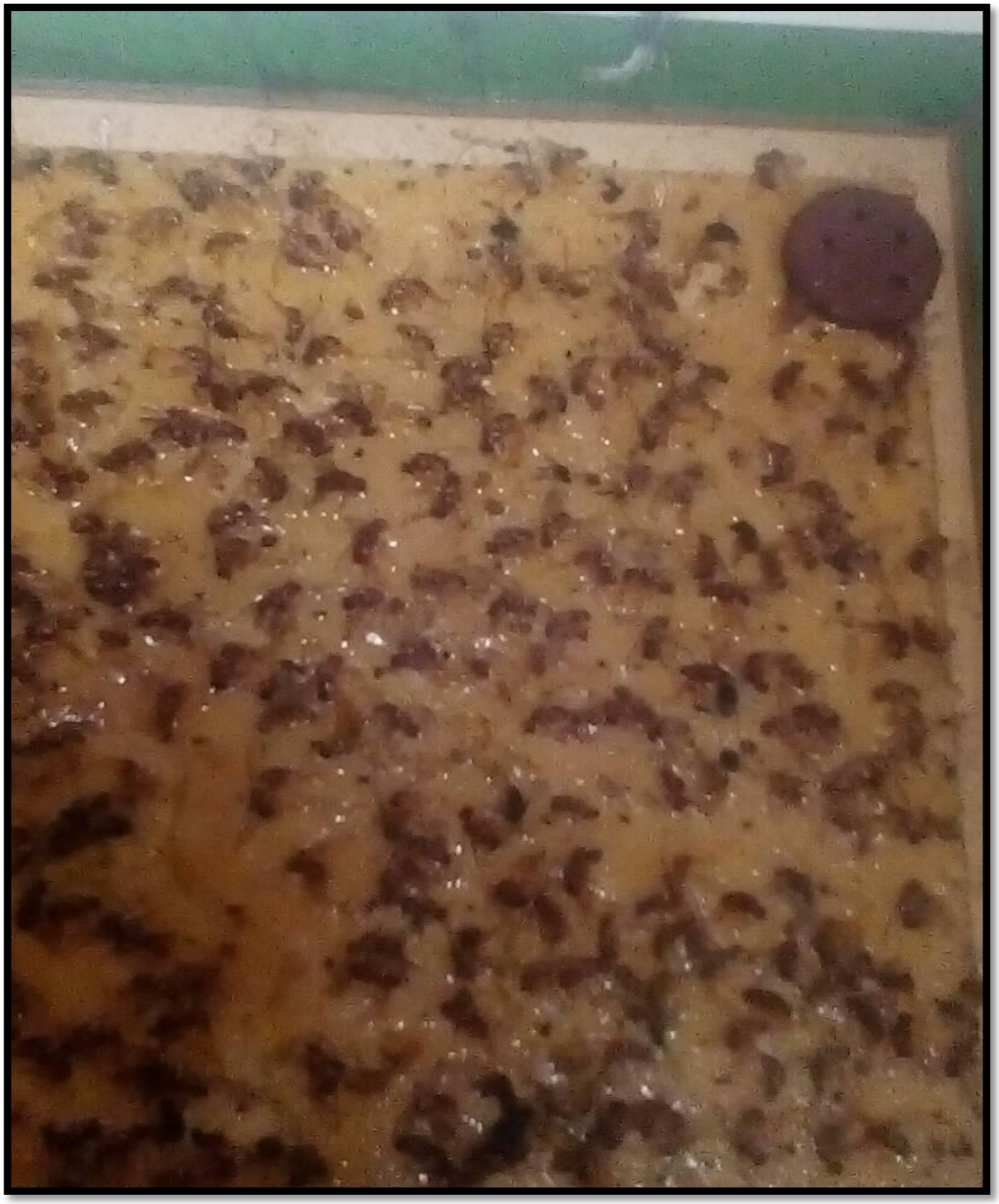


Plate No. 25 A Sticky Trap

3 .3. 4 Evaluation of Efficacy of Two Types of Locally Manufactured Traps:

For this experiment, two types of locally manufactured traps were used, and with 5 replicates for each:

1. Locally manufactured Lynfield trap, baited with 3 ml of Methyl Eugenol mixed with pesticide Malathion (57%) at ratio of 4: 1, respectively.

2. 2 Liter Plastic Bottle, with the top of the bottle cut and fixed to the bottle , upside down with a piece of gauze, cylindrical in shape, with a diameter of 1-2cm and length of 4 cm, attached to the bottom of the bottle by a thin wire, (**Plate No.26**), baited with 3 ml of Methyl Eugenol mixed with pesticide Malathion (57%) at a ratio of 4: 1, respectively.

The experiments were carried out at Al-kadroo site from Septemper to Octoper 2017.

3. 3. 5 Species Monitoring Using Different Sex Pheromones:

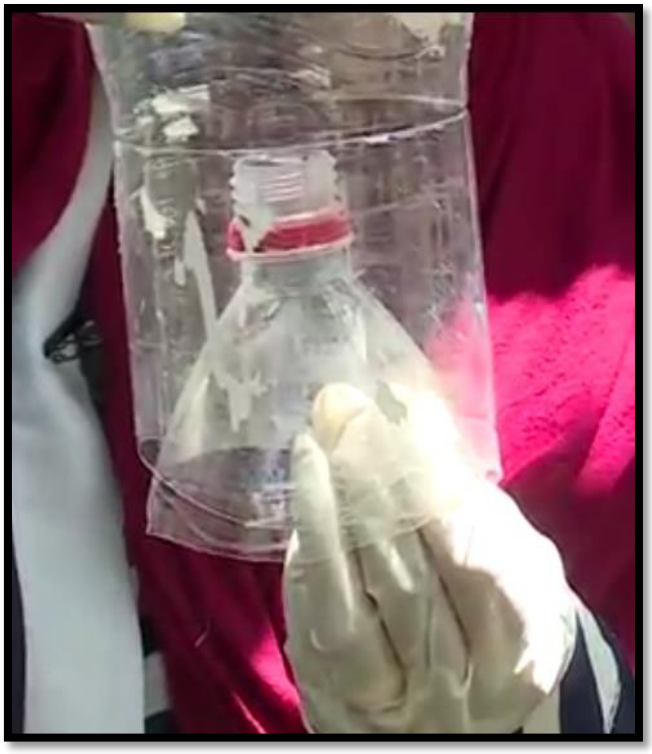
This study was carried out in 3 different farmers' orchards of guava in Al-kadaroo through May 2018, using Tephri-Traps (**Plate No. 27**) baited with 4 types of Sex Pheromones:

1. Methyl Eugenol, 2 grams plug, (**Plate No. 28**).
2. Cuelure, 2 grams plug, (**Plate No. 29**).
3. Trimedlure, 2 grams plug, (**Plate No. 30**).
4. Tryterpenile Acetate, 3ml.

Four traps were used as replicates, and every trap was provided with stripes of the pesticide.

3. 4 Statistical analysis:

1- Randomized Block Design (RBD) for laboratory experiments.
2- A complete random block Design (CRBD) for field experiments
3- ANOVA to analyze the laboratory and semi-field experiments.



A

B

Plate No. 26 A & B 2 Litter Plastic Bottle Trap

Plate No. 27 Tephri-Trap

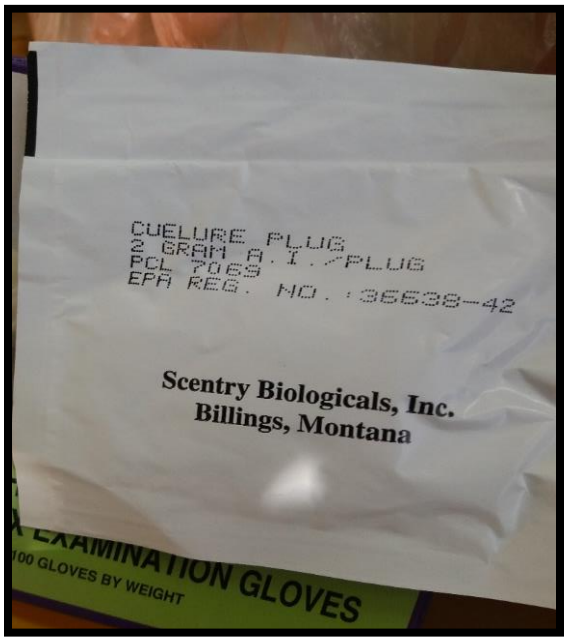


Plate No. 28 Methyl Eugenol Plugs

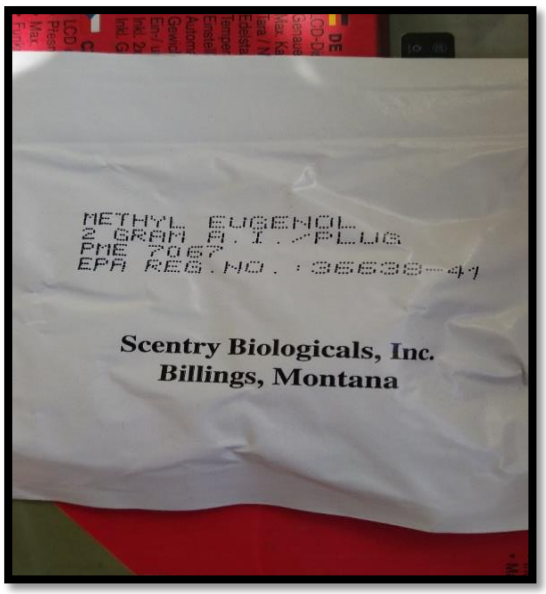


Plate No. 29 Cuelure Plugs

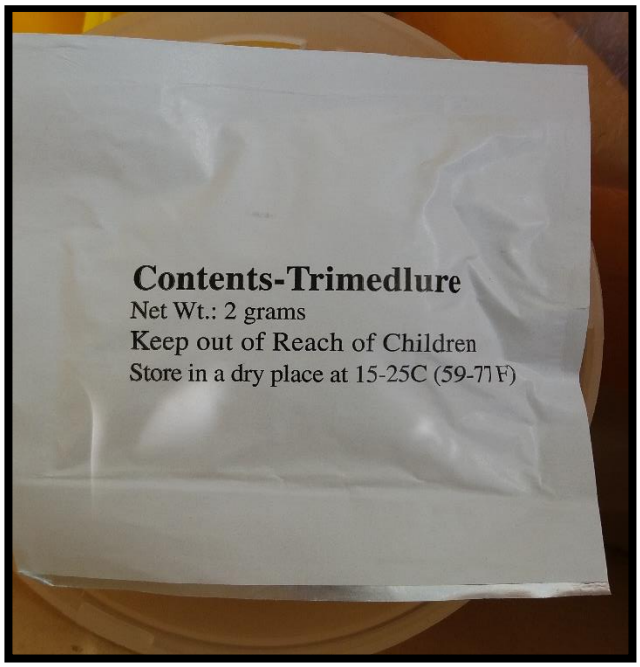


Plate No. 30 Trimedlure Plugs

CHAPTER FOUR

RESULTS

4. RESULTS

4.1. Species Identification:

Results of the specific identification of the fruit flies emerged from collections made from the different sites, during the years 2010 and 2011, and determination of the predominant species are shown in **Tables No. 1, 2 and 3, Appendices 1, 2 and 3 and Fig 5,6 and 7**.

Results of the Identification indicated that, four fruit fly species, belong to the Family Tephritidae, were found in all sites of the Three States. These include: 1. the Asian fruit fly, *Bactrocera invadens* (Drew), 2. Mango fruit fly, *Ceratitis cosyra* (Walker), 3. The Mediterranean fruit fly, *Ceratitis capitata* (Wiedmann) and 4. The Rhodesian fruit fly, *Ceratitis quinaria* (Bezzi)

Also, these results showed that, the Asian fruit fly, *Bactrocera invadens* was found to be the dominant species in all sites during the study periods, attacking mango and guava, followed by *C. cosyra,* associated with mango, while *C. quinaria* and *C. capitata*, were found associated mainly with guava and in small number with mango **(fig No. 8, 9,10,11,12,13 & No. 14)**.

Table No. 1 Mean percentage (%) of fruit fly species emerged from fruits collected from the different sites (17th of February to 20th of March 2010)

Site	Mean % B. invedens	SD	Mean % C. cosyra	SD	Mean % C. capitata	SD	Mean % C. quinaria	SD
Bahri Central Market (Guava)	58±4.73	8.19	5±1.15	2	27.67±1.20	2.08	9.33±5.39	5.03
Elkadaro (Guava)	60.67±2.73	4.73	10±0.58	1	22.67±2.19	3.79	6.67±3.85	3.06
Elfaki Hashim (Guava)	83.67±2.03	3.52	15±2.89	5	0±0	0	1.33±0.77	1.53
Khartoum Central Market (Guava)	82±3.46	6	5±2.89	5	13±0.58	1	0±0	0
Kasala (Guava)	96.67±1.33	2.31	0±0	0	3.33±1.33	2.31	0±0	0
Singa (Mango)	91.33±1.85	3.21	7±1	1.73	1.67±0.88	1.53	0±0	0
Singa (Guava)	84.67±6.49	11.24	3.33±1.67	2.89	12±4.93	8.54	0±0	0
Sennar (Mango)	91.33±2.33	4.04	6.67±1.67	2.89	2±1	1.73	0±0	0

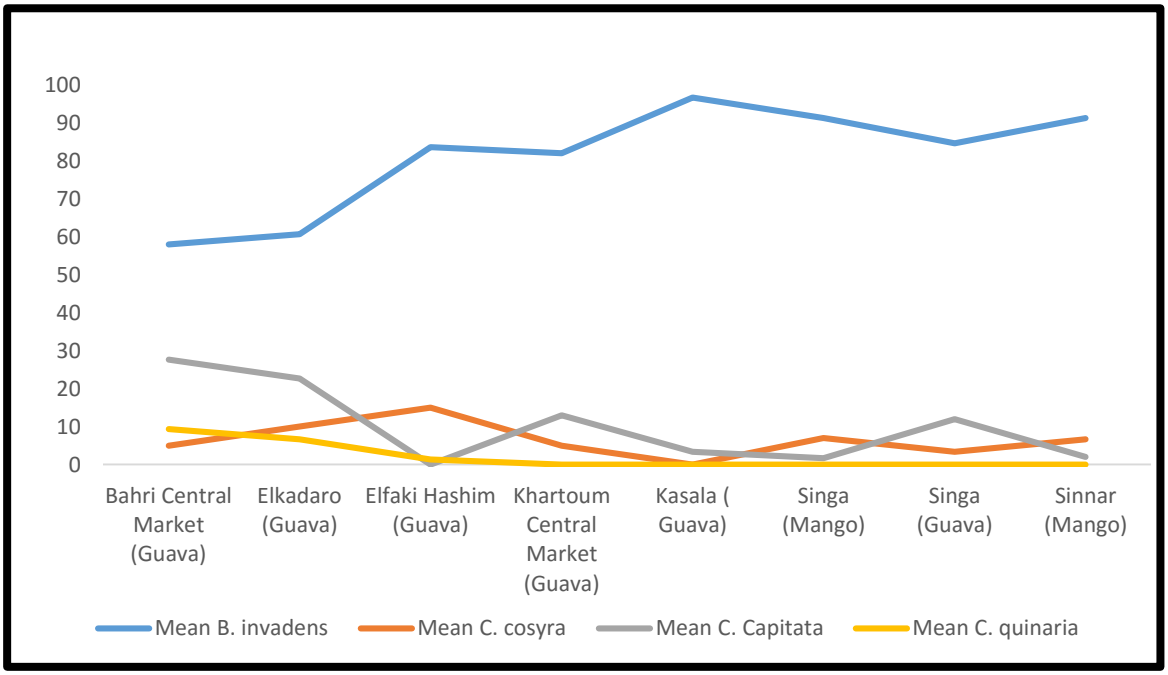


Figure (5) Mean percentage of fruit fly species emerged from fruits collected from different sites (17th of February to 20th of March 2010)

Table No. 2 Mean percentage (%) of fruit fly species emerged from fruits collected from the different sites (19th of October to 23 of November 2010)

Site	Mean % *B. invedens*	SD	Mean % *C.* cosyra	SD	Mean % *C. capitata*	SD	Mean % *C. quinaria*	SD
Bahri Central Market (Guava)	81.67±1.76	3.06	0±0	0	13±1.53	2.65	5.33±0.88	1.53
Elkadaro (Guava)	83±4.93	8.54	0±0	0	13±4.16	7.21	4±1	1.73
Elfaki Hashim (Guava)	81±2.65	4.58	15±4.04	7	0±0	0	4±2.08	3.61
Khartoum Central Market (Guava)	88.33±3.84	6.66	0.67±0.67	1.15	10.67±4.41	7.64	0.33±0.33	0.58
Kasala (Guava)	91.33±2.03	3.51	0±0	0	6.67±1.67	2.89	2±1.15	2
Singa (Guava)	84±2.89	5	1±0.58	1	14±4.16	7.21	1±1	1.73
Sennar (Guava)	91.33±3.48	6.03	1±1	1.73	7.67±3.71	6.43	0±0	0

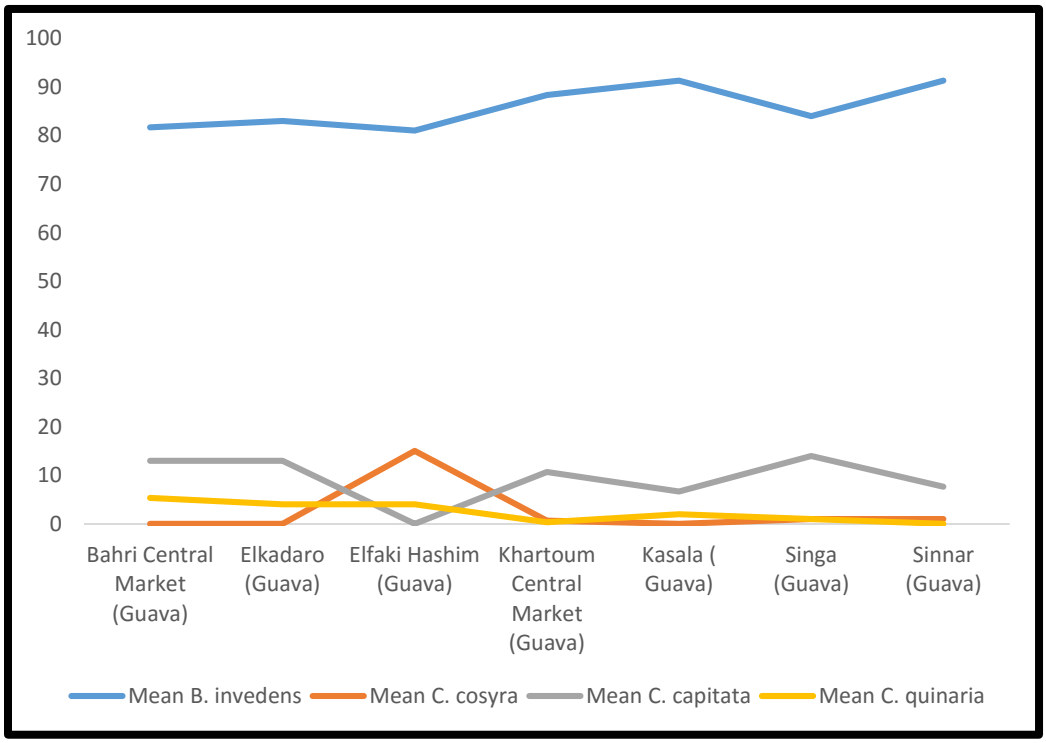


Figure (6) Mean percentage of fruit fly species emerged from fruits collected from different sites (19th of October to 23 of November 2010)

Table No. 3 Mean percentage (%) of fruit fly species emerged from fruits collected from the different sites (15th of February to 19th of March 2011)

Site	Mean % B. invadens	SD	Mean % C. cosyra	SD	Mean % C. capitata	SD	Mean % C. quinaria	SD
Bahri Central Market (Guava)	82.33±6.44	11.15	0±0	0	7±3.61	6.24	7.33±3.38	5.86
Elkadaro (Guava)	86.67±5.04	8.74	0.33±0.33	0.58	7.33±1.45	2.52	2.33±1.20	2.08
Elfaki Hashim (Guava)	88.33±2.03	3.51	0.67±0.67	1.16	8±0.58	1	3±1.15	2
Khartoum Central Market (Guava)	87.67±7.88	13.65	0.33±0.33	0.58	6.33±3.38	5.86	5±5	8.66
Kasala (Guava)	92.33±4.81	8.33	0±0	0	7±4.16	7.21	0.67±0.67	1.15
Singa (Mango)	88.33±3.84	6.66	9.33±3.48	6.03	2.33±1.20	2.08	0±0	0
Singa (Guava)	88.33±8.69	15.04	0±0	0	11.67±8.69	15.04	0±0	0
Sennar (Mango)	89.67±1.76	3.06	9±1.15	2	1.33±1.33	2.30	0±0	0

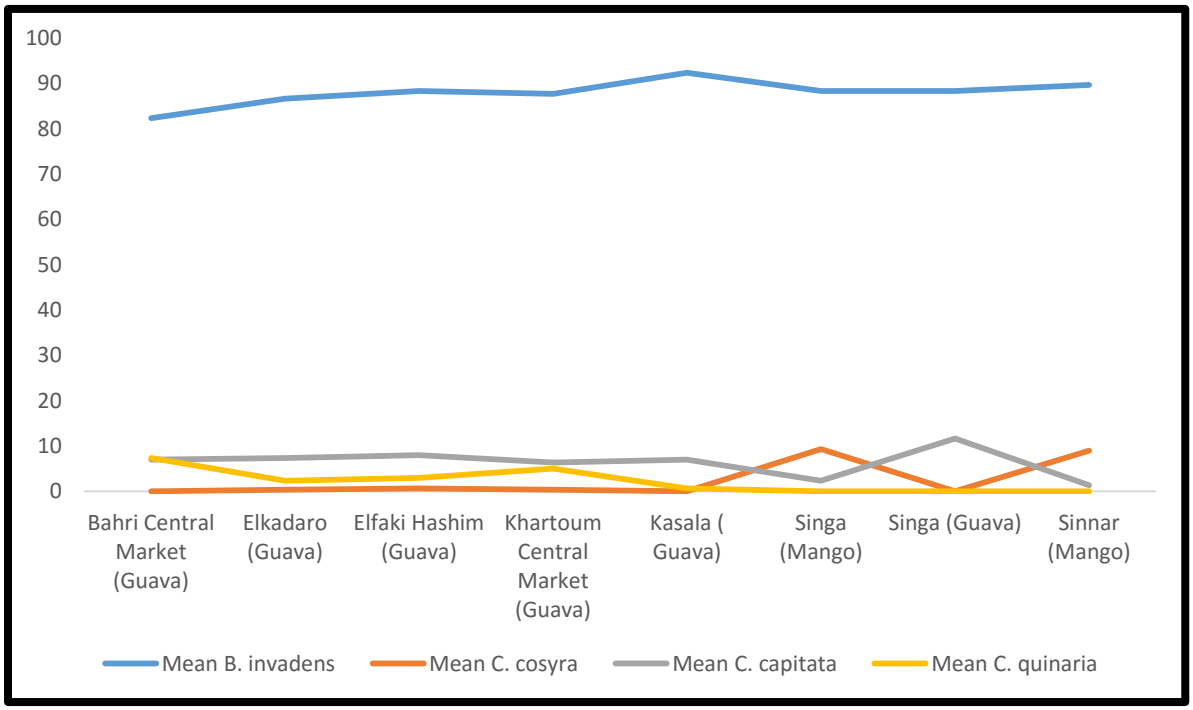
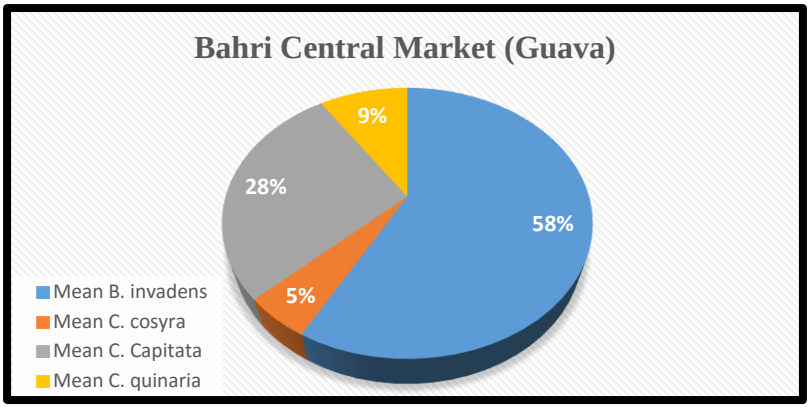
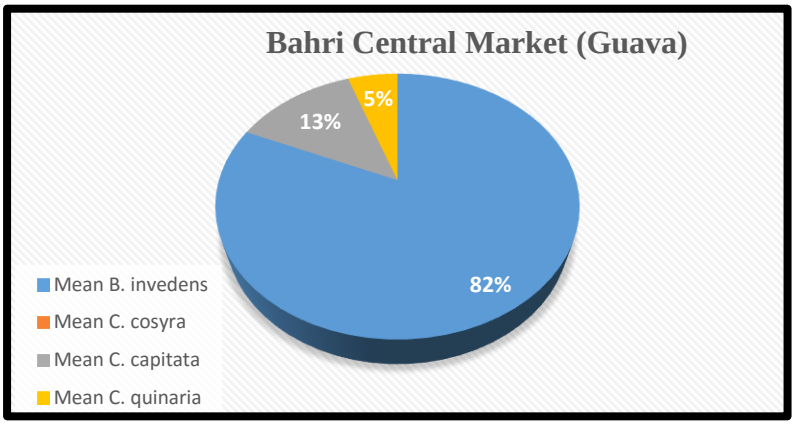


Figure (7) Mean percentage of fruit fly species emerged from fruits collected from different sites (15th of February to 19th of March 2011

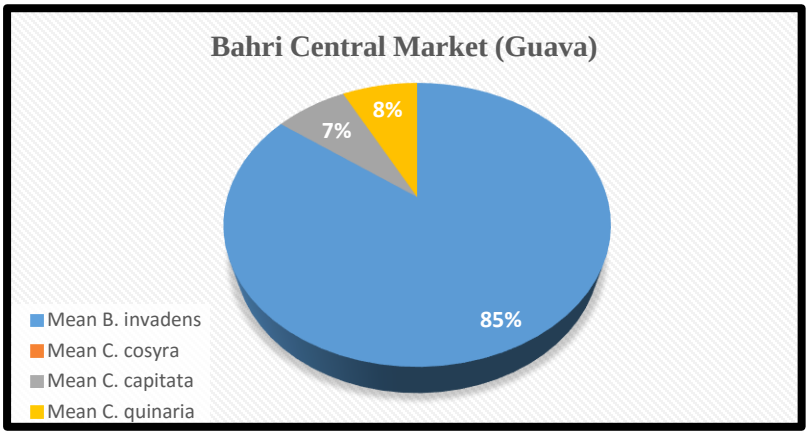
Figure (8) Mean percentage of fruit fly species emerged from Guava fruits collected from Bahri Central Market



Period (A) from 17th of February to 20th of March 2010

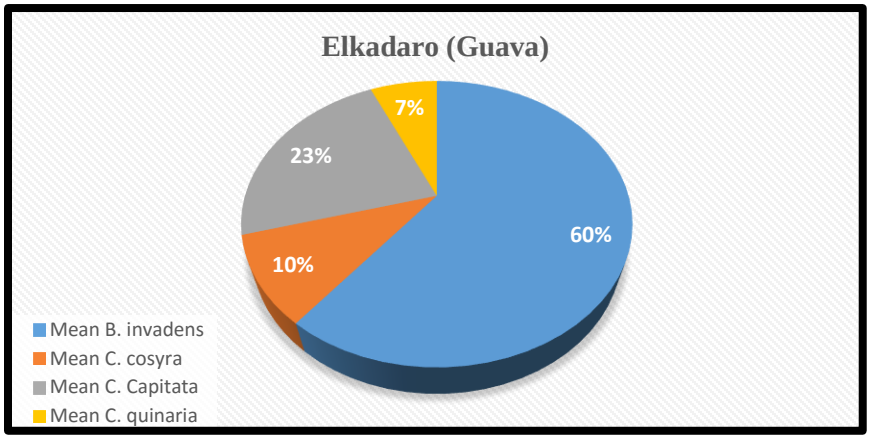


Period (B) from 19th of October to 23 of November 2010

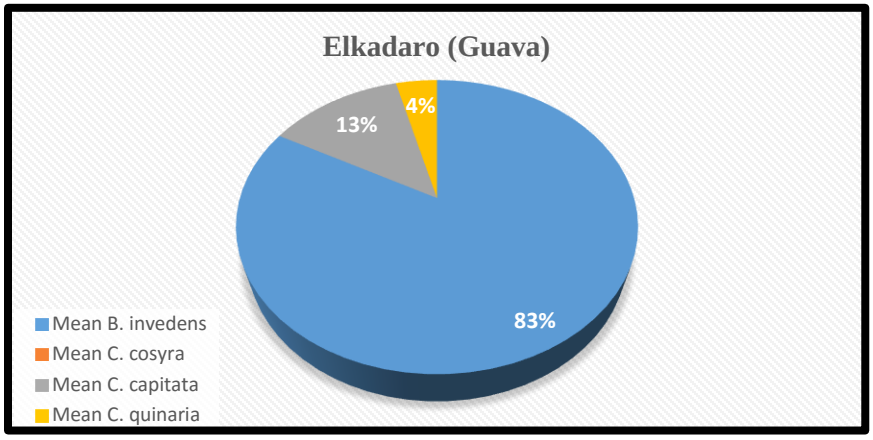


Period (C) from 15th of February to 19th of March 2011

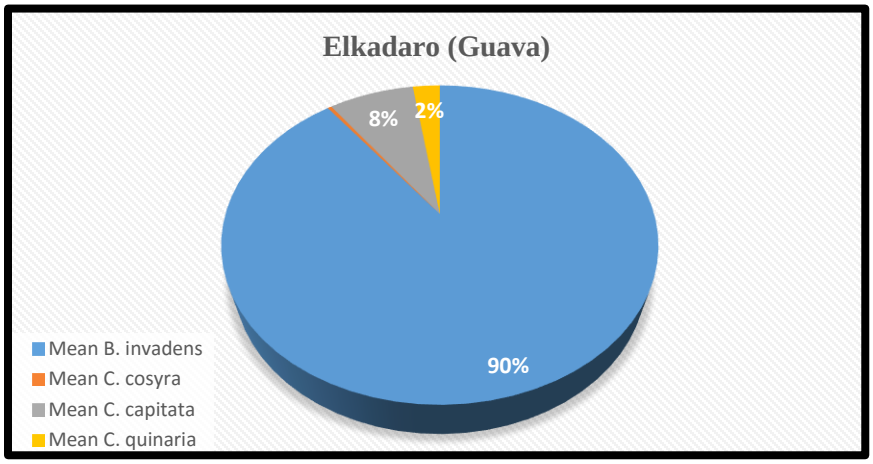
Figure (9) Mean percentage of fruit fly species emerged from Guava fruits collected from Elkadaro



Period (A) from 17th of February to 20th of March 2010

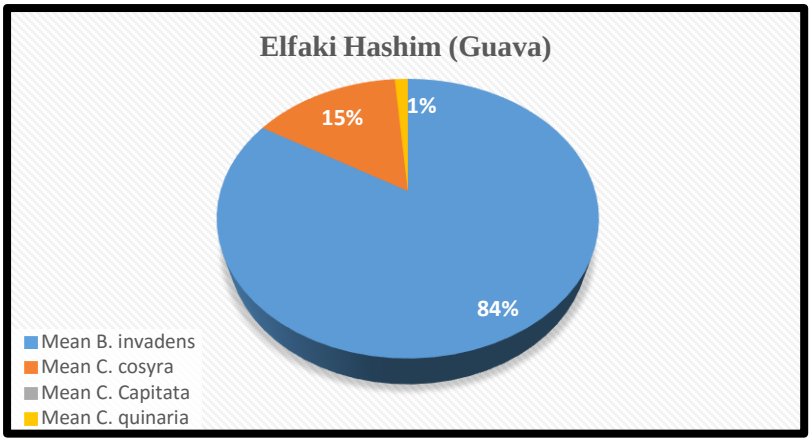


Period (B) from 19th of October to 23 of November 2010

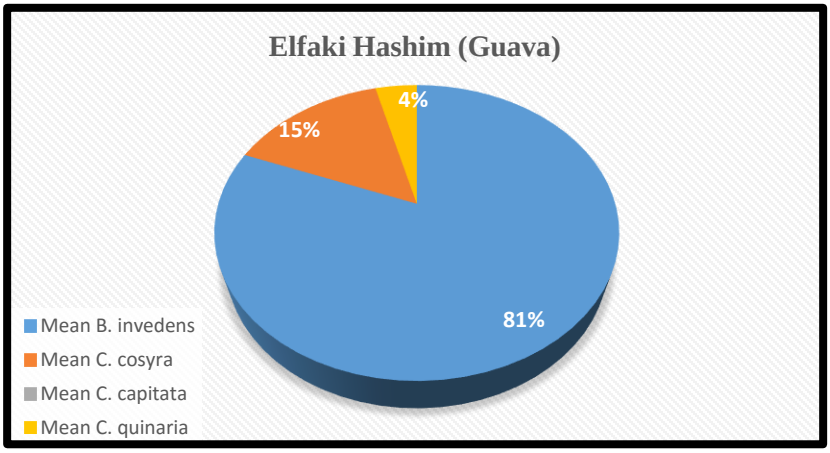


Period (C) from 15th of February to 19th of March 2011

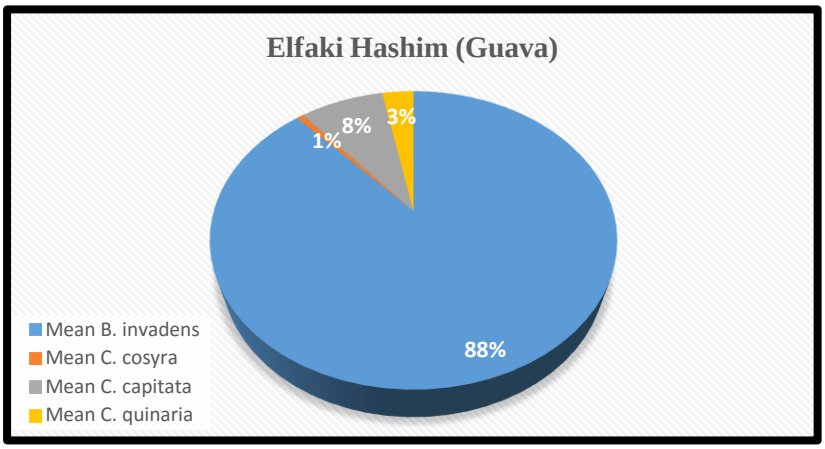
Figure (10) Mean percentage of fruit fly species emerged from Guava fruits collected from Elfaki Hasim



Period (A) from 17th of February to 20th of March 2010

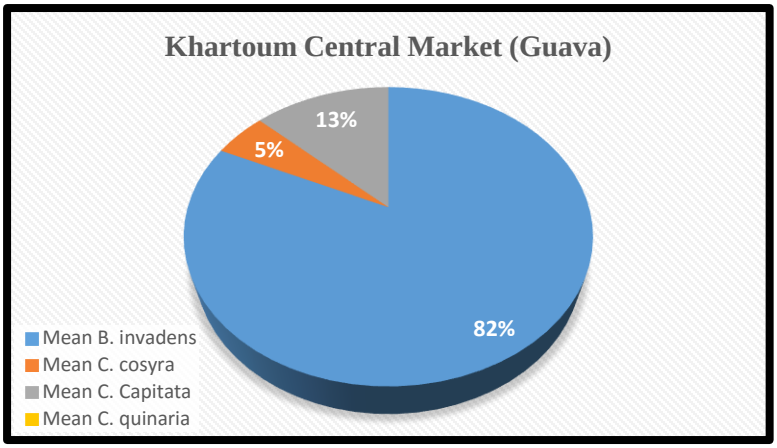


Period (B) from 19th of October to 23 of November 2010

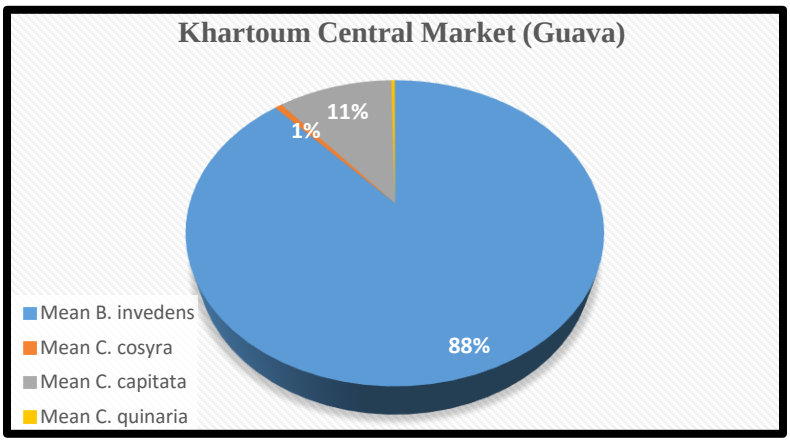


Period (C) from 15th of February to 19th of March 2011

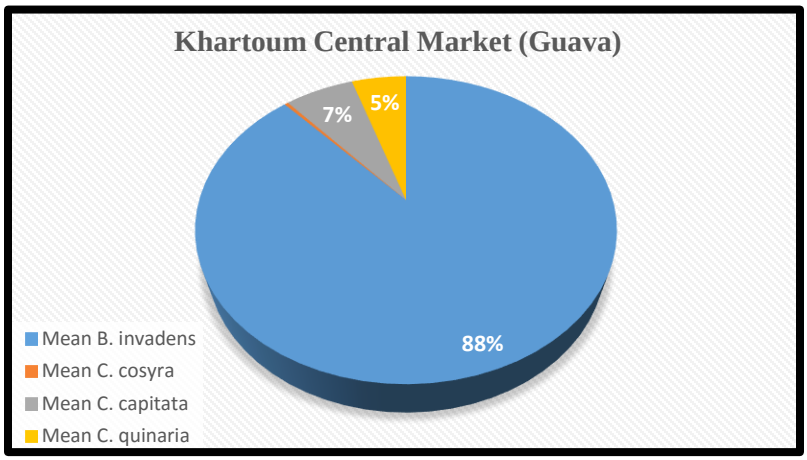
Figure (11) Mean percentage of fruit fly species emerged from Guava fruits collected from Khartoum Central Market



Period (A) from 17th of February to 20th of March 2010

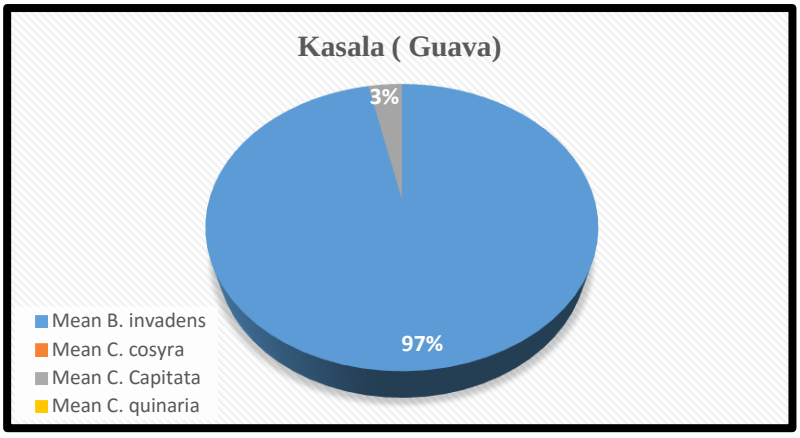


Period (B) from 19th of October to 23 of November 2010

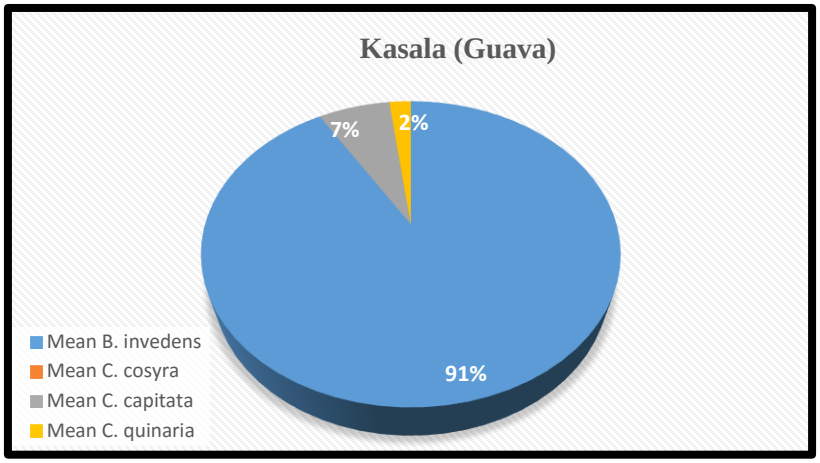


Period (C) from 15th of February to 19th of March 2011

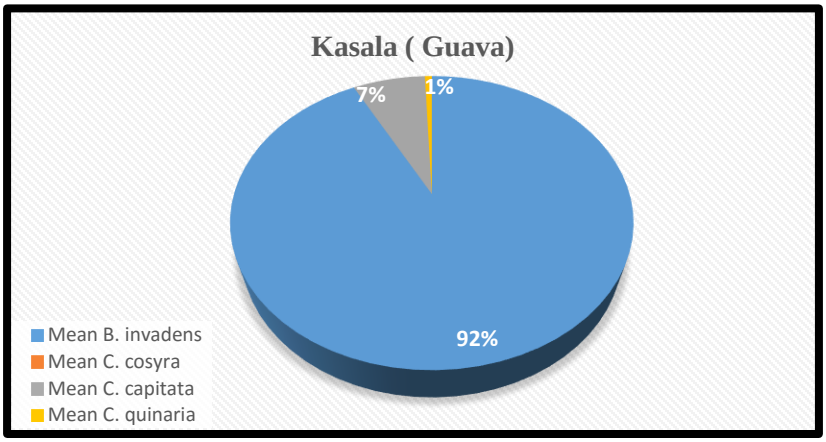
Figure (12) Mean percentage of fruit fly species emerged from Guava fruits collected from Kasala



Period (A) from 17th of February to 20th of March 2010

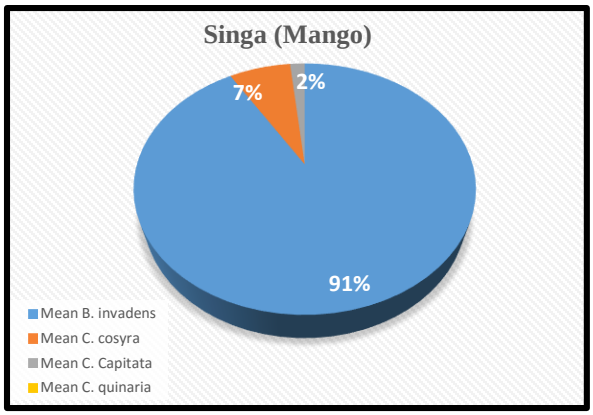


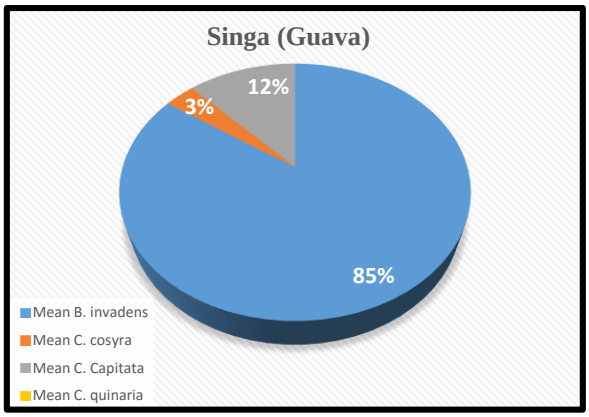
Period (B) from 19th of October to 23 of November 2010



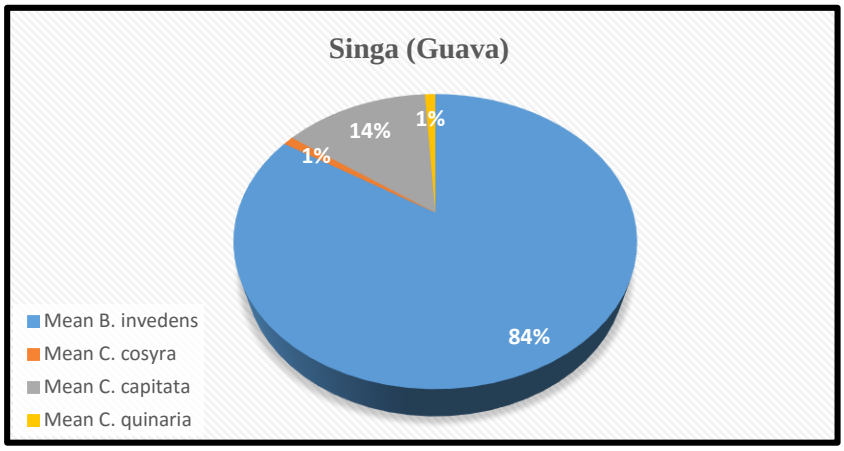
Period (C) from 15th of February to 19th of March 2011

Figure (13) Mean percentage of fruit fly species emerged from Mango and Guava fruits collected from Singa

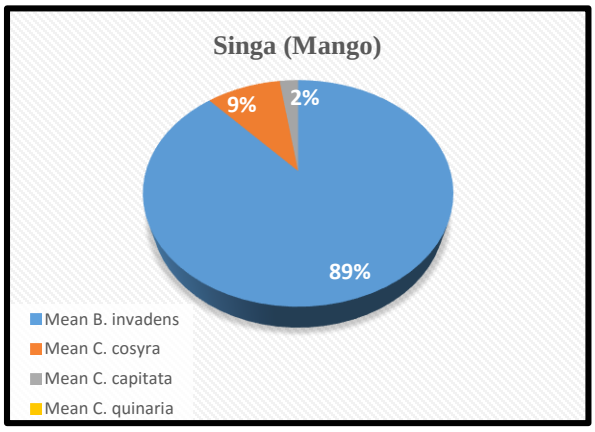


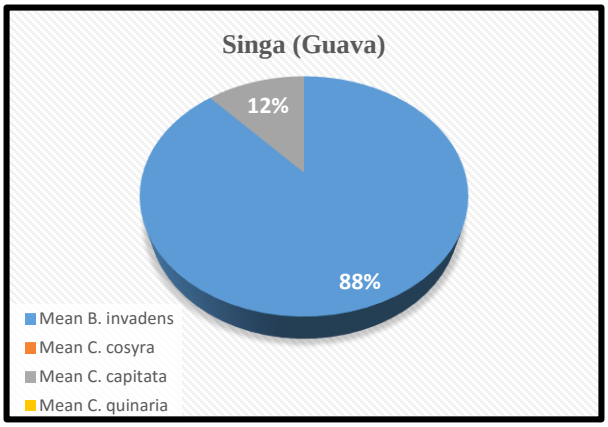


Period (A) from 17th of February to 20th of March 2010



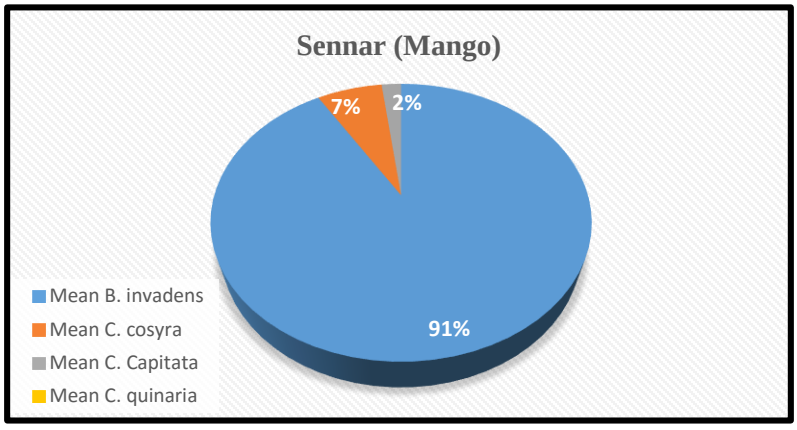
Period (B)from 19th of October to 23 of November 2010



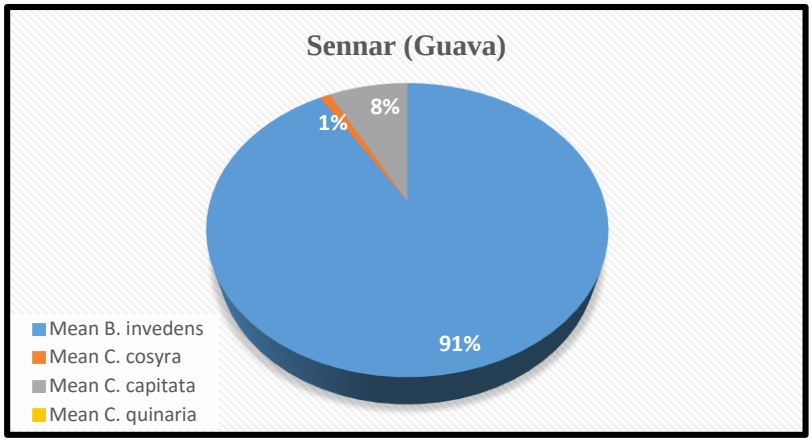


Period (C) from 15th of February to 19th of March 2011

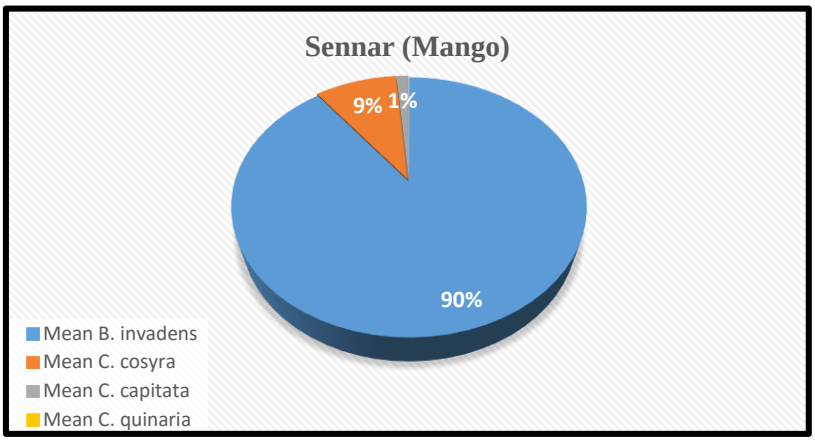
Figure (14) Mean percentage of fruit fly species emerged from Mango and Guava fruits collected from Sennar



Period (A) from 17th of February to 20th of March 2010



Period (B) from 19th of October to 23 of November 2010



Period (C) from 15th of February to 19th of March 2011

4.1.1 Characteristics of the Fruit Fly Species in the Study Sites:

4.1.1.1 The Asian fruit fly, *Bactrocera invadens* (Drew):

The main distinctive characters of the adults of *B. invadens* are:

scutum brown to black, but with high degree of variation from dark brown to complete black. Scutellum yellow with yellow lateral stripes, no medial stripes. Males with pectin, **Plate No. 31 and 32**.

4.1.1.2 The Mango fruit fly, *Ceratitis cosyra*:

The main distinctive characters of the adults of *Ceratitis cosyra* are:

C.cosyra female characterized by the three black areas in the apical half of the scutellum. In the male, orbital setae are not expanded at the apex, **Plate No. 33 and 34**.

4.1.1.3. The Mediterranean fruit fly *Ceratitis capitata*:

The main distinctive characters of the adults of *Ceratitis capitata* are**:**

C.capitata, female characterized by yellow wing pattern and the apical half of the scutellum being entirely black with wavy yellow band across the base of the scutellum. The males are characterized by the black pointed expansion at the apex of the anterior pair of the orbital setae, **Plate No. 35 and 36**. The pattern of grey flecks in the basal wing cells distinguishes *Ceratitis* spp. from most other genera of Tephritid fruit flies.

Also, More specific external characteristics of these species are shown in the Appendices **(e.g., Appendix No.4 , Appendix No.5 , Appendix No.6 , Appendix No.7 , Appendix No.8 and Appendix No.9)**.

4.1.1.4. The Rhodesian fruit fly, *Ceratitis quinaria*:

The main distinctive characters of the adults of *Ceratitis quinaria* are:

C. quinaria, like other Ceratitis spp., has banded wings, and a swollen scutellum which is marked yellow and black. The adult is similar to that of C. cosyra in that the males lack the spatulate frontal setae and feathered mid-tibia, and in

having only one an episternal seta. However, the black markings on the scutum and scutellum are very much smaller **Plate No. 9 and 10**.

4. 1.2 Species Abundance and Sex ratio:

According to the results shown in Tables 1 – 3, mean percentage adults of *Bacterocera invadens* emerged from rearing cages of the different fruits, always exceeds that of *C. cosyra*, C. *quinaria* and *C. capitata*. The sex ratio of the species ranged from 3:1- 4:1(female to male) in mango and guava for *B. invadens*, and 2:1-5:1 (female to male) in mango and guava for *C.cosyra, C.quinqaria* , and *C. capitata*, respectively **(Table No. 4)**.

Plate No. 31 The Asian Fruit fly, *Bactrocera invadens* (Female)

Plate No. 32 The Asian Fruit fly, *Bactrocera invadens* (Male)

Plate No. 33 The Mango fruit fly, *Ceratitis cosyra (*Female)

Plate No. 34 The Mango fruit fly, *Ceratitis cosyra* (Male)

Plate No. 35 The Mediterranean fruit fly *Ceratitis capitata* (Female)

Plate No. 36 The Mediterranean fruit fly *Ceratitis capitata* (Male)

Plate No. 37 The Rhodesian fruit fly, *Ceratitis quinaria* (Female)

Plate No. 38 The Rhodesian fruit fly, *Ceratitis quinaria* (Male)

Table No. 4 Mean number percentage and sex ratio of fruit fly species, reared out of Mango and Guava fruits collected from different Sites (17th of April – 20th of May 2010)

Insects		*B.invadens*		*C.cosyra*		*C.capitata*		*C. quinaria*	
Area	Crop	Species %	Sex ratio	Species %	Sex ratio	Species %	Sex ratio	Species %	Sex ratio
Bahri Central Market	Guava	51	4:1	5	4:1	30	5:1	14	4:1
Elkadaro	Guava	57	3:1	12	3:1	25	4:1	6	2:1
Elfaki Hashim	Mango	87	3:1	13	3:1	0	-	0	-
Khartoum Central Market	Guava	82	3:1	5	4:1	13	3:1	0	-
Kassala	Guava	94	4: 1	6	5:1	0	-	0	-
Singa	Mango	89	4: 1	11	5:1	0	-	0	-
Singa	Guava	97	4:1	0	-	3	2:1	0	-
Sinnar	Mango	75	3:1	12	3:1	13	3:1	0	-

4. 2.Tests of Feaces Extract Containing Natural Pheromone:

Treating of mango and guava fruits with different concentrations of Bacterocera *invadens* faeces phermonal extracts significantly reduced fruit infestation (number of larvae per fruit ($P < 0.0001$). The effectiveness of the faeces phermonal extract was surprisingly uniform.

4. 2. 1 Pheromones' Tests (First Season – 2011):

4. 2. 1. 1 Laboratory Experiments:

Effects of Different Concentrations of Natural Pheromone Extracts on *Bacterocera invadens* Oviposition in Mango (First Season – 2011)

The results shown in **Table No. 5, Figure No. 15**, **and Appendix No. 10 and 11** indicated that, effects of all concentrations of the extract were significantly different from the control.

Also, the results showed no significant difference between the lower concentrations (2.5% and 5%) and between the higher concentrations, 10% and 15%.

4. 2. 1. 2 Semi-Field Experiments:

Effects of Different Concentrations of Natural Pheromone Extracts on *Bacterocera invadens* Oviposition in Mango ((First Season – 2011)

These results are shown in **Table No. 6, Figure No. 16 and Appendix No. 12 and 13.** The results showed that, effects of all concentrations were significantly lower from the control.

There was a significant difference between concentrations (2.5% and 5%), while there was no significant difference between effects of the higher concentrations.

Table No. 5 Effects of Different Concentrations of Natural pheromone on *B. invadens* oviposition in mango

(Laboratory Experiment – Season 1)

Treatments	Mean No. of larvae	SD
2.5	16 b ± 0.23	0.82
5	12.5 b ± 0.54	1.91
10	0 c ± 0	0
15	0 c ± 0	0
Control	25.5 a ± 1.35	4.80

Means followed by the same letter are not significantly different at ($p \leq 0.0001$) According to LSD (3.52)

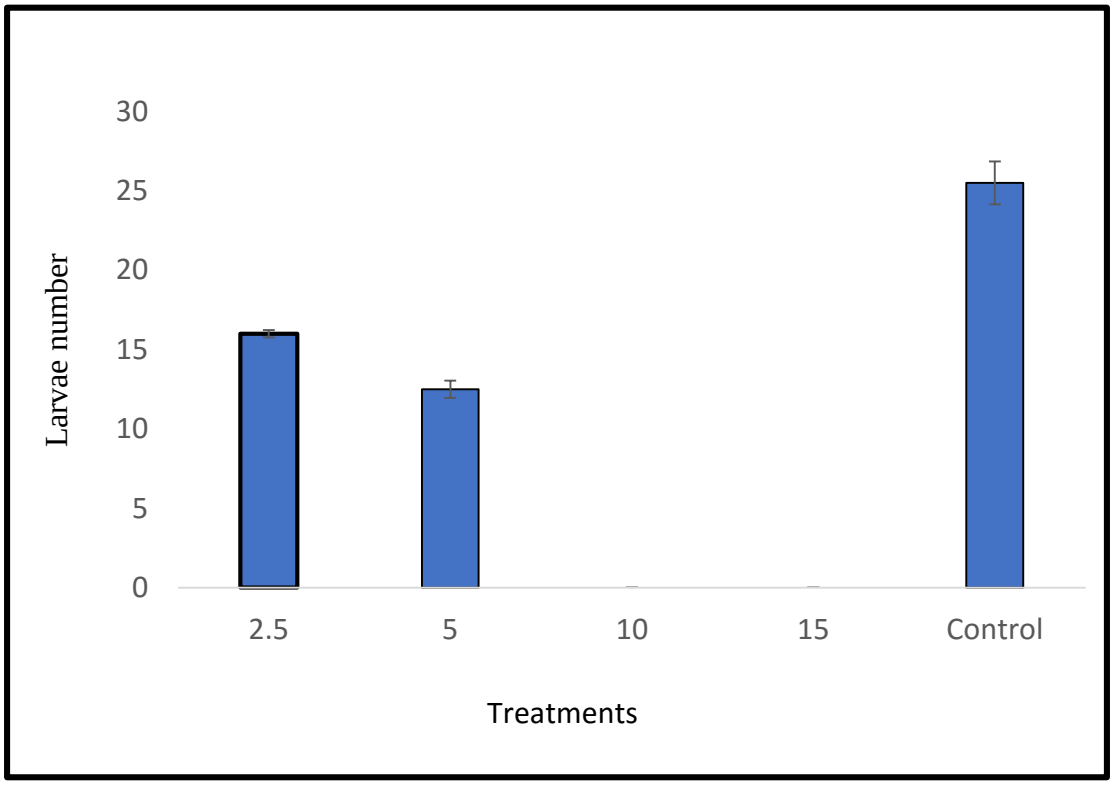


Figure No. 15 Effects of Different Concentrations of Natural pheromone on *B. invadens* oviposition in Mango
(Laboratory Experiment – Season 1)

Table No. 6 Effects of Different Concentrations of Natural pheromone on *B. invadens* oviposition in mango

(Semi field Experiment-Season 1)

Treatments	Mean No. of larvae	SD
2.5	12.5 b ± 0.96	1.91
5	9 c ± 0.41	0.82
10	0 d ± 0	0
15	0 d ± 0	0
Control	17.5 a ± 0.96	1.91

Means followed by the same letter are not significantly different at ($p \leq 0.0001$) According to LSD (1.93)

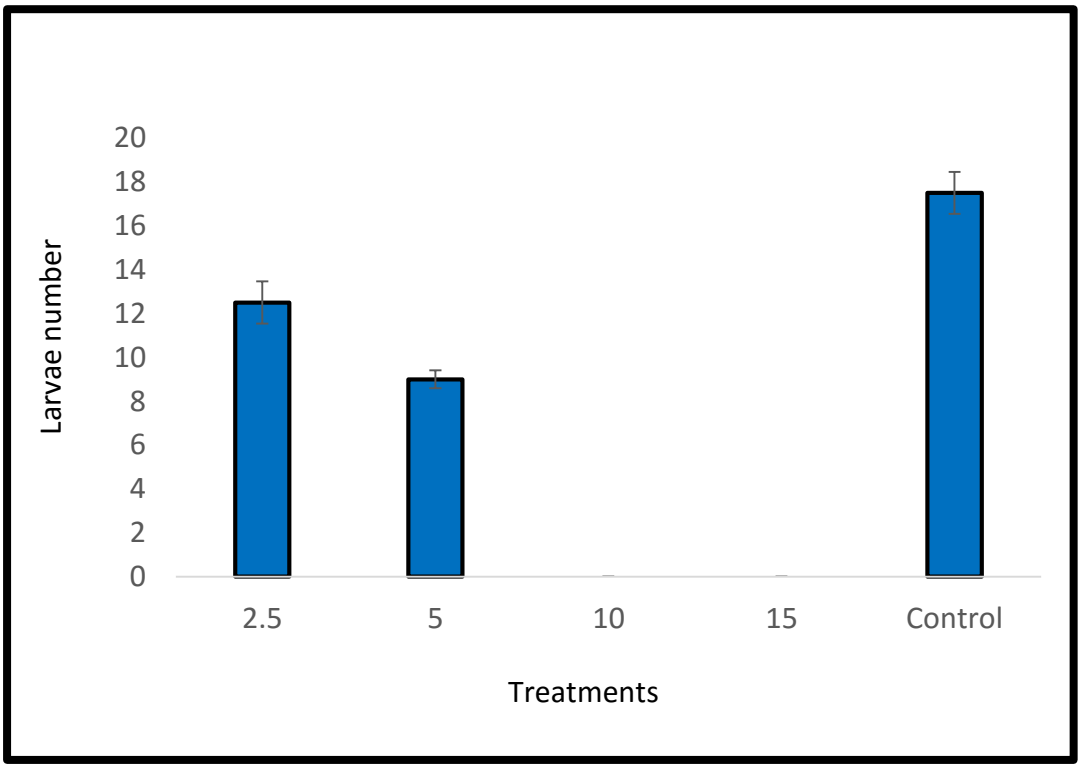


Figure No. 16 Effects of Different Concentrations of Natural pheromone on *B. invadens* oviposition in mango
(Semi field Experiment -Season 1)

4. 2. 2 Pheromones' Tests (Second Season – 2012):

4. 2. 2. 1 Laboratory Experiments:

Effects of Different Concentrations of Natural Pheromone Extracts on *Bacterocera invadens* Oviposition in Mango (Second Season – 2012)

The data relating to the effects of the different treatments on oviposition are illustrated in **Table No. 7, Figure No. 17 Appendix No. 14 and 15**. All concentrations were significantly lower from control. Also, there was a significant difference between 2.5% and 5% concentrations, while there was no significant different between 10% and 15% concentrations. The higher concentrations were significantly different from 2.5% and 5% concentrations.

4. 2. 2. 2 Semi-Field Experiments:

Effects of Different Concentrations of Natural Pheromone Extracts on *Bacterocera invadens* Oviposition in Mango (Second Season – 2012)

As shown in **Table No. 8, Figure No. 18 Appendix No. 16 and 17**, all treatments significantly reduced female oviposition. The most effective treatments were 10% and 15%, followed by 5% and 2.5%. All concentrations were significantly lower from the control, also there was a significant difference between the concentrations 2.5% and 5%, while there was no significant difference between 10% and 15% concentrations.

Table No. 7 Effects of Different Concentrations of Natural pheromone on *B. invadens* oviposition in mango (Laboratory Experiments – Season 2)

Treatments	Mean No. of larvae	SD
2.5	17.5 b ± 0.65	1.29
5	12.5 c ± 0.87	1.73
10	0 d ± 0	0
15	0 d ± 0	0
Control	28 a ± 0.91	1.83

Means followed by the same letter are not significantly different at ($p \leq 0.0001$) According to LSD (1.91)

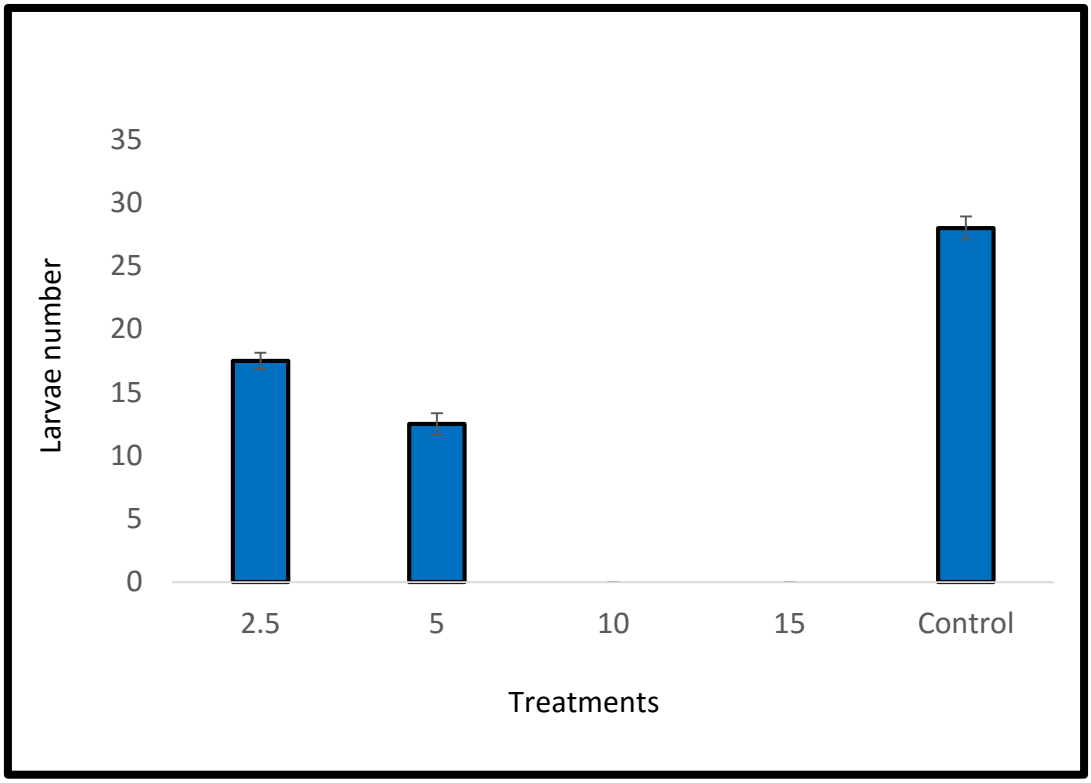


Figure No. 17 Effects of Different Concentrations of Natural pheromone on *B. invadens* oviposition in mango (Laboratory Experiment -Season 2)

Table No. 8 Effects of Different Concentrations of the Natural pheromone on *B. invadens* oviposition in mango (Semi field Experiment-Season 2)

Treatments	Mean No. of larvae	SD
2.5	12.75 b ± 0.48	0.96
5	8 c ± 0.41	0.82
10	0 d ± 0	0
15	0 d ± 0	0
Control	18.5 a ± 0.65	1.29

Means followed by the same letter are not significantly different at ($p \leq 0.0001$) According to LSD (1.26)

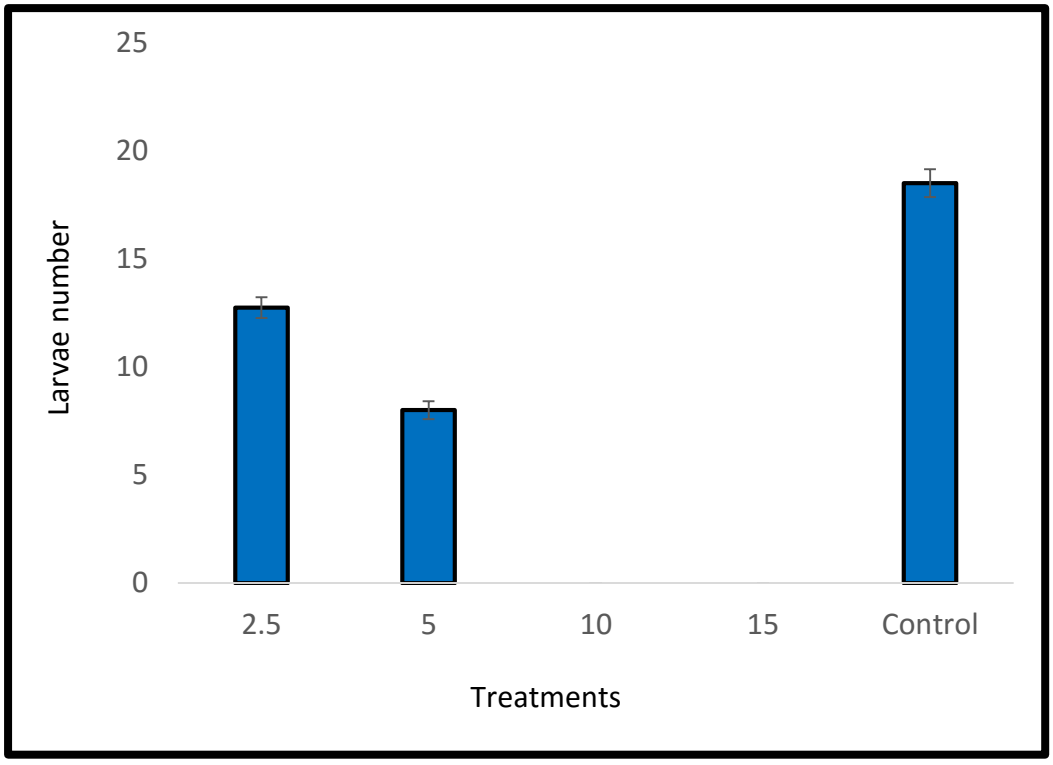


Figure No. 18 Effects of Different Concentrations of Natural pheromone on *B. invadens* oviposition in mango (Semi field Experiment-Season 2)

4. 2. 2. 3 Laboratory Experiments:

Effects of Different Concentrations of Natural Pheromone Extracts on *Bacterocera invadens* Oviposition in Guava (Second Season – 2012)

The mean number of larvae per Guava fruit treated with different concentrations of natural pheromone shown in **Table No. 9 , Figure No. 19 and Appendix No. 18 and 19**.

Mean number of larvae was significantly higher in the control, followed by 2.5% and 5% concentrations, while the lowest mean number of larvae was laid in fruits treated with 15% and 10% concentrations. All concentrations were significantly lower from control. Also, there was a significant difference between the concentrations 2.5% and 5%, while there was no significant different between 10% and 15% concentrations.

4. 2. 2. 4 Semi-Field Experiments:

Effects of Different Concentrations of Natural Pheromone Extracts on *Bacterocera invadens* Oviposition in Guava (Second Season – 2012)

Table No. 10, Figure No. 20 and Appendix No. 20 and 21 show the effect of treatments under investigation on the mean number of larvae per Guava fruit under semi-field condition. It has been observed that, the mean number of larvae significantly decreased with increasing doses of the natural pheromone.

All concentrations were significantly lower from control. Also, there was significant difference between the concentrations 2.5% and 5%, while there was no significant difference between 10% and 15% concentrations.

Table No. 9 Effects of Different Concentrations of Natural pheromone on *B. invadens* oviposition in Guava (Laboratory Experiments-Season 2)

Treatments	Mean No. of larvae	SD
2.5	14.25 b ± 0.25	0.5
5	10.75 c ± 0.75	1.5
10	0 d ± 0	0
15	0 d ± 0	0
Control	21.25 a ± 0.75	1.5

Means followed by the same letter are not significantly different at ($p \leq 0.0001$) According to LSD (1.47)

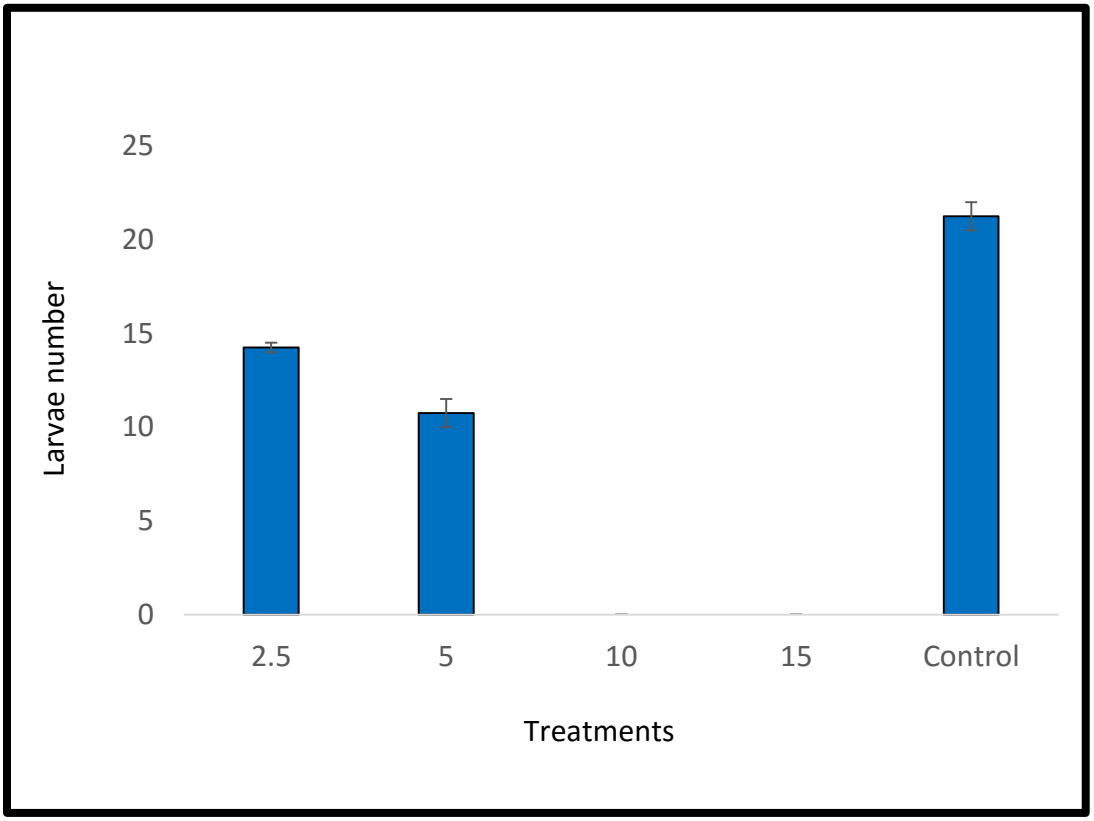


Figure No. 19 Effects of Different Concentrations of Natural pheromone on *B. invadens* oviposition in Guava (Laboratory Experiments-Season 2)

Table No. 10 Effects of Different Concentrations of Natural pheromone on *B. invadens* oviposition in Guava (Semi field Experiment -Season 2)

Treatments	Mean No. of larvae	SD
2.5	12 b ± 0.41	0.82
5	7.25 c ± 0.48	0.96
10	0 d ± 0	0
15	0 d ± 0	0
Control	14.25 a ± 0.85	1.71

Means followed by the same letter are not significantly different at ($p \leq 0.0001$) According to LSD (1.52)

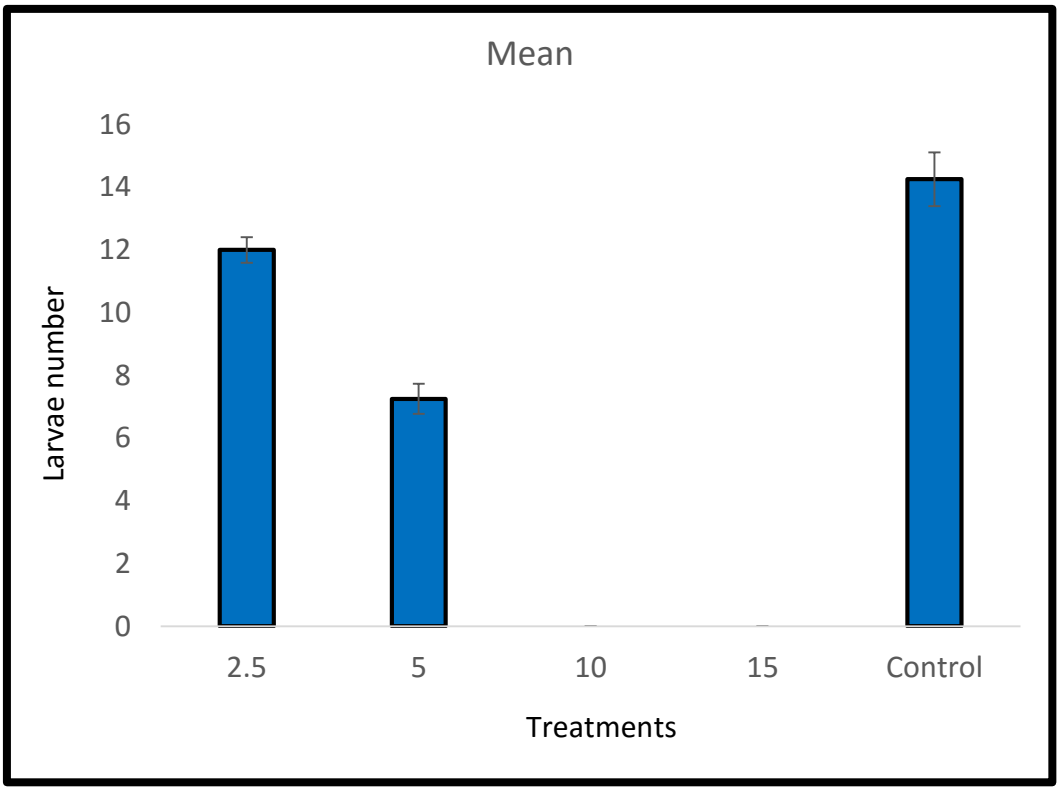


Figure No. 20 Effects of Different Concentrations of Natural pheromone on *B. invadens* oviposition in Guava (Semi field Experiments-Season 2)

4. 3. 3 Structural Elucidation of Host Marking Pheromone Experiments at JICA Laboratory:

After injection of the extracted and purified natural pheromone samples in HPLC column, the fractions were collected according to peak development of chromatograph. [UV- Detection : 220nm. Electro – physiological activity 47- 60 min., resulted in 17 peaks], and [UV – Detection : 200nm. Electro - physiological activity 47-60 min resulted in 32 peaks].

Tables No. 11 and No. 12, Figures No. 21 and No. 22 show: the retention time, area, height, area percentage and height percentage for every peak.

4.2.4 Experiments at Chemical Laboratories, University of Medical Sciences and Technology:

Other natural pheromone samples injected in Gas Chromatography-Mass Spectrometry (GC.MS), using mass spectrometer, column: Rtx-5MS, Length (30 m), Diameter (0.25mm) and thickness (0.25µl), and carrier gas Helium resulted in 42 peak that's to say 42 compound using GC-MS libraries.

Table No. 13 and Figure No. 23 and Appendix No. 22-42 show: the retention time, area, area percentage, compound name and compound formula for every peak.

Table No.11 Results of the Injection of Sample 1 in HPLC

PeakTable

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.846	2779162	160242	2.148	5.662
2	5.492	222553	23813	0.172	0.841
3	5.796	268532	20828	0.208	0.736
4	6.803	14401595	1254265	11.130	44.318
5	7.507	418323	24775	0.323	0.875
6	7.982	657991	31962	0.509	1.129
7	8.832	2778409	86592	2.147	3.060
8	10.199	9515097	182921	7.354	6.463
9	10.669	6687397	191818	5.168	6.778
10	11.435	11378133	215117	8.794	7.601
11	14.986	39004329	188286	30.145	6.653
12	15.696	8360809	160313	6.462	5.664
13	17.350	13772509	125765	10.644	4.444
14	19.486	6929756	66023	5.356	2.333
15	23.052	10098470	51434	7.805	1.817
16	23.783	1214732	31240	0.939	1.104
17	24.550	902589	14758	0.698	0.521
Total		129390387	2830153	100.000	100.000

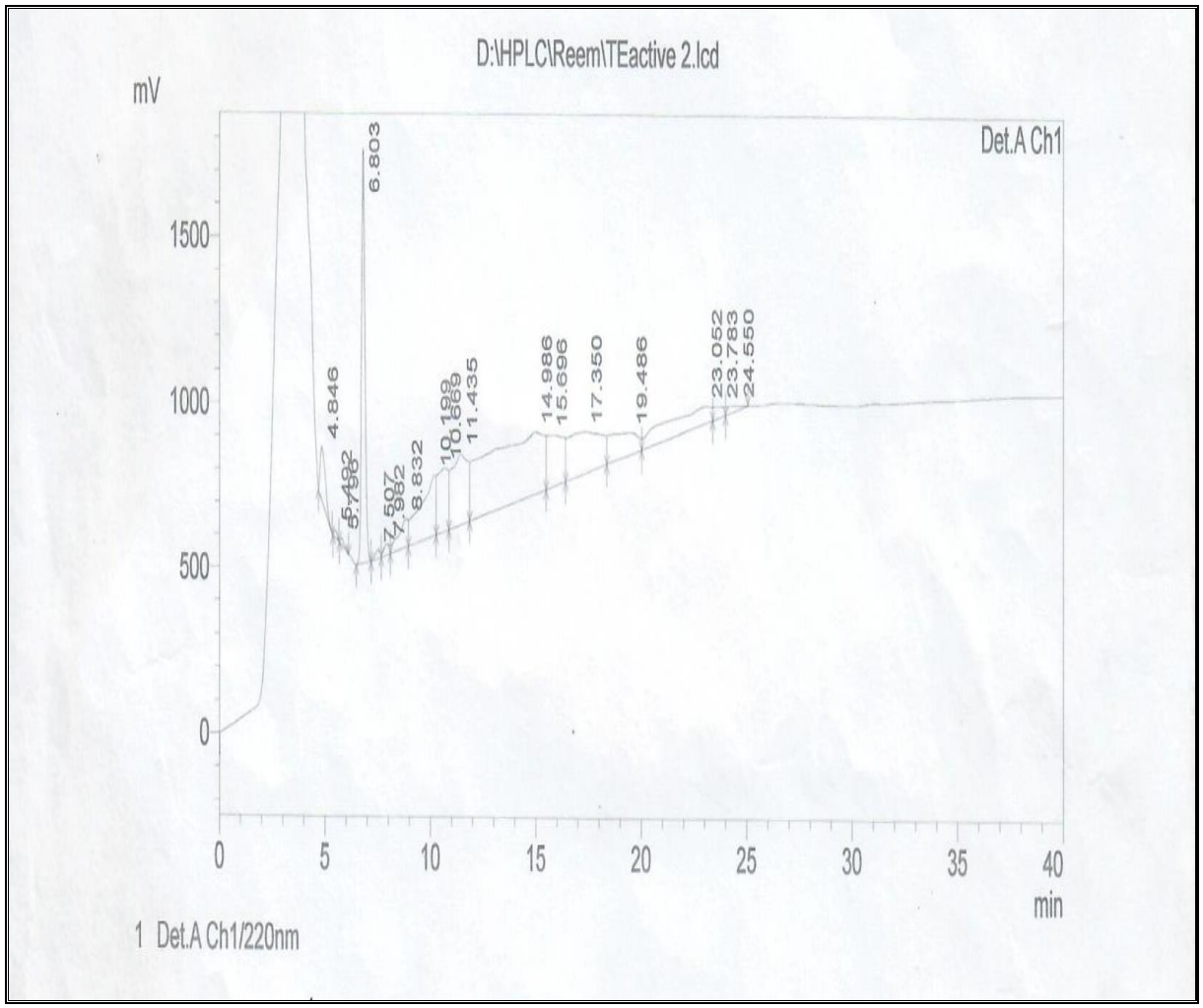


Figure No. 21 Results of the Injection of Sample 1 in HPLC

Table No. 12 Results of the Injection of Sample 2 in HPLC

PeakTable

Detector A Ch1 200nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.867	56089345	2342510	15.178	26.296
2	4.008	31962506	2566094	8.649	28.806
3	4.751	3473042	376083	0.940	4.222
4	5.726	608372	61970	0.165	0.696
5	6.131	15681095	1573766	4.243	17.667
6	7.003	2197414	79232	0.595	0.889
7	7.789	259948	32048	0.070	0.360
8	8.349	38462	4658	0.010	0.052
9	8.830	13088	1747	0.004	0.020
10	9.296	69747	4743	0.019	0.053
11	9.762	13623	1226	0.004	0.014
12	10.226	694646	53437	0.188	0.600
13	11.009	93957	6717	0.025	0.075
14	11.215	125503	8073	0.034	0.091
15	11.484	138028	9231	0.037	0.104
16	11.790	272884	11773	0.074	0.132
17	12.262	729712	13322	0.197	0.150
18	13.725	1107063	23491	0.300	0.264
19	14.038	436547	25150	0.118	0.282
20	14.520	1408506	35689	0.381	0.401
21	15.039	1014324	32410	0.274	0.364
22	15.591	5401121	34488	1.462	0.387
23	20.462	121177	3831	0.033	0.043
24	21.338	2102	127	0.001	0.001
25	21.902	8465	471	0.002	0.005
26	36.175	23797946	34397	6.440	0.386
27	36.723	10458628	33134	2.830	0.372
28	50.574	21339	271	0.006	0.003
29	68.659	16086923	101533	4.353	1.140
30	69.303	4162831	87791	1.126	0.986
31	73.348	44525675	369800	12.049	4.151
32	75.391	148525123	978947	40.192	10.989
Total		369539143	8908161	100.000	100.000

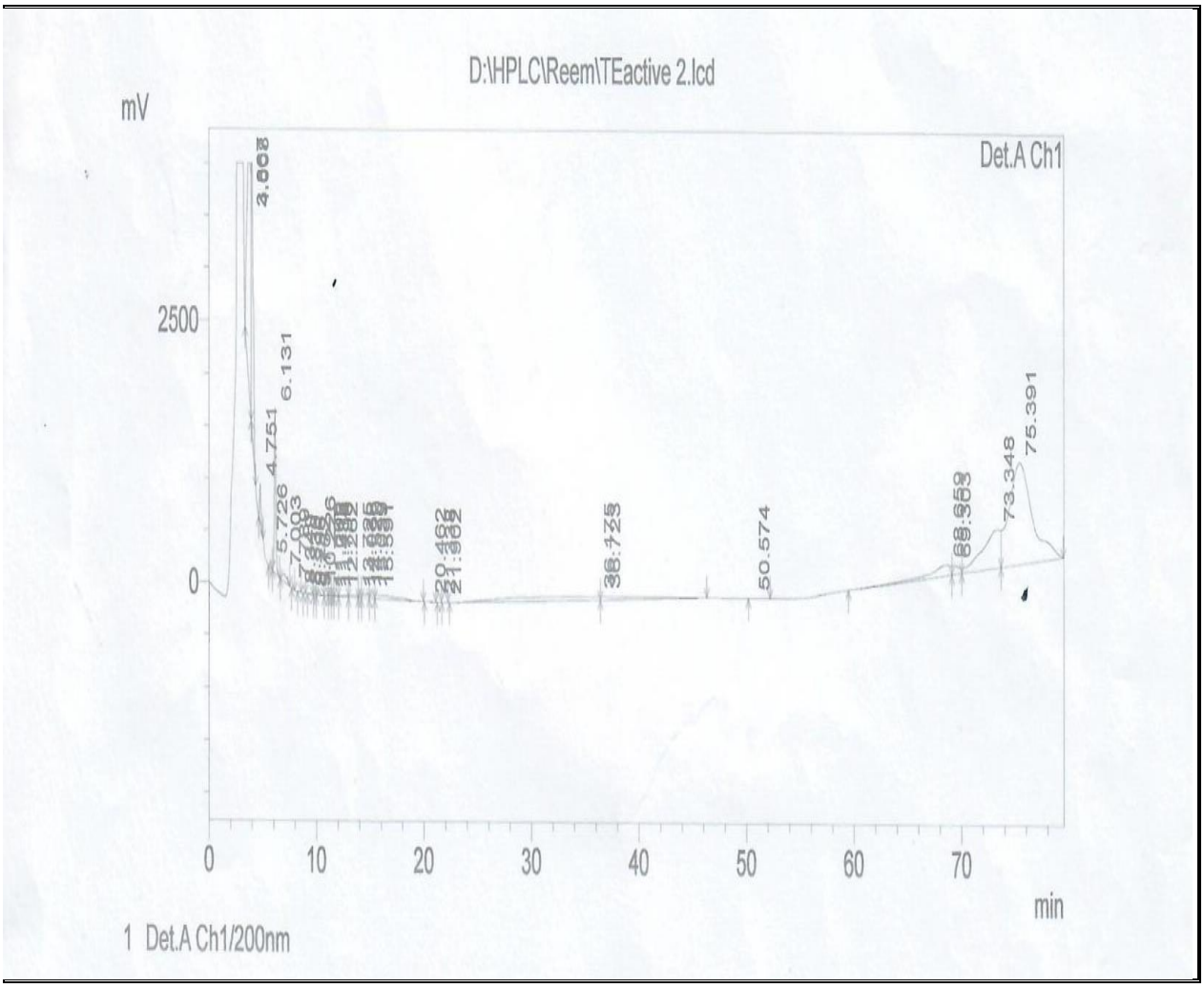


Figure No. 22 Results of the Injection of Sample 2 in HPLC

Table No.13 Results of the Injection of samples in "GC-MS "

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	3.046	140228	0.28	4-Cyclopentene-1,3-dione
2	3.161	232616	0.47	Piperidin-4-ol, 2,5-dimethyl-
3	3.456	614893	1.23	2-Cyclopenten-1-one, 2-hydroxy-
4	4.313	787407	1.58	2-Hydroxy-gamma-butyrolactone
5	4.588	316458	0.63	4(H)-Pyridine, N-acetyl-
6	4.835	334994	0.67	Pantolactone
7	5.242	76220	0.15	1-Cyclopentene-1-carboxylic acid
8	5.446	317325	0.64	1,3,5-Triazine-2,4,6-triamine
9	5.656	163485	0.33	Piperazine, 2-methyl-
10	6.339	678417	1.36	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydrox
11	6.702	1059232	2.12	Benzoic acid
12	7.361	851726	1.71	Divinyl sulfide
13	8.306	1733602	3.48	Isosorbide
14	8.336	1281299	2.57	Guanidine, (4-aminobutyl)-
15	8.871	170946	0.34	2-Methyl-2-(4-nitro-butyryl)-cyclohexanor
16	9.007	269476	0.54	1,2-Cyclopentanedione, 3-methyl-
17	9.944	514831	1.03	Acexamic acid
18	11.153	455405	0.91	D-Allose
19	11.466	174789	0.35	3,6-Nonadecadione
20	11.564	3447018	6.91	.alpha.-L-lyxo-Hexopyranoside, methyl 3-
21	13.039	582090	1.17	1,2-Octanediol
22	13.872	164933	0.33	7-Phenylheptanoic acid
23	13.969	620291	1.24	Tetradecanoic acid
24	14.853	254394	0.51	2-Hydrazino-2-imidazoline
25	15.028	1310114	2.63	2,4-Imidazolidinedione, 5-(2-methylpropyl
26	15.456	543766	1.09	9-Hexadecenoic acid, methyl ester, (Z)-
27	15.648	272828	0.55	Hexadecanoic acid, methyl ester
28	15.854	1244222	2.50	cis-9-Hexadecenoic acid
29	16.026	4258301	8.54	n-Hexadecanoic acid
30	17.180	5047276	10.12	n-Nonadecanol-1
31	17.343	546653	1.10	9-Octadecenoic acid (Z)-, methyl ester
32	17.560	219208	0.44	Methyl stearate
33	17.716	1712223	3.43	Oleic Acid
34	17.757	1868484	3.75	9-Octadecenoic acid, (E)-
35	17.909	1715213	3.44	Octadecanoic acid
36	18.291	334189	0.67	Tetracosyl acetate
37	18.987	3301262	6.62	Behenic alcohol
38	21.567	5944148	11.92	2-methylhexacosane
39	21.936	1354296	2.72	.gamma.-Sitosterol
40	23.068	902573	1.81	Tetrapentacontane, 1,54-dibromo-
41	23.628	1146447	2.30	1-Heptacosanol
42	25.232	2891009	5.80	Tetrapentacontane
		49854287	100.00	

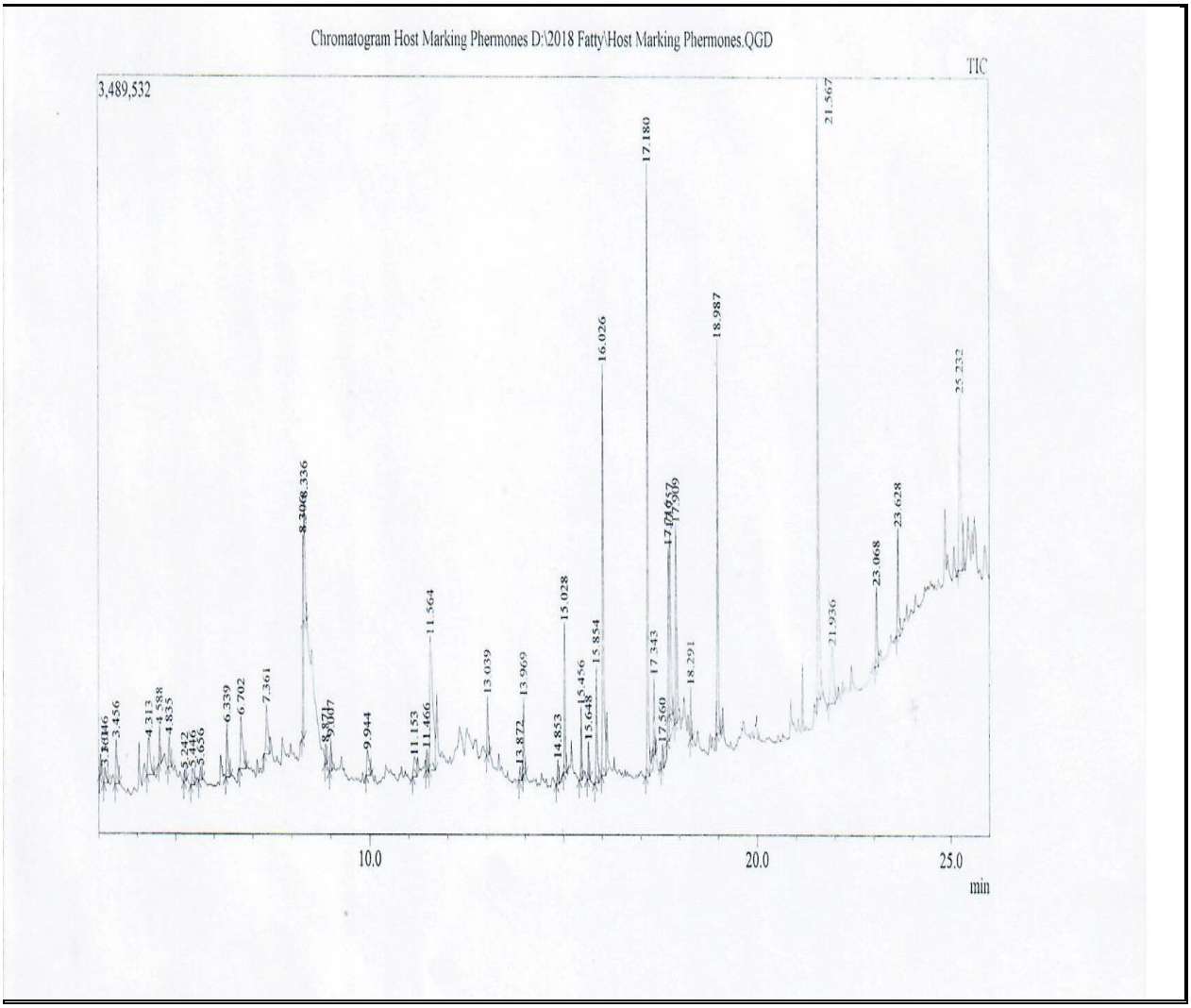


Figure No. 23 Results of the Injection of samples in "GC-MS "

4.3 Evaluation of fruit fly traps:

4.3.1 Sex Pheromone Traps Catchability

Catchability of locally manufactured Lynfield trap located in Al-kadroo from June to July 2016 showed no significant difference in the first and second weeks, while catchability in the third week was significantly different (**Table No. 14 and Figure No. 24**). More results has been observed using the same traps at Elfaki-Hasim from June to July 2016, which also showed no significant difference between the first, second and third weeks (**Table No. 15 and Figure No. 25)**

The mean number of adults trapped by locally manufactured Lynfield trap in Al-kadroo from February to March 2017 showed significant difference for the first week only and no significant difference between second, third and fourth week (**Table No. 16 and Figure No. 26**)., while the same traps in Al-khoglab from February to March 2017 showed significant difference between first and fourth week (**Table No. 17 and Figure No. 27**).

4. 3. 2 Evaluation of Different Traps Efficacy:

As shown in **Table No. 18 and Figure No. 28** , the Sticky Traps was highly significantly different from other traps, Lynfield trap baited by Methyl Eugenol mixed with Malathion , with added Guava fruit juice to the bottom of the trap was also significantly different from other traps.

4. 3. 3 Evaluation of Two types of Locally Manufactured Traps Efficacy:

Trap two show high significant different from trap one in the 4 weeks of experiment as shown in **Table No. 19 and Figure No. 29**.

4. 3. 4 Species Monitoring Using Different Sex Pheromones:

In the Three Sites, through the month period of the experiment, Methyl Eugenol was the only pheromone showed significant difference ,while there was no significant difference between Cuelure, Trimedlure and Terbenile Acetate. (The results of these experiments are shown in (**Tables No. 20 -31 and Figures No. 30- 41**)

Table No. 14 Catchability of the Locally manufactured lynfield Traps baited with Methyl Eugenol (Al-kadaroo site: 22.6.2016 - 13.7.2016)

Treatments	Mean No. Of adult fruit flies	CTD	SD
1st week	52 b ± 4.92	7	11.01
2nd week	46 b ± 5.76	7	12.87
3rd week	440 a ± 93.35	63	208.74

Means followed by the same letter are not significantly different at ($p<0.0013$)
According to LSD (178.87)

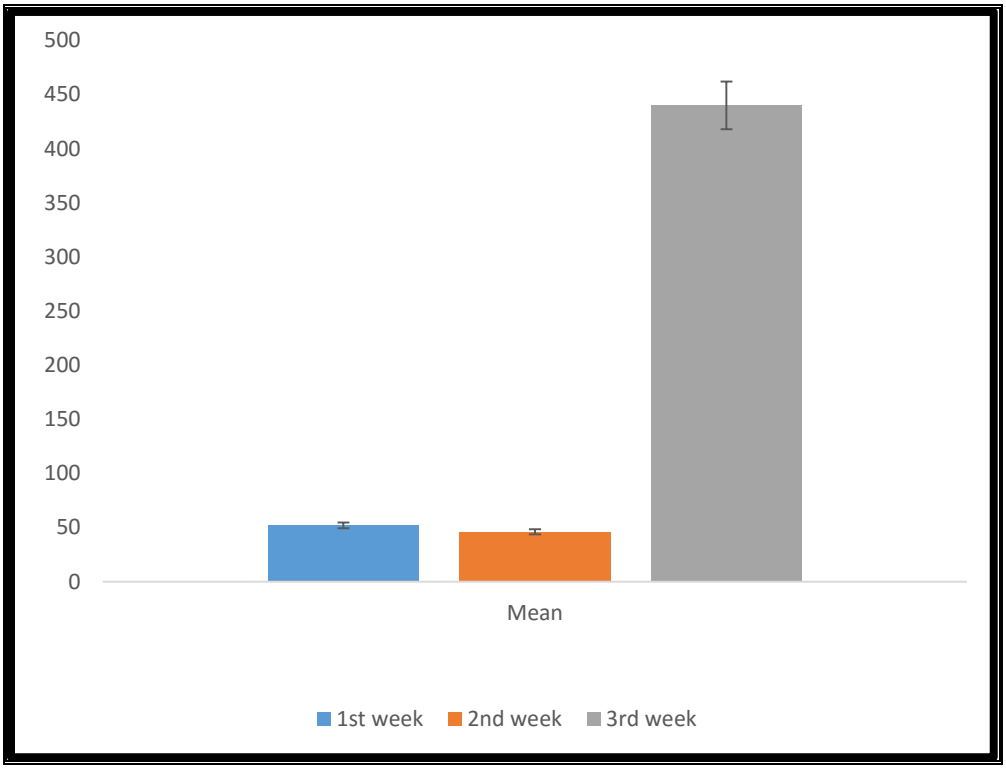


Figure No. 24 Catchability of the Locally manufactured lynfield Traps baited with Methyl Eugenol (Al-kadaroo site: 22.6.2016 - 13.7.2016)

Table No. 15 Catchability of the Locally Manufactured lynfield Traps baited with Methyl Eugenol (Al-Faki-Hashim Site: 22.6.2016 - 13.7.2016)

Treatments	Mean No. Of adult fruit flies	CTD	SD
1st week	20 a ± 5.50	3	12.30
2nd week	23 a ± 3.59	3	8.02
3rd week	35 a ± 6.15	5	13.75

Means followed by the same letter are not significantly different at (p<0.1855)
According to LSD (18.33)

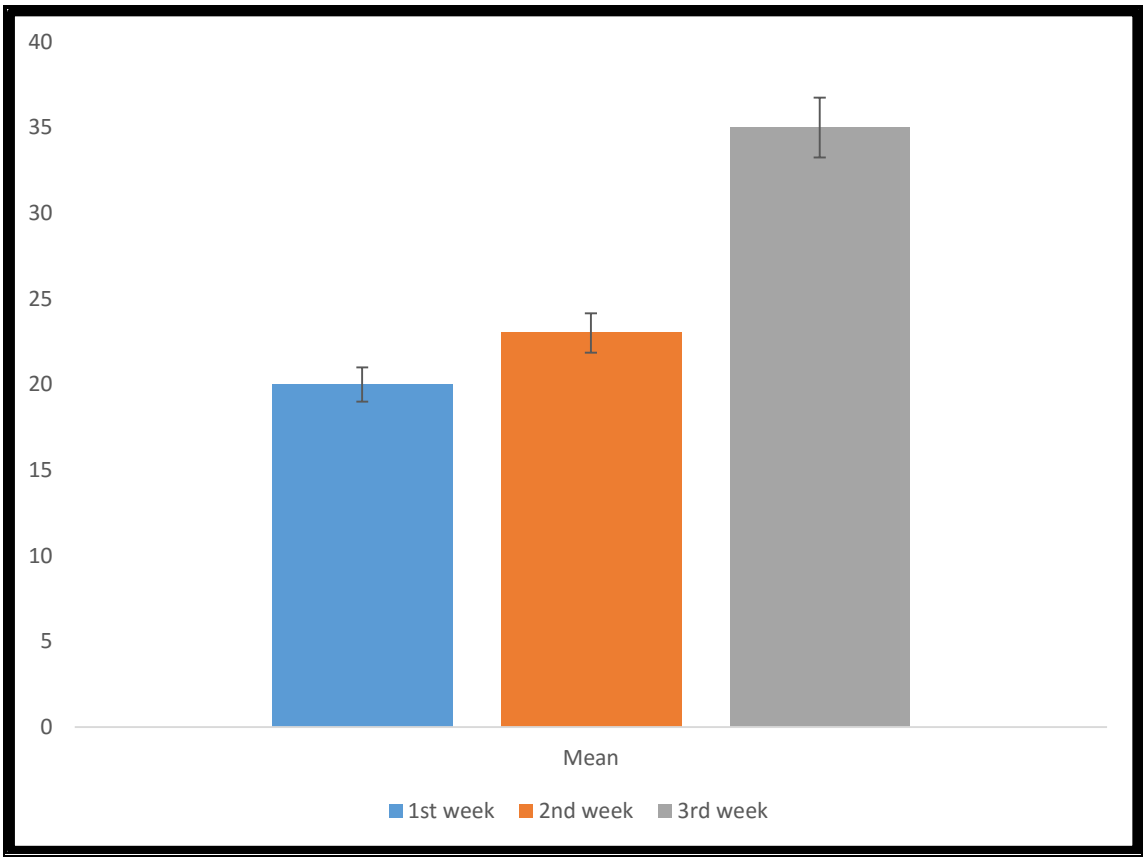


Figure No. 25 Catchability of the Locally Manufactured Lynfield Traps baited with Methyl Eugenol (Alfaki-Hashim site: 22.6.2016 - 13.7.2016)

Table No. 16 Catchability of the Locally manufactured lynfield traps baited with Methyl Eugenol (Al-kadaroo Site : 6.2.2017 - 5.3.2017

Treatments	Mean No. Of adult fruit flies	CTD	SD
1st week	42 a ± 5.38	6	12.02
2nd week	10 b ± 3.62	1	8.09
3rd week	7 b ± 2.98	1	6.67
4th week	4 b ± 1.14	-	2.55

Means followed by the same letter are not significantly different at ($p<0.0000$) According to LSD (11.81)

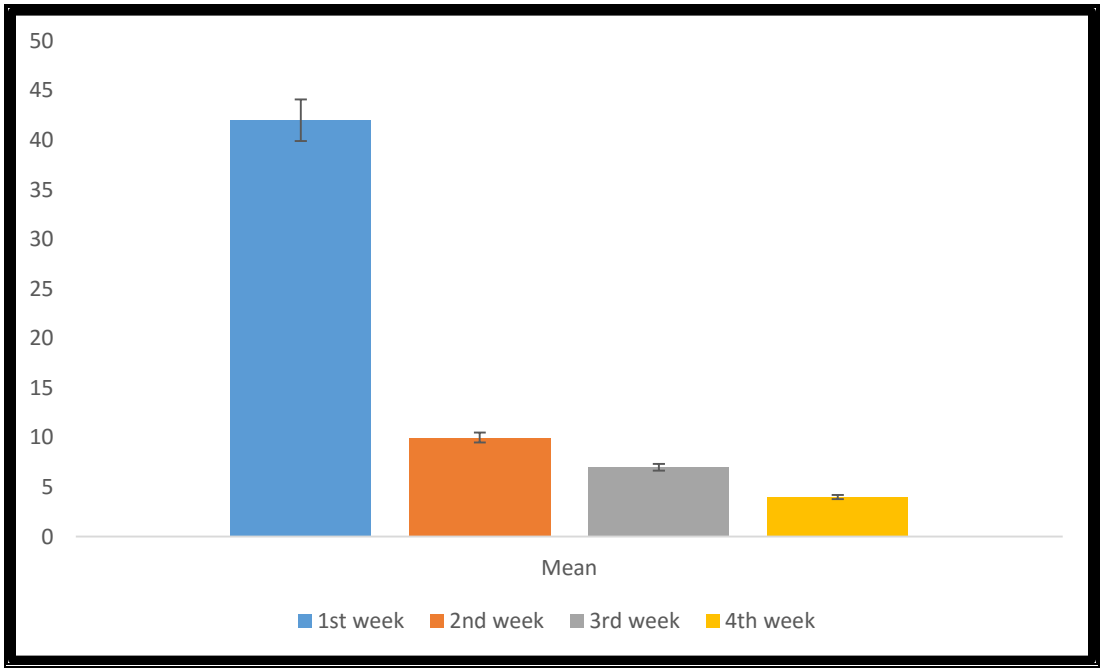


Figure No. 26 Catchability of the Locally Manufactured lynfield Traps baited with Methyl Eugenol (Al-kadaroo Site : 6.2.2017 - 5.3.2017)

Table No. 17 Catchability of the Locally Manufactured lynfield Traps baited with Methyl Eugenol (Al-khojalab Site : 6.2.2017 - 5.3.2017)

Treatments	Mean No. Of adult fruit flies	CTD	SD
1st week	43 a ± 17.15	6	38.35
2nd week	20 ab ± 5.42	3	12.12
3rd week	15 ab ± 4.56	2	10.20
4th week	12 b ± 4.22	2	9.43

Means followed by the same letter are not significantly different at ($p<0.1479$) According to LSD (29.59)

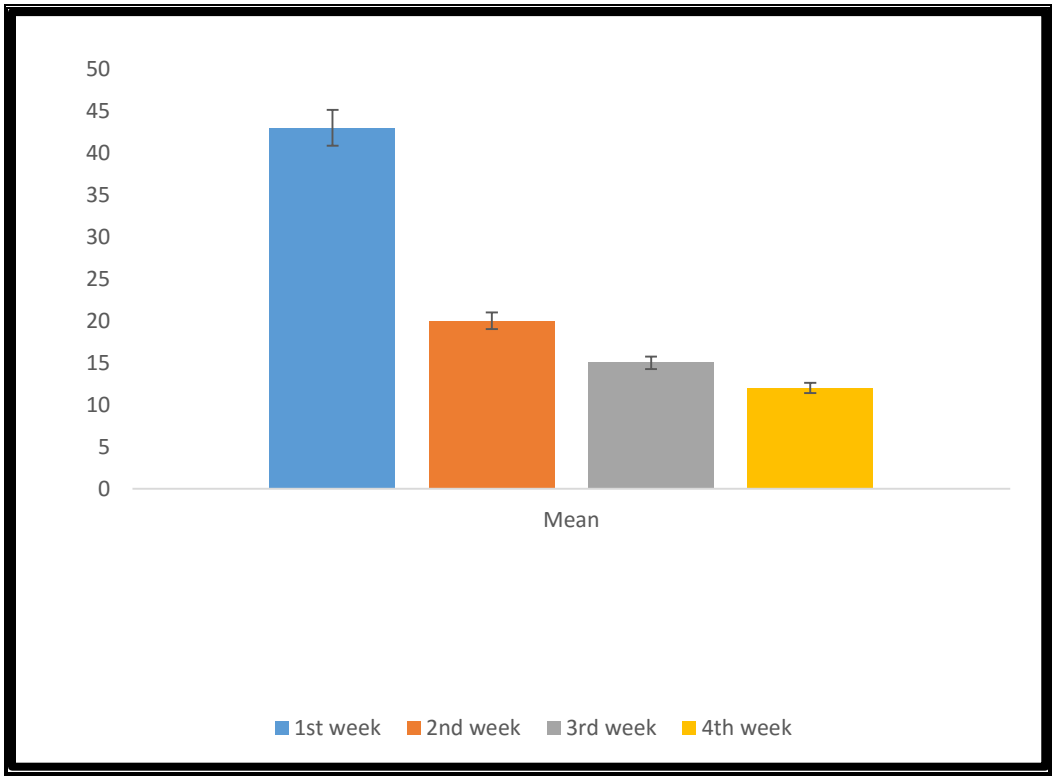


Figure No. 27 Catchability of the Locally manufactured lynfield Traps baited with Methyl Eugenol (Al-khojalab Site : 6.2.2017 - 5.3.2017)

Table No. 18 Mean No. of Adult Fruit Flies Caught by Different Traps (Al-Halfaia Site :16.5.2017 - 6.6.2017)

Treatments	Mean No. of adult fruit flies	CTD	SD
ME + Cyper	59 c ± 5.76	8	11.52
ME + Malation	151 c ± 17.16	22	34.31
Sticky Trap + ME	800 a ±185.65	144	371.30
Food Trap + Cyper	210 bc ± 23.35	39	46.70
Food Trap +Malation	478 b ± 36.34	68	72.69

Means followed by the same letter are not significantly different at (p<0.0005) According to LSD (274.86)

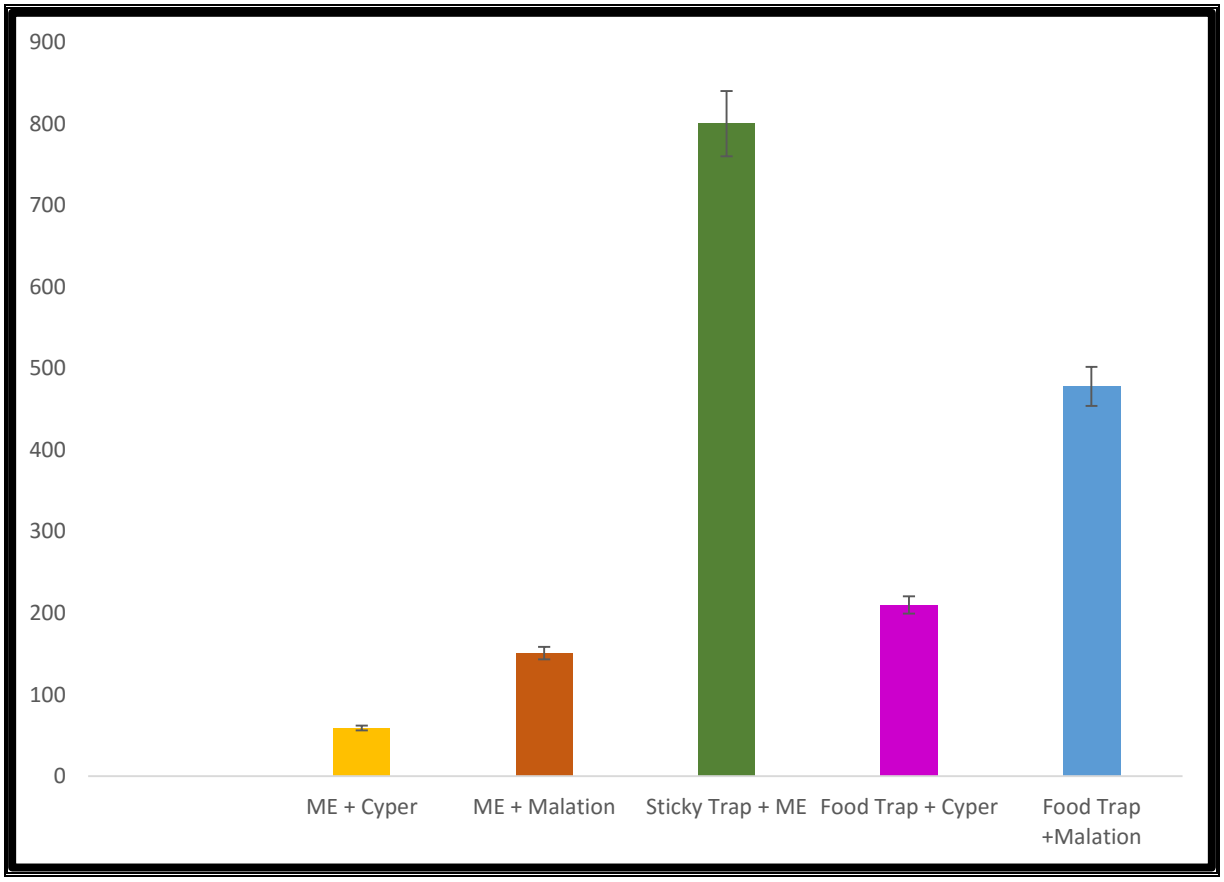


Figure No. 28 Evaluation of Different Traps Efficacy (Al-Halfaia Site :16.5.2017 -6.6.2017)

Table No. 19 Mean No. of Adult Fruit Flies Caught by Two Types of Locally Manufactured Traps Efficacy (Al- kadaroo Site (27.9.2017 - 18.10.2017)

		Mean No. of adult fruit flies	CTD	SD
Trap1	1st week	75 b ± 6.02	11	13.46
	2nd week	28 b ± 4.45	4	9.94
	3rd week	41 b ± 4.08	6	9.12
	4th week	44 b ± 6.44	6	14.39
Trap2	1st week	863 a ± 113.60	123	254.01
	2nd week	1044 a ± 201.18	149	449.85
	3rd week	1482 a ± 253.53	212	566.92
	4th week	1659 a ± 483.52	237	1081.18

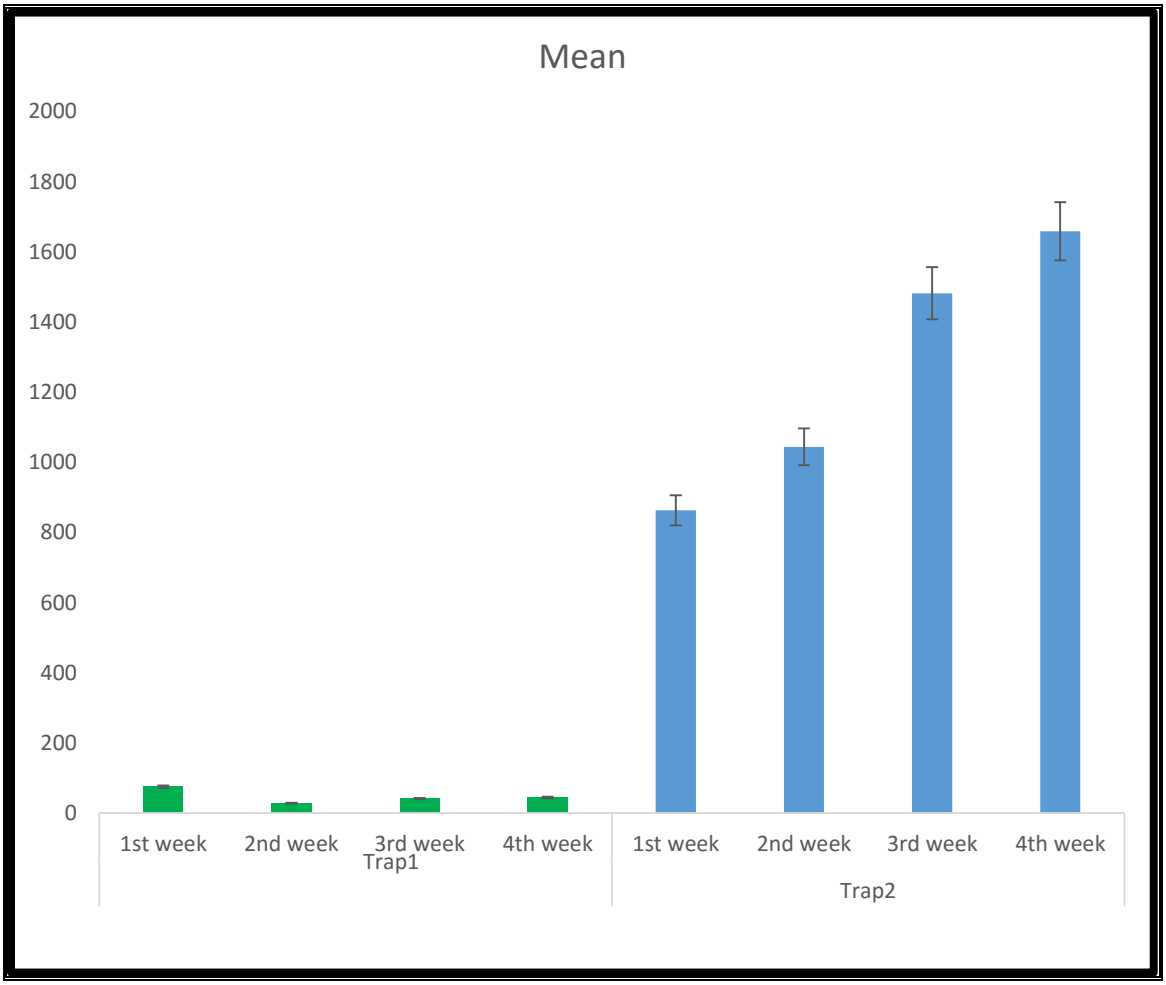


Figure No. 29 Evaluation of Two Types of Locally Manufactured Traps Efficacy (Al- kadaroo Site (27.9.2017 - 18.10.2017)

Table No. 20 Species monitoring using different types of pheromones in three sites - Site 1: (7. 3. 2018)

Treatment	Mean No. of adult fruit flies	CTD	SD
ME	105 a ± 36.68	15	73.36
C	0 b ± 0	0	0
T	0 b ± 0	0	0
TA	41 b ± 15.94	6	31.89

Means followed by the same letter are not significantly different at ($p<0.0077$)
According to LSD (57.50)

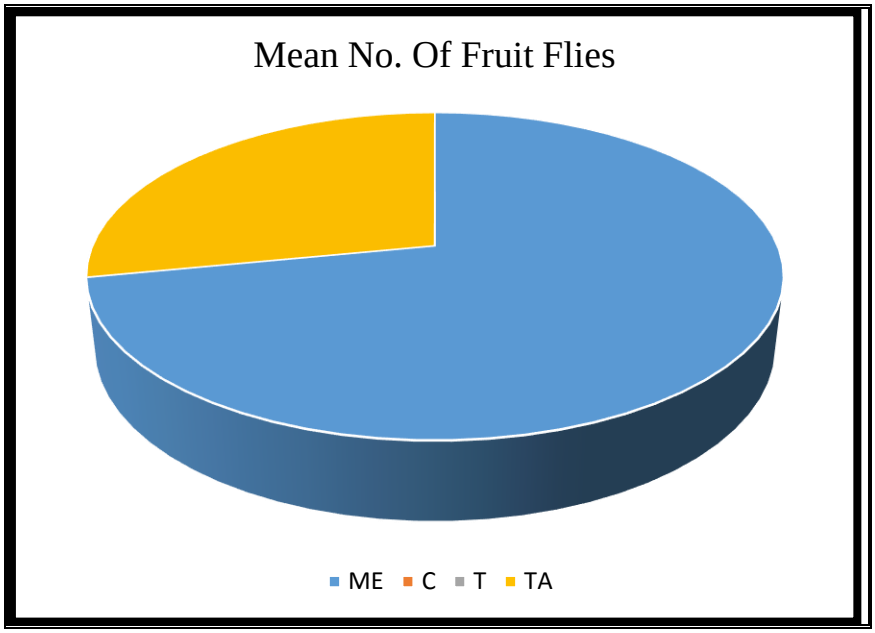


figure No. 30 Pie Chart Showing Species monitoring using different types of pheromones in three sites: Site 1: (7. 3. 2018)

Table No. 21 Species monitoring using different types of pheromones in three sites - Site 2 (7. 3. 2018)

Treatment	Mean No. of adult fruit flies	CTD	SD
ME	1514 a ± 549	216	1098
C	0 b ± 0	0	0
T	0 b ± 0	0	0
TA	48 b ± 5.52	7	11.05

Means followed by the same letter are not significantly different at ($p<0.0083$)
According to LSD (879.33)

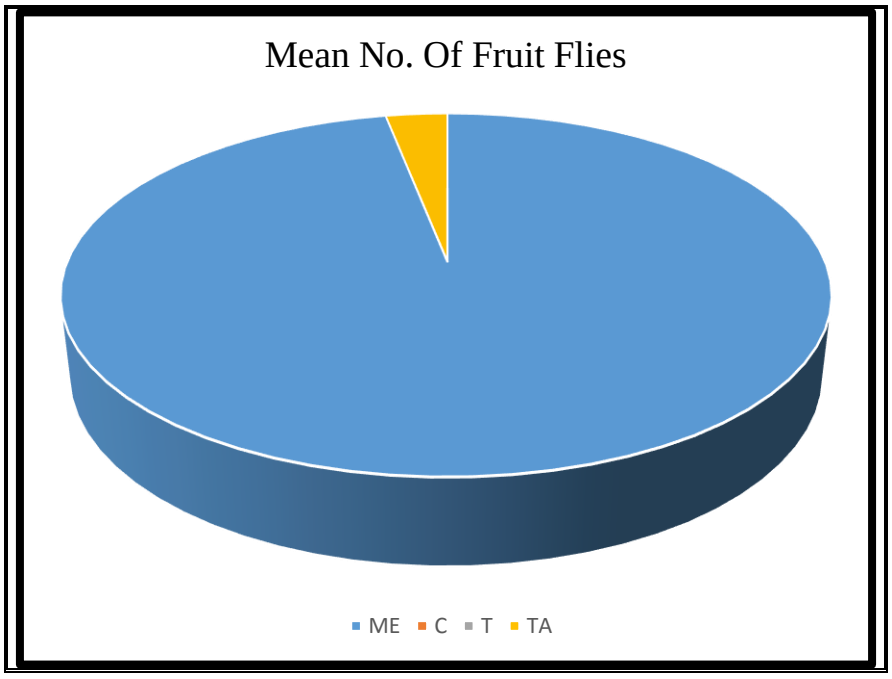


Figure No. 31 Pie Chart Showing Species Monitoring Using Different Types of Pheromones in Three Sites - Site 2 (7. 3. 2018)

Table No. 22 Species monitoring using different types of pheromones in three sites - Site 3: (7. 3. 2018)

Treatment	Mean No. of adult fruit flies	CTD	SD
ME	778 a ± 167.62	111	335.23
C	1 b ± 0.48	-	0.96
T	0 b ± 0	0	0
TA	25 b ± 9.23	4	18.46

Means followed by the same letter are not significantly different at ($p<0.0002$)
According to LSD (268.76)

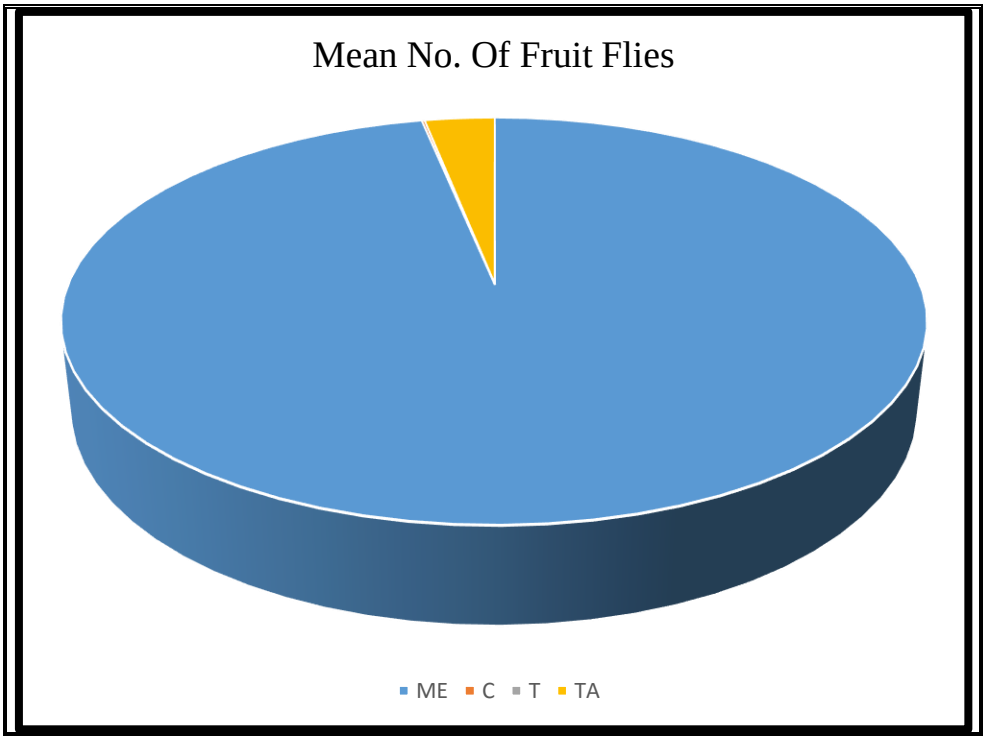


Figure No. 32 Pie Chart Showing Species Monitoring Using Different Types of Pheromones in Three Sites - Site 3:(7. 3. 2018)

Table No. 23 Species monitoring using different types of pheromones in three sites - Site 1: (13. 3. 2018)

Treatment	Mean No. of adult fruit flies	CTD	SD
ME	157 a ± 22.49	22	44.97
C	0 b ± 0	0	0
T	0 b ± 0	0	0
TA	27 b ± 9.43	4	18.86

Means followed by the same letter are not significantly different at (p<0.0000)
According to LSD (43.05)

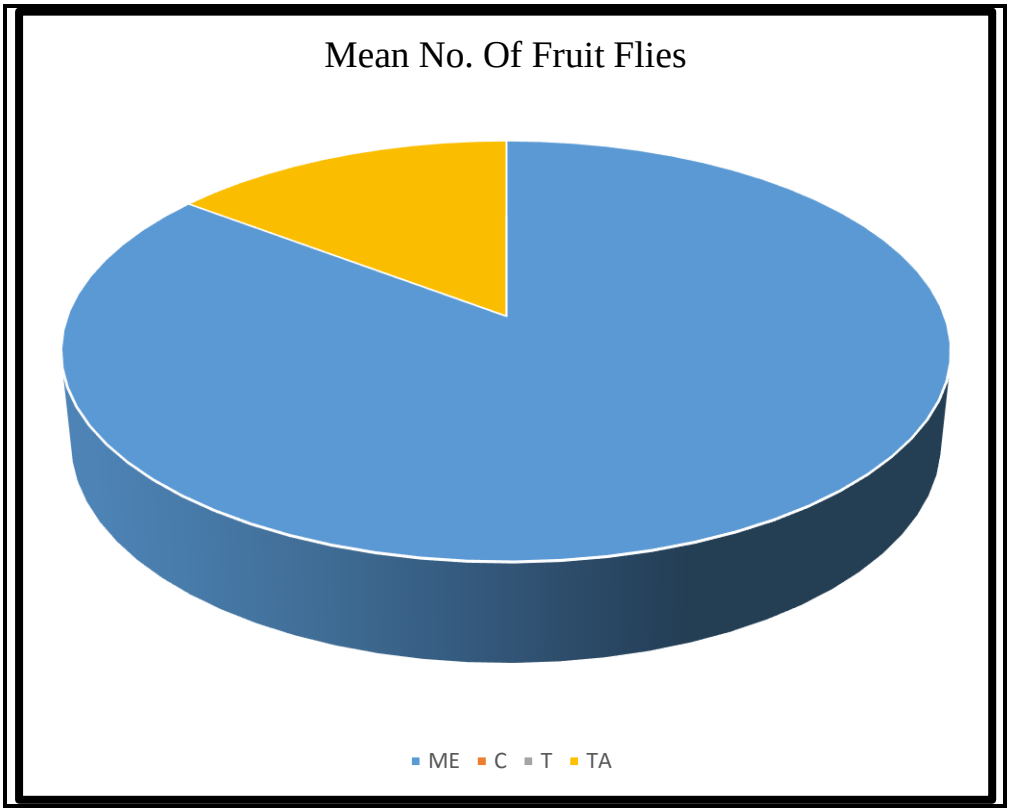


Figure No. 33 Pie Chart Showing Species Monitoring Using Different Types of Pheromones in Three Sites - Site 1: (13. 3. 2018)

Table No. 24 Species monitoring using different types of pheromones in three sites - Site2: (13. 3. 2018)

Treatment	Mean No. of adult fruit flies	CTD	SD
ME	1193 a ± 199.71	170	399.41
C	0 b ± 0	0	0
T	0 b ± 0	0	0
TA	17 b ± 6.24	2	12.49

Means followed by the same letter are not significantly different at (p<0.0000)
According to LSD (318.47)

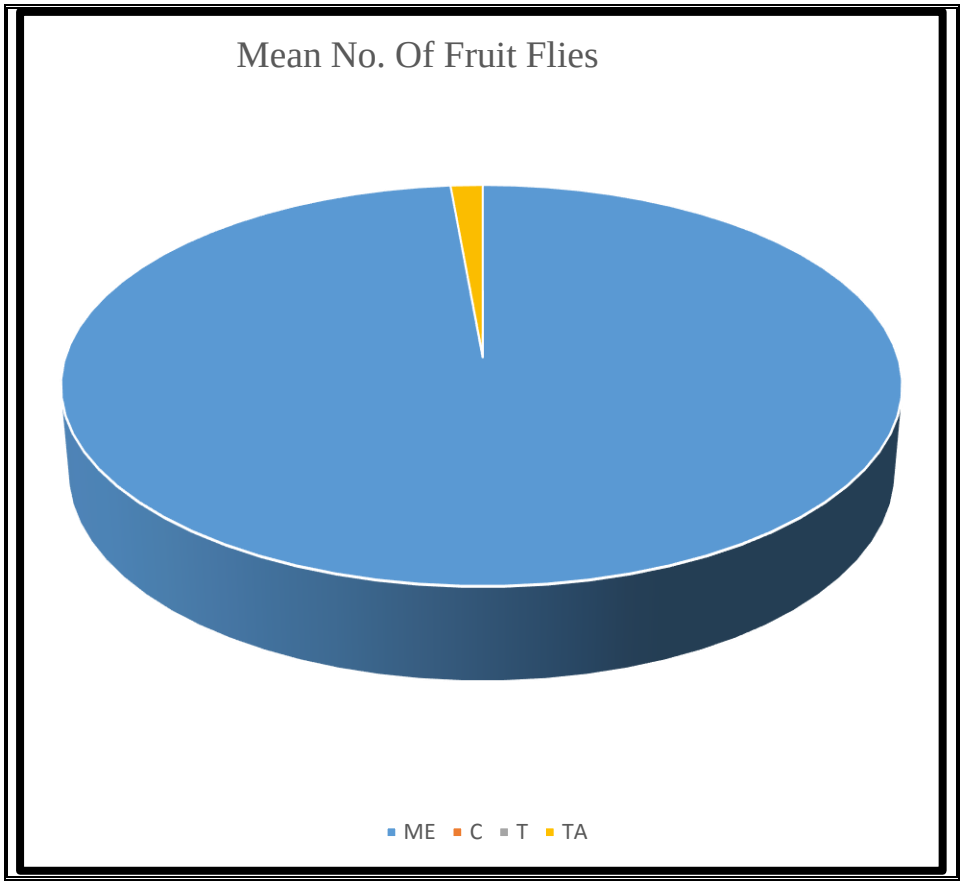


Figure No. 34 Pie Chart Showing Species Monitoring Using Different Types of Pheromones in Three Sites - Site2: (13. 3. 2018)

Table No. 25 Species monitoring using different types of pheromones in three sites - Site 3: (13. 3. 2018)

Treatment	Mean No. of adult fruit flies	CTD	SD
ME	280 a ± 39.73	40	79.46
C	0 b ± 0.25	0	0.5
T	0 b ± 0	0	0
TA	7 b ± 5.46	1	10.92

Means followed by the same letter are not significantly different at (p<0.0000)
According to LSD (64.82)

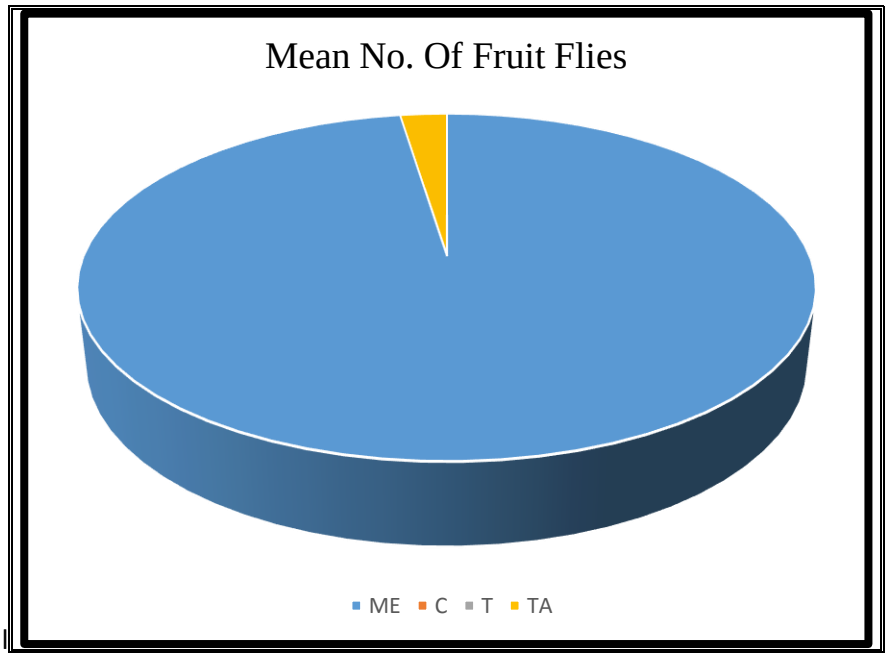


Figure No. 35 Pie Chart Showing Species Monitoring Using Different Types of Pheromones in Three Sites - Site 3: (13. 3. 2018)

Table No. 26 Species monitoring using different types of pheromones in three sites - Site 1: (20. 3. 2018)

Treatment	Mean No. of adult fruit flies	CTD	SD
ME	130 a ± 44.98	19	89.95
C	0 b ± 0.5	0	1
T	0 b ± 0	0	0
TA	30 b ± 12.39	4	24.77

Means followed by the same letter are not significantly different at ($p<0.0060$)
According to LSD (68.65)

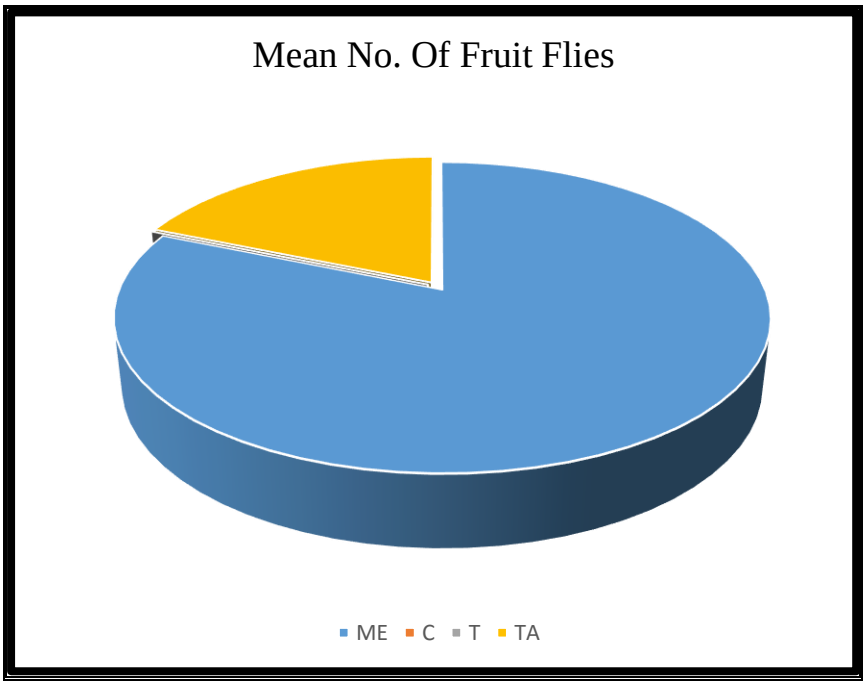


Figure No. 36 Pie Chart Showing Species Monitoring Using Different Types of Pheromones in Three Sites - Site 1: (20. 3. 2018)

Table No. 27 Species monitoring using different types of pheromones in three sites - Site 2: (20. 3. 2018)

Treatment	Mean No. of adult fruit flies	CTD	SD
ME	909 a ± 127.08	130	254.16
C	0 b ± 0	0	0
T	0 b ± 0	0	0
TA	21 b ± 10.66	3	21.32

Means followed by the same letter are not significantly different at ($p<0.0000$)
According to LSD (199.51)

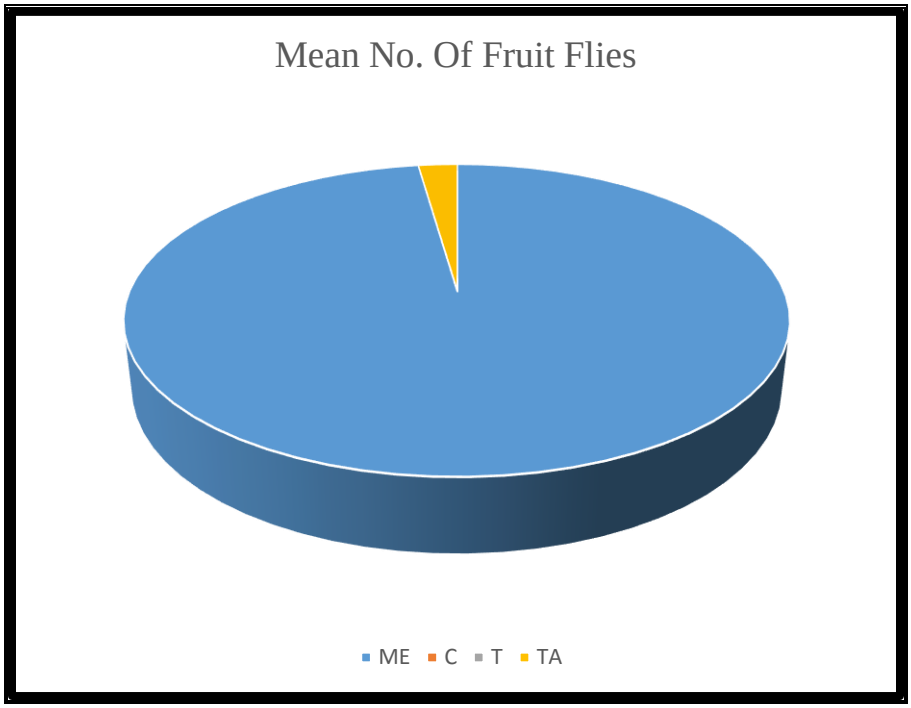


Figure No. 37 Pie Chart Showing Species Monitoring Using Different Types of Pheromones in Three Sites - Site 2: (20. 3. 2018)

Table No. 28 Species monitoring using different types of pheromones in three sites - Site 3: (20. 3. 2018)

Treatment	Mean No. of adult fruit flies	CTD	SD
ME	388 a ± 71.29	55	142.58
C	1 b ± 0.75	-	1.5
T	0 b ± 0	0	0
TA	11 b ± 5.43	2	10.86

Means followed by the same letter are not significantly different at ($p<0.0001$)
According to LSD (115.73)

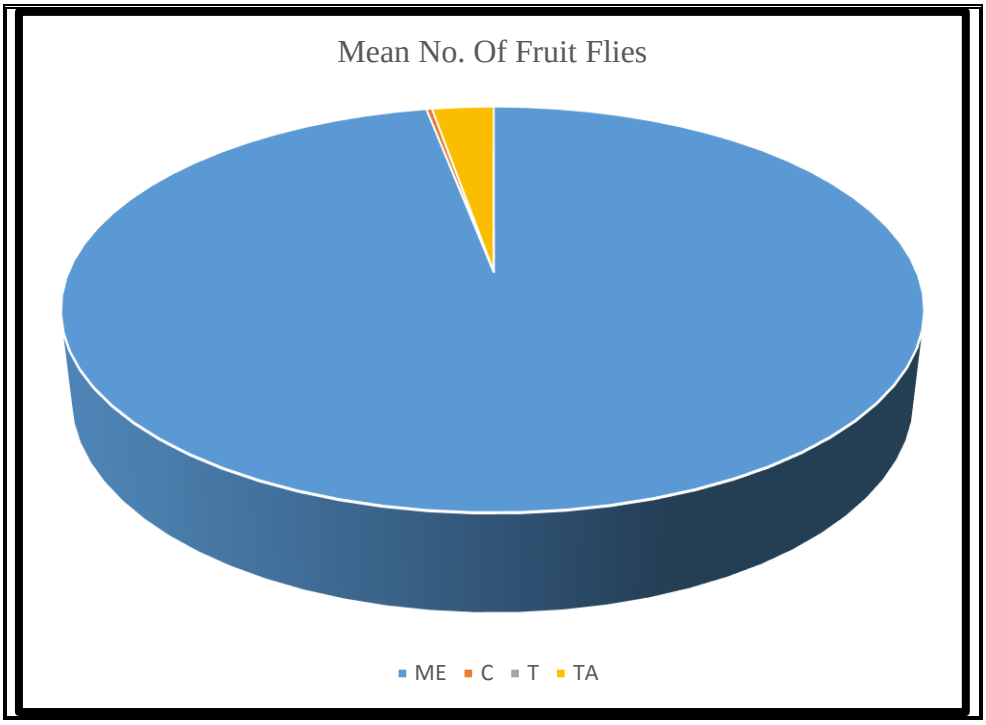


Figure No. 38 Pie Chart Showing Species Monitoring Using Different Types of Pheromones in Three Sites - Site 3: (20. 3. 2018)

Table No. 29 Species monitoring using different types of pheromones in three sites - Site 1: (27. 3. 2018)

Treatment	Mean No. of adult fruit flies	CTD	SD
ME	165 a ± 68.99	24	137.97
C	0 b ± 0	0	0
T	0 b ± 0	0	0
TA	20 b ± 12.82	3	25.63

Means followed by the same letter are not significantly different at ($p<0.0181$) According to LSD (106.65)

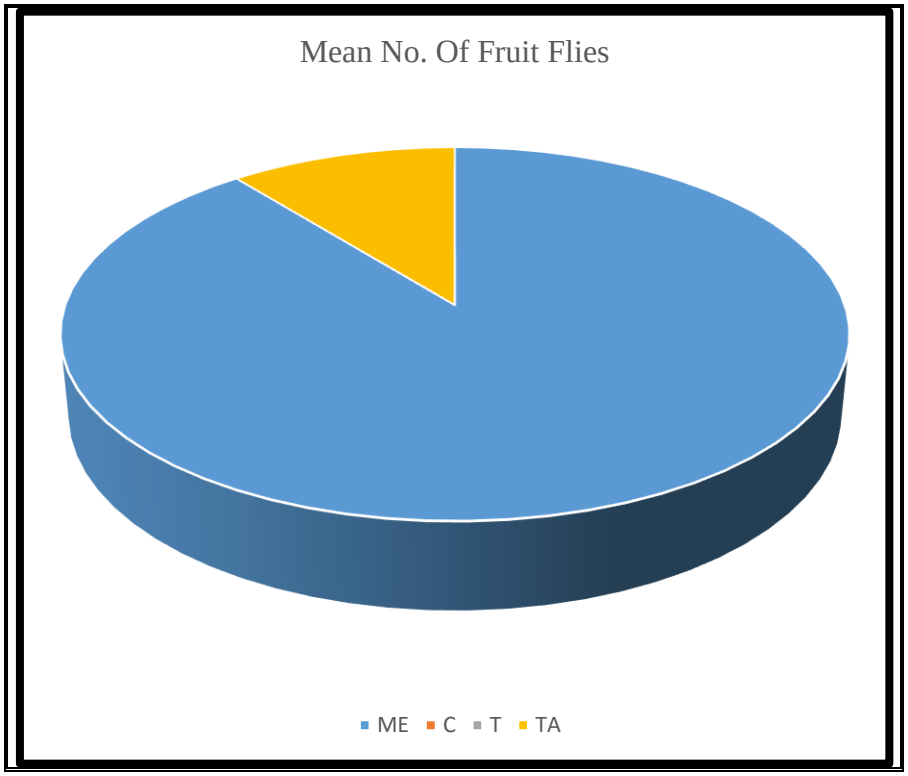


Figure No. 39 Pie Chart Showing Species Monitoring Using Different Types of Pheromones in Three Sites - Site 1: (27. 3. 2018)

Table No. 30 Species monitoring using different types of pheromones in three sites - Site 2: (27. 3. 2018)

Treatment	Mean No. of adult fruit flies	CTD	SD
ME	668 a ± 72.85	95	145.70
C	0 b ± 0	0	0
T	0 b ± 0	0	0
TA	15 b ± 2.63	2	5.26

Means followed by the same letter are not significantly different at ($p<0.0000$) According to LSD (115.96)

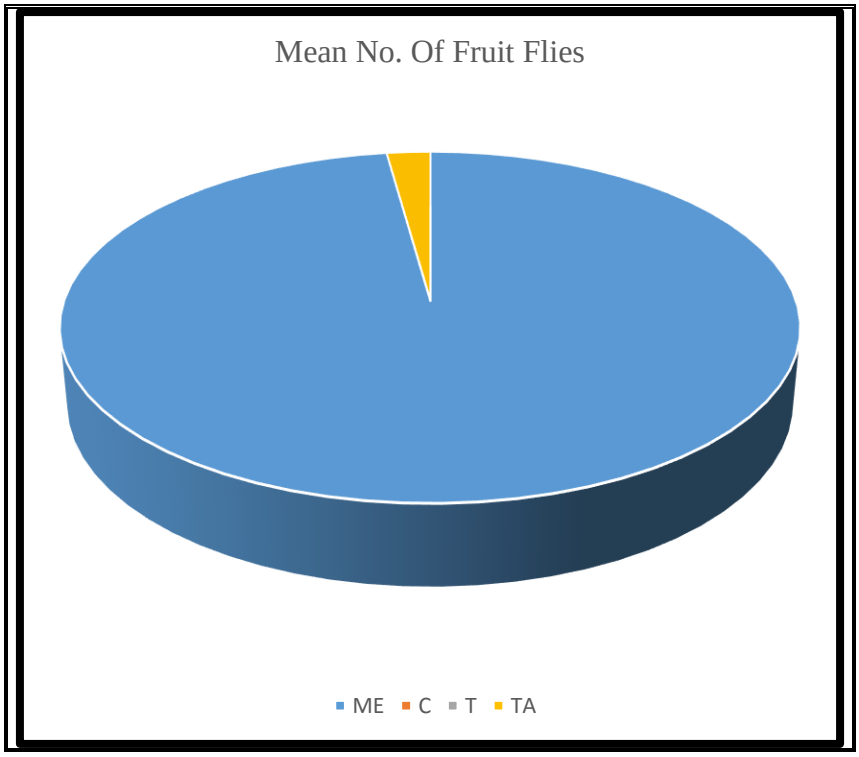


Figure No. 40 Pie Chart Showing Species Monitoring Using Different Types of Pheromones in Three Sites - Site 2: (27. 3. 2018)

Table No. 31 Species monitoring using different types of pheromones in three sites - Site 3: (27. 3. 2018)

Treatment	Mean No. of adult fruit flies	CTD	SD
ME	268 a ± 52.61	38	105.21
C	0 b ± 0	0	0
T	0 b ± 0	0	0
TA	6 b ± 5.09	-	10.18

Means followed by the same letter are not significantly different at ($p<0.0001$) According to LSD (86.60)

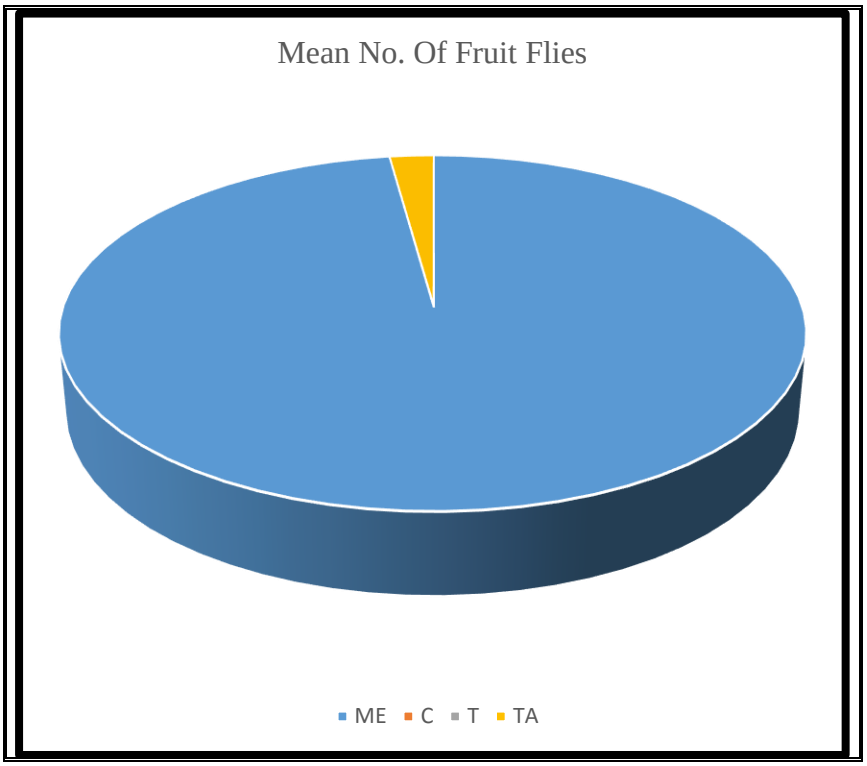


Figure No. 41 Pie Chart Showing Species Monitoring Using Different Types of Pheromones in Three Sites - Site 3: (27. 3. 2018)

ME: Methyl Eugenol, C: Cuelure, T: Trimedlure, TA: Trypenil Acetate, CTD: Number of captured flies/Trap/Day

CHAPTER FIVE

DISCUSSION

5. **DISCUSSION**

The Horticultural Production Sector in Sudan has its potential part in the economy through fruit export. During many years, this sector did not face any problem in fruit production and pest control. However, by the beginning of this century, the problems of fruit flies infestation come to be the main enigma of fruit farmers in many parts of the country.

5.1 Species Identification:

5.1.1 Identification of the fruit flies species found in the study area:

Up to 2005, there were about 40 species of fruit flies recorded in Sudan. In 2005, a new species was recorded invading many areas of fruit production in the country and was identified as the Asian (or the Invasive) fruit fly, *Bactrocera invadens* **(Drew *et al.*, 2005)**. Since its discovery in 2005 and within few years, it was spreading fast and replacing the already existing species **(Mohammed and Ali, 2008)**. In the following years, the fruit fly infestation was increasing, and the Asian fruit fly became the dominant species in many areas of fruit production in the country **(Elaraky *et al.*, 2012 and Mahmoud *et al.*, 2016)**. In 2008, a National Workshop on Fruit flies was held in Khartoum, and fruit flies were considered one of the Main National Pests in the Country.

The present study revealed that, four species were found in association with mango and guava in the study sites. Them were, *Bacterocera invadens*, *Ceratitis cosyra, C. quinaria* and *C. capitata.* Among these, *B. invadens* was found to be the dominant in all study sites in the different States.

In a previous study, **Magid (2010)** reported that, *B. invadens* was the dominant species in mango, guava and citrus production areas of the River Nile State (Shendi area). Also, in another study in River Nile State, **Keikha (2011)** indicated that, the fruit flies species found in Shendi, Al Zaidab, Al ketiab and Al Bawga areas were, *B. invadens*, *C.cosyra* and *C. capitata*, with *B. invadens* as the dominant one. In a study in Khartoum State , **Bashir (2010)** reported that, *B. invadens* and *C. cosyra* were the main species found infesting mango fruits in Al Faki-Hashim area (Khartoum North), with *B. invadens* as the most prevalent species. Also, **El-Araky *et al.*, (2012)** in their study revealed that, B. *invadens* was the most dominant species of fruit flies in Gazira State. In a study in the Blue Nile State, **Fadlelmula and Ali (2014)** indicated that, both collection of infested fruits and trapping with "Methyl Eugenol (ME)" were used to monitor and detect the fruit flies in Five Localities in the State (e.g., Damazeen, Rouseries, Baw, Kurmuk and Geissan). Five species of fruit flies were found, viz., *B. invadens, C.cosyra, B. cucurbitae, Dacus ciliatus* and *D. Longistylus.* In all areas, *B. invades*, was the dominant species. The main host plants recorded in the State were mango (*Mangifera indica*), guava (*Psidium guajava*), grape fruit (*Citrus paradise*), banana (*Musa* spp.), papaya (*Carica papaya*), cantaloupe (*Cucumismelo*), brazilia (*Terminalia braziliansis*), Usher (*Calotropis procera*) and wild strawberry (*Fragaria vesca*).

Although *B. invadens* was recorded and identified in Sudan in recent years **(Drew *et al.*, 2005)**, it seems that, this species is spreading fast and replacing the already existed species in many States, as shown in this study, and as indicated in the above mentioned studies. These results illustrate the phenomena of displacement of fruit flies to each other as a result of competition.

In the last century, the most notable examples of these phenomena were the displacement of *C. capitata* by the Queensland fruit fly *Bacterocera tryoni*

(Froggatt) around Sydney area in Australia **(Debach, 1966)**, and displacement of the same species by *Bacterocera dorsalis* (Hende) from the Coastal areas in Hawaii in 1945 **(Duyck and Quilici, 2002)**. Recently, **Ekesi *et al.*, (2009)** also referred to this phenomenon, as they stated that, within 4 years of invasion, *B. invadens* displaced C. *capitata* and C. *cosyra*, and became the dominant fruit fly pest of mango in Kenya.

In their review on the relationship between competition and invasion of fruit flies, **Duyck *et al.*, (2004)** indicated that, the fruit flies of *Bacterocera* species are polyphagous and encounter interspecific competition with other polyphagous Tephritid flies that already well-established in an area. In the case of the displacement of *B. invadens* in Kenya, **Ekesi *et al.*, (2009)** suggested that, there are two possible mechanisms responsible for the displacement, namely, resource competition by the larvae within the mango fruits and the aggression behavior noticed between the adult flies. Also, here in Sudan, **Khair *et al.*, (2015)** stated that, the predominance of *B. invadens* over other species was related mainly to its polyphagous nature, short life cycle, high fecundity and high sex ratio.

In conclusion, it can be stated that, the recent studies on fruit flies, in River Nile State by **Khair *et al.*, (2015)**, in Khartoum, Kassala and South Kordofan States by **Mahmoud *et al.*, (2016)** and in Khartoum State, by **Sidahmed *et al.*, (2017)**, in addition to the present study, again confirmed the gradual displacement of the invasive fruit fly *B. invadens* for all fruit fly species common in these States.

5.1.2 Sex Ratio's of Fruit flies Identified species:

In the present study, the females of *B. invadens* found to outnumber the males by three to four folds (i.e., the sex ratio ranged between 3:1- 4:1), while females of other Ceratitis species were two to five (2:1- 5:1), folds of the males. This finding was

supported by **Ahmed (2001)**, who reported that, the sex ratio of *C. cosyra* was found to be 4:1. Also, **Mohamed (2005)** reported a similar sex ratio of 4: 1 for *B. invadens*. **Rendel *et al.*, (1995)** stated that, females of the fruit fly *C. cosyra* outnumbered the males by five times (i.e., 5:1). **Magid (2010)** reported that, females of *B. invadens* and *C. cosyra* always found to outnumber males by at least four folds (i.e., 4:1), compared to three folds (i.e., 3:1) for *C. capitata* males.

5.2 Feaces Extract Tests:

5. 2. 1 Effect of Natural Pheromone (Faeces extract) on Fruit Fly Oviposition:

In search for the proper methods for fruit fly control, a number of studies in some parts of the world (e.g., **Aluja *et al.*, 2003**) pointed to the pheromone(s) deposited by some females in order to prevent oviposion by other females in the same fruit, as a means of protection for their progeny. The studies indicated the importance of these pheromones, called host marking pheromones (HMP), their isolation and identification, and the possibility of their application as a method of fruit fly control (e.g., **Aluja *et al.*, 2009**).

Accordingly, the present study was initiated during the last period in order to identify the HMP of the main species in the country, the invasive fruit fly *B. invadens*, and its application as a means of fruit fly control. As indicated in Part II of the study, the results of the preliminary application of the crude extracts of the pheromone indicated that, it has a positive effects on reducing the oviposition of *B. invadens* females on the treated fruits, compared with the control (**Tables 5-10 and figures 14-19**).

At that time, and according to those promising results, the main objectives were to continue the study for the isolation and identification of the HMP of *B. invadens*.

These results are not surprising, since the application of extracts of *Ceratitis capitata* faeces to coffee berries generate similar oviposition deterrent effects (**Arredondo and Dı´az-Fleischer, 2006**). In that study, the laboratory and field treatments of halves of coffee bushes with methanolic extracts containing 0.1, 1.0 and 10mg faeces ml^{-1} resulted in a significant reduction of infestation by *C. capitata* only at the highest concentrations. The authors concludded that, these results indicated a clear potential for the use of this infochemical in integrated management programmes targeted at this pest. In another study, **Aluja *et al.*, (2009)** demonstrated that, spraying parts of tropical plum and mango trees with faeces extracts significantly reduced fruit infestation by *Anastrepha oblique* by 94.1% when measured 8 days after application. Also, application of the synthetic analogue the HMP of *A. ludens*, Anastrephamide, resulted in fruit loss cut by half and an 80% reduction in numbers of fly larvae per fruit.

Kachigamba *et al.*, (2012) investigated conspecific and heterospecific oviposition host discrimination among four economically important fruit fly pests of mango in Africa. Observations were done on mango slices marked by the flies and treated with aqueous solutions of faecal matter of the flies, respectively. In both host-marking and fecal matter experiments, *C. cosyra*, which is the most destructive species of the four on mango, was exceptional. It only discriminated against hosts treated with its fecal matter but with lower sensitivity, while *C. capitata* and *C. fasciventris* discriminated against hosts marked by it or treated with its fecal matter and with higher sensitivity. The authors suggested that, these results provide evidence for potential of managing some of the major fruit fly species infesting mango in Africa using the host-marking pheromone of the mango fruit fly, *C. cosyra*.

5. 2. 2 Structural Elucidation of Feaces Containing Host Marking Pheromone:

The results of the experiments concerning this part of the study, using HPLC and GS-MS analyses demonstrated that, the feaces extract contain many compounds,

according to the development of chromatographs. In the first analysis, sample one showed 17 peaks, and sample 2 showed 32 peaks. The samples analysis by GS-MS showed the presence of 42 compounds.

The HMP of the African fruit fly, *Ceratitis cosyra*, was identified by **Cheseto *et al.*, (2017)** as glutathione (GSH). GSH which was isolated from the aqueous extract of adult female fecal matter. Extracts of the fecal matter were collected from both males and females of *C. cosyra* at different ages. Analysis by HPLC revealed the presence of a female-specific peak at retention time 4.5 min with UV absorption at λ max 220 nm. Moreover in bioassays, synthetic GSH reduced oviposition responses in conspecifics of *C. cosyra* and the heterospecific species, *C. rosa, C. fasciventris, C. capitata, and Zeugodacus cucurbitae*.

In another study by **Cheseto *et al.*, (2018)**, using a bioassay-guided approach, the HMP of the Natal fruit fly species *Ceratitis rosa* was identified as glutamic acid from the aqueous fecal matter extract of ovipositing females. This was done by "Liquid Chromatography−Quadrupole Time-Of Flight−Mass Spectrometry" (LC-QTOF-MS) from the column at 1.7 min with a molecular ion peak [M+H] + at m/z 148.0607, corresponding to a molecular formula of $C_5H_{10}NO_4$. The amino acid identity was supported by the presence of the expected fragments with mono isotopic mass ions at m/z 102.0549 [M+H−HCOOH] + and 130.0503 [M+H−H2O] +,26. The identity was confirmed by comparison of mass spectrometric data, retention time, and co injection of the natural extract with an authentic standard. Glutamic acid levels were 10−20 times higher in fecal matter than in the ovipositor or hemolymph extracts of the females.

Chemical analysis of the aqueous fecal matter extracts of both females and males, by LC-QTOF-MS, identified glutamic acid, 1, as specific to the fecal matter extract of females.

In the previous results of this part of the study, the extracts of the natural pheromone demonstrated clear efficiency in reducing oviposition by *Bacterocera invadens* females.

As indicated above, the natural pheromone extracts contain many compounds. The results of **Cheseto *et al.*, (2018)**, identified 11 compouns in the faecal extracts of the males and females of *Ceratitis rosa.* At this point, it was obvious that, for the present study, there was a need for more advanced chemical analysis of the pheromone extracts, to identify the specific compounds responsible for reducing oviposition responses.

5.3 Fruit fly Traps Evaluation:

5. 3 . 1 Sex pheromone traps catchability:

In 2015, as pointed out in the study, the farmers in the study sites were facing a problems through increasing fruit fly infestation and a reduction in fruit production.

The mean number of fruit flies trapped showed no significant difference in all traps in the different sites except Elkadaroo. The range of cached flies per trap per week was " 4 to 440" from June to July 2016 and February to March 2017. In all sites, the catchability was generally low and the damage was high.

Sabah (2015) , working in the same sites (Al-kadaroo, Al-khogalab and Alfaki-Hashim) using the same type of traps , from January to April 2015, catched flies per trap per week between 305 to 5952. More results were reported by **Hassan (2015)** in Shendi Area during the period of April 2012- April 2014, where the catcha bility per month ranged between 654-1893. The present results showed a

decrease in fruit flies numbers trapped by locally manufactured Lynfield trap baited with Methyl Eugenol, mixed with Malathion (57%). The reasons for reduction in catchability could be due to either a resistance to Malathion, which has been used since 2008, or an invasion of a new species of fruit flies in the study sites.

5. 3 . 2 Evaluation of Different Traps Efficacy:

In the investigation for solutions of these problems, and through the continuous monitoring of fruit flies, a new species was found invading the study sites in large numbers. The identification showed that, the species is the peach fruit fly, *Bactrocera zonata*, which was recorded for the first time in that area. In addition, the continuous application of different pheromone and food traps indicated that, this new species became the dominant in all study sites, compared with the few numbers found other species of fruit flies (**Tables No. 20 -31 and Figures No. 30- 41**)

These experiments were carried out to investigate the reasons of the decrease in traps catch- ability. The results showed that, the Sticky Traps catched the highest mean number of trapped fruit flies (800), followed by Lynfield trap baited with Methyl Eugenol mixed with Malathion (57%) and Guava fruit juice (478 flies), Lynfield trap baited with Methyl Eugenol mixed with Cypermethrin with Guava fruit juice (210 flies), Lynfield trap baited with Methyl Eugenol mixed with malathion (57%) (151 fies) and Lynfield trap baited Methyl Eugenol mixed with

Cypermethrin, which catched 59 flies. During checking of the traps and counting the numbers of flies, a new species of fruit flies was noticed, as its numbers were larger in every catch. It was then taken for identification. By the assistance of the Entomology researchers at the ARC, this species was identified as *Bacterocera zonata*. This was the first time for *B.* zonata to be recorded in the study sites.

The first report of *B. zonata* in Sudan was in July 2011, as the flies were detected in 3 locations , in Wad Medani, Elkamlin and Singa; where the species was caught in small numbers, in addition *B. invadens* , which was also caught in those sites **(Salah *et al*., 2012)**. Other studies undertaken by Mahmoud et. al., (2016) , also, reported that, *B. zonata* was recorded in the Northern, River Nile, Khartoum, Kassala, Gezira, Gedarif and White Nile States, co-existing with *B. dorsalis* in surveys carried out from 2014 to 2015.

5. 3 . 3 Evaluation of two types of locally manufactured Traps Efficacy:

In these experiments, the comparison of the " 2 litter plastic bottle trap (2^{nd} Trap) " with the " locally manufactured Lynfield trap (1^{st} Trap)" resulted in a range of catched flies per trap per week, which were between (28 to 75) for the 1^{st} trap and (863 to 1659) for the 2^{nd} trap.

The new design of the "2 litter plastic bottle trap", with one upside down hole make it difficult for the flies to get out of the trap so be exposed to the pesticide for

long time. *Bactrocera zonata* was reported to be resistant to Malation in some studies and this is why the catchability of traps decreased.

Nadeem *et al*., (2014) Insecticides resistance against fourteen field populations of *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) from Chichawatni, District Sahiwal, Pakistan to six insecticides *viz.*, trichlorfon, malathion (Organophosphates), bifenthrin, lambda-cyhalothrin (Pyrethorids) , methomyl (Carbamate) and spinosad (Microbial) was assessed by topical assay under laboratory conditions. In insecticides bioassay, trichlorfon was observed susceptible to high resistance level (1.01-fold to 41.13-fold), bifenthrin and malathion were found susceptible to moderate resistance level (1.00-fold to 14.27-fold and 1.00-fold to 20.37-fold), lambda-cyhalothrin and spinosad were showed susceptible to low resistance (1.00-fold to 9.57- fold and 1.20 -fold to 9.95-fold), while effect of methomyl were remained as susceptible to all the tested populations.

From the results it is concluded that methomyl was remained susceptible to all the tested populations, while other five tested insecticides have developed the resistance against *B. zonata* populations, which required adopting new strategies to overcome resistance in this pest.

Also **Radwan (2012)** reported that, the management of *B. zonata* has been based on the use of malathion (organophosphate insecticide), a practice that induced resistance. The high resistance ratio (RR=30.47 fold) and resistance coefficient (RC=75.33) to malathionwere detected in a field population of *B. zonata* compared with the laboratory susceptible strain. More results by **Ahmad *et al* (2010)** showed that *B. zonata* from Multan and Faisalabad zones were resistant to trichlorfon, malathion, lambda-cyhalothrin and bifenthrin ranging 3-19 fold, however,

population from these places were susceptibility to spinosad. Malathion registered resistances ratio (3-6 fold) less than bifenthrin (8-11 fold), trichlorofon (10-19 fold) and cyhalothrin (4-9 fold). The data suggest that B. zonata has developed resistance to trichlorfon, and indicate a danger for its use as cover spray and in baits.)

5. 3 . 4 Species monitoring using different sex pheromones:

This issue represented a big problem for the present study, which was carried out during two years, aiming to identify the HMP of the *B. invadens*, which lead to the stop of the research at that point.

It is worth here to mention that, this problem of displacement of fruit flies is a continuous matter and was recorded in some areas in the world during the last decades (e.g., **Duyck and Quilici, 2002, Ekesi *et al*., 2009**). Therefore, in Sudan, this phenomenon was clearly represented previously, by the displacemrnt of many species in sudan (for example, *Ceratitis cosyra*) by *B. invadens* since its discovery in the country in 2005 (e.g., **Sideamed *et al* 2017**). At present, again the matter is repeated by the displacement of *B. invadens* by *B. zonata*. Also, the dominance of *B. zonata* was recorded in other areas in Sudan (e.g., **Alarky *et al*., 2012; Mahmoud *et al*. 201 and Taha, *et al*., 2018**-Unpublished Report)

Methyl Eugenol plugs baited traps showed the highest number of trapped insects followed by Trypenil Acetate, while Cuelure and Trimedlure traps catched nothing. Trapped insects were *Bactrocera zonata, Ceratitis quiaria* and *C. cosyra.* The dominant species which has been found in traps baited with Methyl Eugenol plugs was *B. zonata*. these results shows the displacement to B. invadens , the dominant species before 2015.

Abdel-gader and Salah (2016) repoted that, a survey was initiated to determine the abundance of *Bactrocera zonata* in relation to *Bactrocera dorsali*s at various periods in three different locations in Wad Madani, Gezira State, Central Sudan. The proportions of *B. zonata* were also recorded in various directions at different dates in one location. The study aimed to investigate any tendency of *B. zonata* to displace *B. dorsalis* in Central Sudan. The results indicated an increase in the proportion of *B. zonata* in the total catch during the mango fruit ripening period (April to June). By the end of June 2012, *B. zonata* represented more than 90% of the catch in northern orchards of the surveyed area. In southern orchards, the proportion was 50% in June. The same trend over time was observed during the same period in 2014, where the proportion was around 70% for *B. zonata* in northern orchards and less than 50% during May and June in southern orchards. The proportion of *B. zonata* was found to be more than 50% by the end of June 2014 in three directions in one of the northern orchards. The results of the study

may indicate the ability of *B. zonata* to displace *B. dorsalis* in some parts of Central Sudan during the mango fruit ripening period (April – June). More results were recorded by **Mahmoud *et al.*, (2016)** in the Northern, River Nile, Khartoum, Kassala, Gezira, Gedarif and White Nile States, co-existing with *B. dorsalis*. The relative abundance of *B. zonata* to the total of both fruit flies in Methyl Eugenol traps in the different Stastes were : 51-100%, 0.4-0.5%, 24.5%, 0.4%, 36.3, 1-66%, 1.7% and 0.2-100% , for each State respectively **(Mahmoud *et. Al.*, 2016)**. Nevertheless, as this matter of fruit fly control is of prime importance in this country, it means that, the whole work should be repeated again and should be directed now to concentrate on the identification of the HMP of *B. zonata*, the dominant species at the present time.

All the results of Part Three of the present study, and as indicated above, refer to the dominance of that species. Therefore, it is better to take that chance in a new study, for identify the HMP of this species, hopefully to obtain a new compound of importance in the management of this invasive pest.

Conclusion

- Monitoring of Tephritid fruit flies in study area revealed the existence of four fruit fly species, under the genus *Ceratitis* and *Bactrocera*. These are: Asian fruit fly, *Bactrocera invadens,* Mango fruit fly, *Ceratitis cosyra,* Mediterranean fruit fly, *Ceratitis capitata* and Rhodesian fruit fly, *Ceratitis quinaria* .
- *B. invadens* is the dominant species in Study area.
- Previous studies revealed that, *Bactrocera invadens* displace *Ceratitis capitata* and *Ceratitis cosyra* and become the predominant fruit fly pest in Sudan.
- *The present study showed that B. invadens displace ceratitis spp. in study site.*
- Methanolic extract of *B. invadens* feaces significantly deterred females from oviposition.
- These results provide evidence for potential of managing some of the major fruit fly species *B. invadens* using the host-marking pheromone.
- Decrease in fruit flies numbers trapped by locally manufactured Lynfield trap baited with Methyl Eugenol, mixed with Malathion (57%) could be ascribed either a resistance to Malathion, which has been used since 2008, or an invasion of a new species of fruit flies in the study sites.

- Evaluation of different traps types showed that, the Sticky Traps catched the highest mean number of trapped fruit flies, followed by Lynfield trap baited with Methyl Eugenol mixed with Malathion (57%) and Guava fruit juice.
- The new design of the "2 litter plastic bottle trap", with one upside down hole make it difficult for the flies to get out of the trap so as be exposed to the pesticide for a longer period of time thats why its catchability is significuntly higher than the localy manufactured Lynfield trap.
- After more investigations in Khartoum state using different pheromone types a new spescies *Bactrocera zonata* was found to be completely displaced *B. invadens*

Recommendations

- The promising results of feaces containing pheromone indicated a clear potential for the use of this infochemical in integrated management programmes targeted at this pest.
- More investigations on the isolation and structure determination of the host marking pheromone specially critical for the dominant species.
- Structure elucidation results varied depending on the method used, at this point, it was obvious that, for the present study, there was a need for more advanced chemical analysis of the pheromone extracts.
- Identification of the active compounds that deter oviposition should studies and determined so as to Synthesize the pheromone.
- Applications of synthesized Pheromones as apart of IPM of fruit flies is highly recommended to get the maximum benefit.
- Monitoring of the fruit flies using Pheromone traps is necessary for detecting the presence of the species found and their abbundance, because the species and their populations are continuously changing.
- 2 Liter Plastic Bottle traps and sticky traps with their simple design and high cach ability are recommended for use by farmers in mass trapping.
- More work in mechanism of competitive displacement of invasive species should be conducted.

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APPENDICES

Appendix No. 1: Percentage of fruit fly species emerged from fruits collected from different sites (17th of February to 20th of March 2010)

Site	*B. invadens*			*C. cosyra*			*C. capitata*			*C. quinaria*		
	1	2	3	1	2	3	1	2	3	1	2	3
1. Bahri Central Market (Guava)	51	67	56	5	3	7	30	26	27	14	4	10
2. Elkadaro (Guava)	57	66	59	10	9	11	27	21	20	6	4	10
3. Elfaki-Hashim (Guava)	87	80	84	10	20	15	0	0	0	3	0	1
4. Khartoum Central Market (Guava)	82	88	76	5	0	10	13	12	14	0	0	0
5. Kasala (Guava)	98	98	94	0	0	0	2	2	6	0	0	0
6. Singa (Mango)	95	89	90	5	8	8	0	3	2	0	0	0
7. Singa (Guava)	97	75	82	0	5	5	3	20	13	0	0	0
8. Sinnar (Mango)	95	87	92	5	10	5	0	3	3	0	0	0

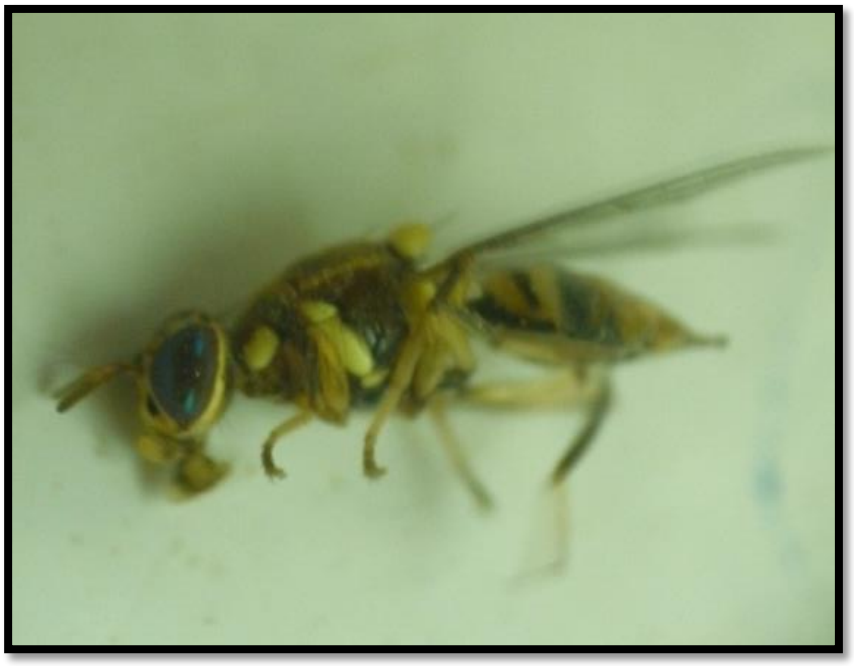
Appendix No. 2: Percentage of fruit fly species emerged from fruits collected from different sites (19th of October to 23 of November 2010)

Site	*B. invadens*			*C. cosyra*			*C. capitata*			*C. quinaria*		
	1	2	3	1	2	3	1	2	3	1	2	3
1. Bahri Central Market (Guava)	85	79	81	0	0	0	10	14	15	5	7	4
2. Elkadaro (Guava)	91	74	84	0	0	0	7	21	11	2	5	5
3. Elfaki-Hashim (Guava)	86	80	77	7	20	18	0	0	0	7	0	5
4. Khartoum Central Market (Guava)	90	81	94	0	0	2	9	19	4	1	0	0
5. Kasala (Guava)	95	91	88	0	0	0	5	5	10	0	4	2
6. Singa (Guava)	89	84	79	2	0	1	6	16	20	3	0	0
7. Sinnar (Guava)	85	97	92	15	3	5	0	0	3	0	0	0

Appendix No. 3: Percentage of fruit fly species emerged from fruits collected from different sites (15th of February to 19th of March 2011)

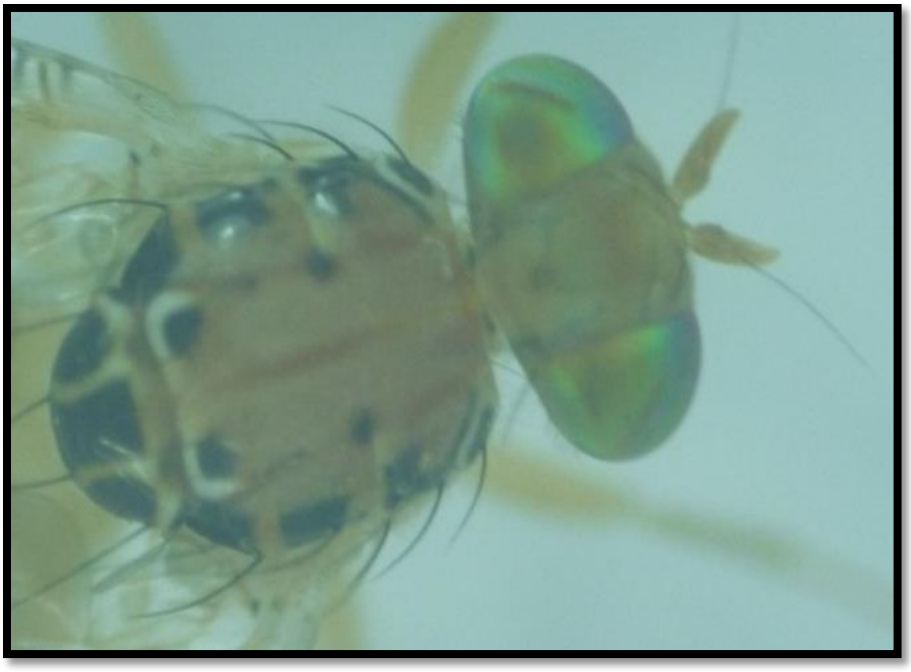
Site	*B. invadens*			*C. cosyra*			*C. capitata*			*C. quinaria*		
	1	2	3	1	2	3	1	2	3	1	2	3
1. Bahri Central Market (Guava)	95	74	78	0	0	0	0	12	9	5	14	3
2. Elkadaro (Guava)	77	89	94	0	0	1	10	7	5	3	4	0
3. Elfaki-Hashim (Guava)	92	85	88	0	2	0	7	8	9	1	5	3
4. Khartoum Central Market (Guava)	97	94	72	1	0	0	2	4	13	0	0	15
5. Kasala (Guava)	99	95	83	0	0	0	1	5	15	0	0	2
6. Singa (Mango)	94	90	81	3	10	15	3	0	4	0	0	0
7. Singa (Guava)	71	96	98	0	0	0	29	4	2	0	0	0
8. Sinnar (Mango)	93	89	87	7	11	9	0	0	4	0	0	0

Appendix No. 4: *Bacterocera invadens*, male and female (ventral view)

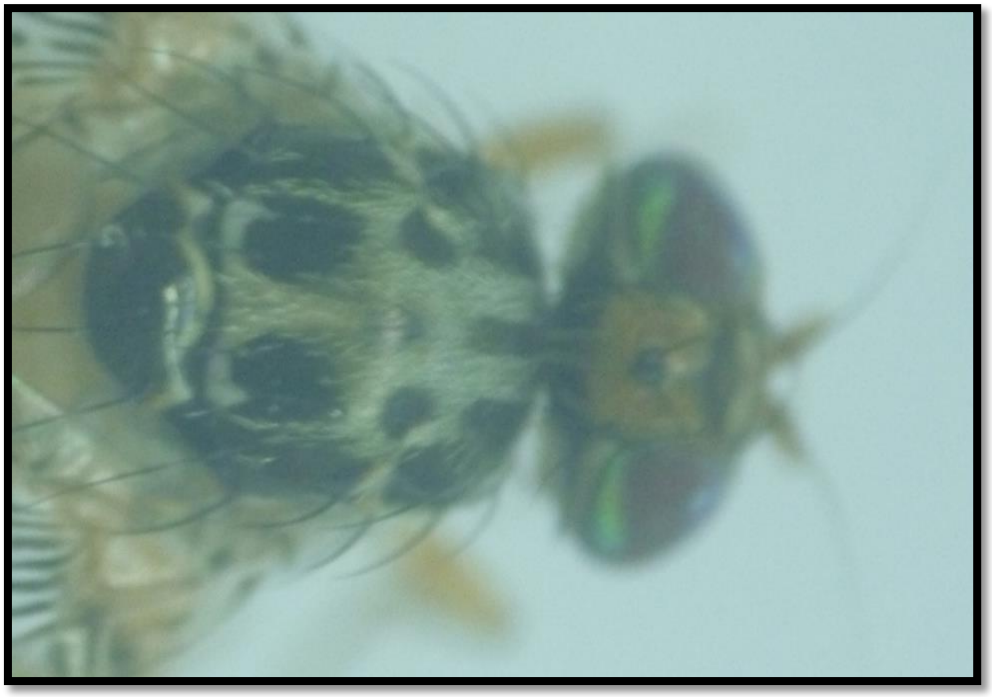


Appendix No. 5: *Bacterocera invadens* Female (Lateral View)

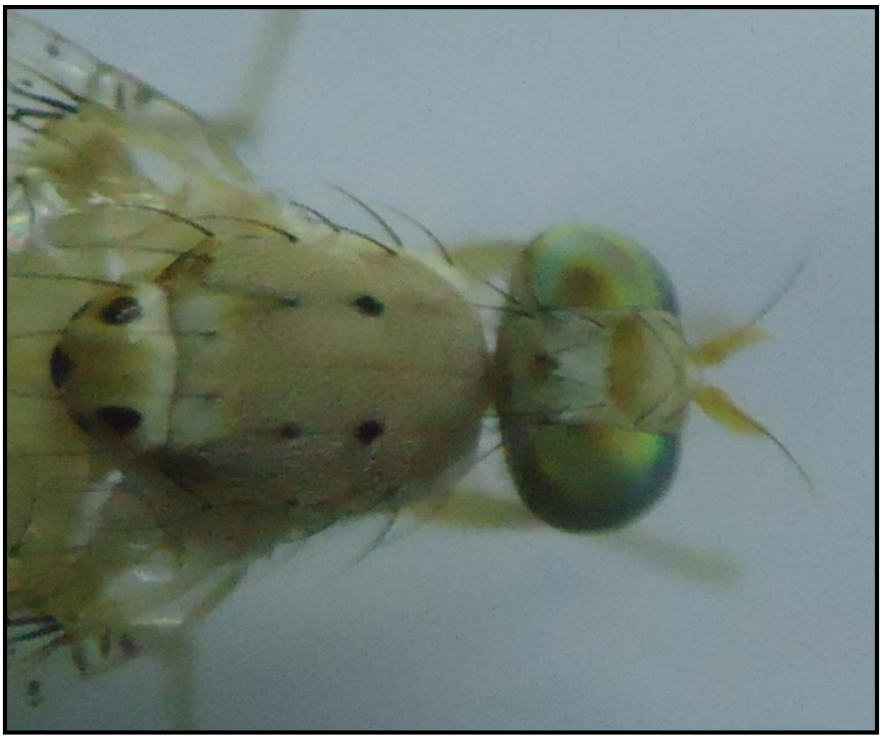
Appendix No. 6: *Ceratitis cosyra* Female (Lateral View)



Appendix No. 7: *Ceratitis cosyra* (Thorax Characteristics)



Appendix No. 8: *Ceratitis capitata* (Thorax Characteristics)



Appendix No. 9: *Ceratitis quinaria* (Thorax Characteristics)

Appendix No. (10): Effect of Natural pheromone in *B. invadens* oviposition in mango (Laboratory Experiments-Season 1)

Treatments	R1	R2	R3	R4	Total	Mean
2.5	17	15	16	16	64	16
5	12	10	14	14	50	12.5
10	0	0	0	0	0	0
15	0	0	0	0	0	0
Control	30	28	25	19	102	25.5

Appendix No. (11): Completely Randomized AOV for RESULTS Effect of Natural pheromone in *B. invadens* oviposition in mango (Laboratory Experiments-Season 1)

Source	DF	SS	MS	F	P
TREATMENT	4	1917.20	479.300	87.7	0.0000
Error	15	82.00	5.467		

Total	19	1999.20		

Grand Mean 10.800 CV 21.65

Appendix No. (12): Effect of Natural pheromone in *B. invadens* oviposition in mango (Semi field Experiment-Season 1):

Treatments	R1	R2	R3	R4	Total	Mean
2.5	14	10	12	14	50	12.5
5	9	10	9	8	36	9
10	0	0	0	0	0	0
15	0	0	0	0	0	0
Control	19	15	19	17	70	17.5

Appendix No. (13): Randomized Complete Block AOV Table for RESULTS Effect of Natural pheromone in *B. invadens* oviposition in mango (Semi field Experiment-Season 1):

Source	DF	SS	MS	F	P
REPLICATI	3	5.200	1.733		
TREATMENT	4	957.200	239.300	152.74	0.0000
Error	12	18.800	1.567		
Total	19	981.200			

Grand Mean 7.8000 CV 16.05

Appendix No. (14): Effect of Natural pheromone in *B. invadens* oviposition in mango (Laboratory Experiments-Season 2)

Treatments	R1	R2	R3	R4	Total	Mean
2.5	16	18	17	19	70	17.5
5	10	13	13	14	50	12.5
10	0	0	0	0	0	0
15	0	0	0	0	0	0
Control	27	29	26	30	112	28

Appendix No. (15): Completely Randomized AOV for RESULTS Effect of Natural pheromone in *B. invadens* oviposition in mango (Laboratory Experiments-Season 2)

Source	DF	SS	MS	F	P

TREATMENT	4	2294.80	573.700	359	0.0000
Error	15	24.00	1.600		
Total	19	2318.80			

Grand Mean 11.600 CV 10.90

Appendix No. (16): Effect of Natural pheromone in *B. invadens* oviposition in mango (Semi field Experiment-Season 2):

Treatments	R1	R2	R3	R4	Total	Mean
2.5	13	12	12	14	51	12.75
5	8	7	9	8	32	8
10	0	0	0	0	0	0
15	0	0	0	0	0	0
Control	20	18	19	17	74	18.5

Appendix No. (17): Randomized Complete Block AOV Table for RESULTS Effect of Natural pheromone in *B. invadens* oviposition in mango (Semi field Experiment-Season 2):

Source	DF	SS	MS	F	P
REPLICATI	3	1.75	0.583		
TREATMENT	4	1042.80	260.700	391.05	0.0000
Error	12	8.00	0.667		
Total	19	1052.55			

Grand Mean 7.8500 CV 10.40

Appendix No. (18): Effect of Natural pheromone in *B. invadens* oviposition in Guava (Laboratory Experiments-Season 2)

Treatments	R1	R2	R3	R4	Total	Mean
2.5	15	14	14	14	57	14.25
5	12	10	9	12	43	10.75
10	0	0	0	0	0	0
15	0	0	0	0	0	0
Control	20	22	20	23	85	21.25

Appendix No. (19): Completely Randomized AOV for RESULTS Effect of Natural pheromone in *B. invadens* oviposition in Guava (Laboratory Experiments-Season 2)

Source	DF	SS	MS	F	P
TREATMENT	4	1369.50	342.375	360	0.0000
Error	15	14.25	0.950		
Total	19	1383.75			

Grand Mean 9.2500 CV 10.54

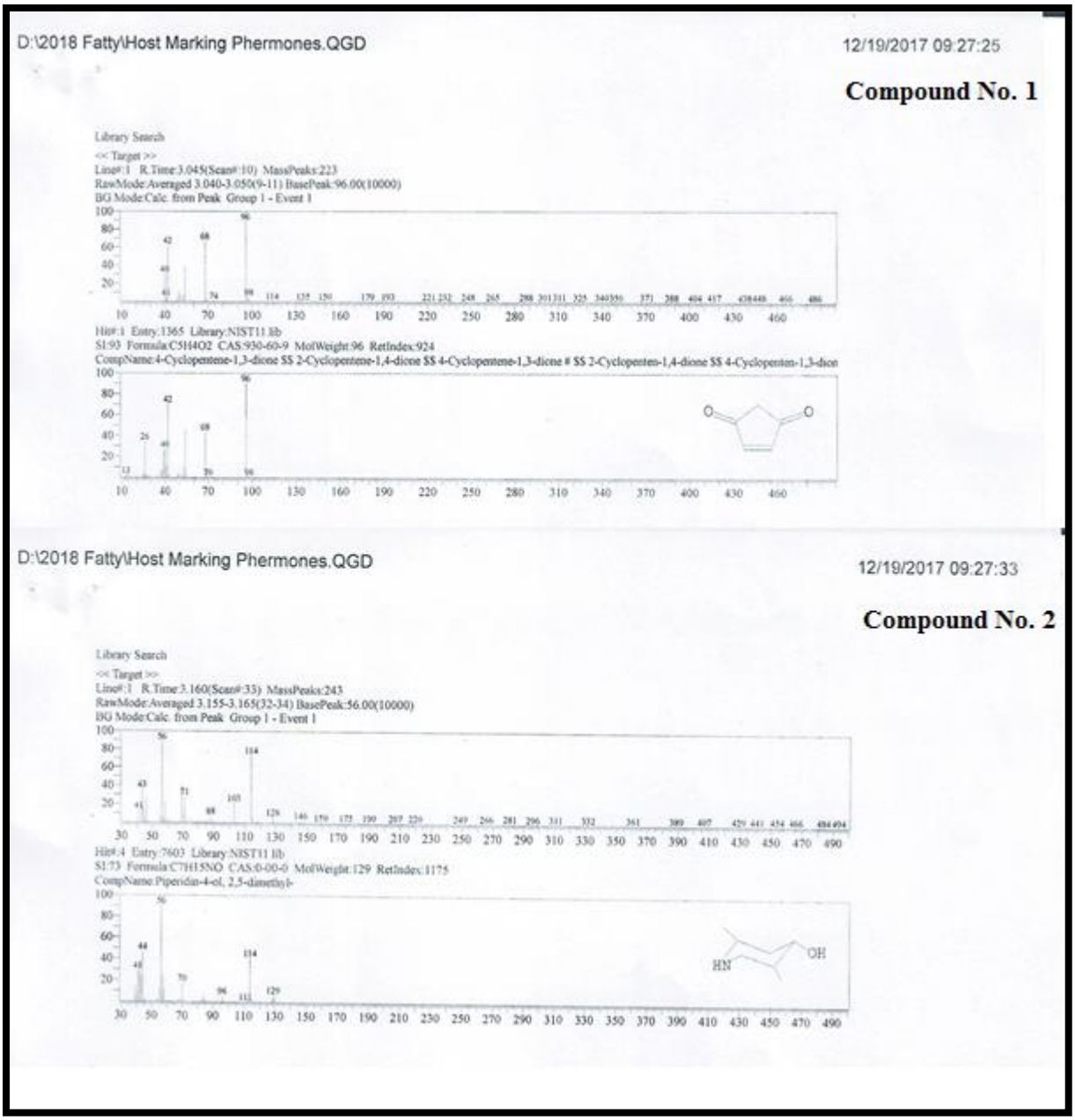
Appendix No. (20): Effect of Natural pheromone in *B. invadens* oviposition in Guava (Semi field Experiment -Season 2)

Treatments	R1	R2	R3	R4	Total	Mean
2.5	12	12	13	11	48	12
5	6	7	8	8	29	7.25
10	0	0	0	0	0	0
15	0	0	0	0	0	0
Control	16	15	14	12	57	14.25

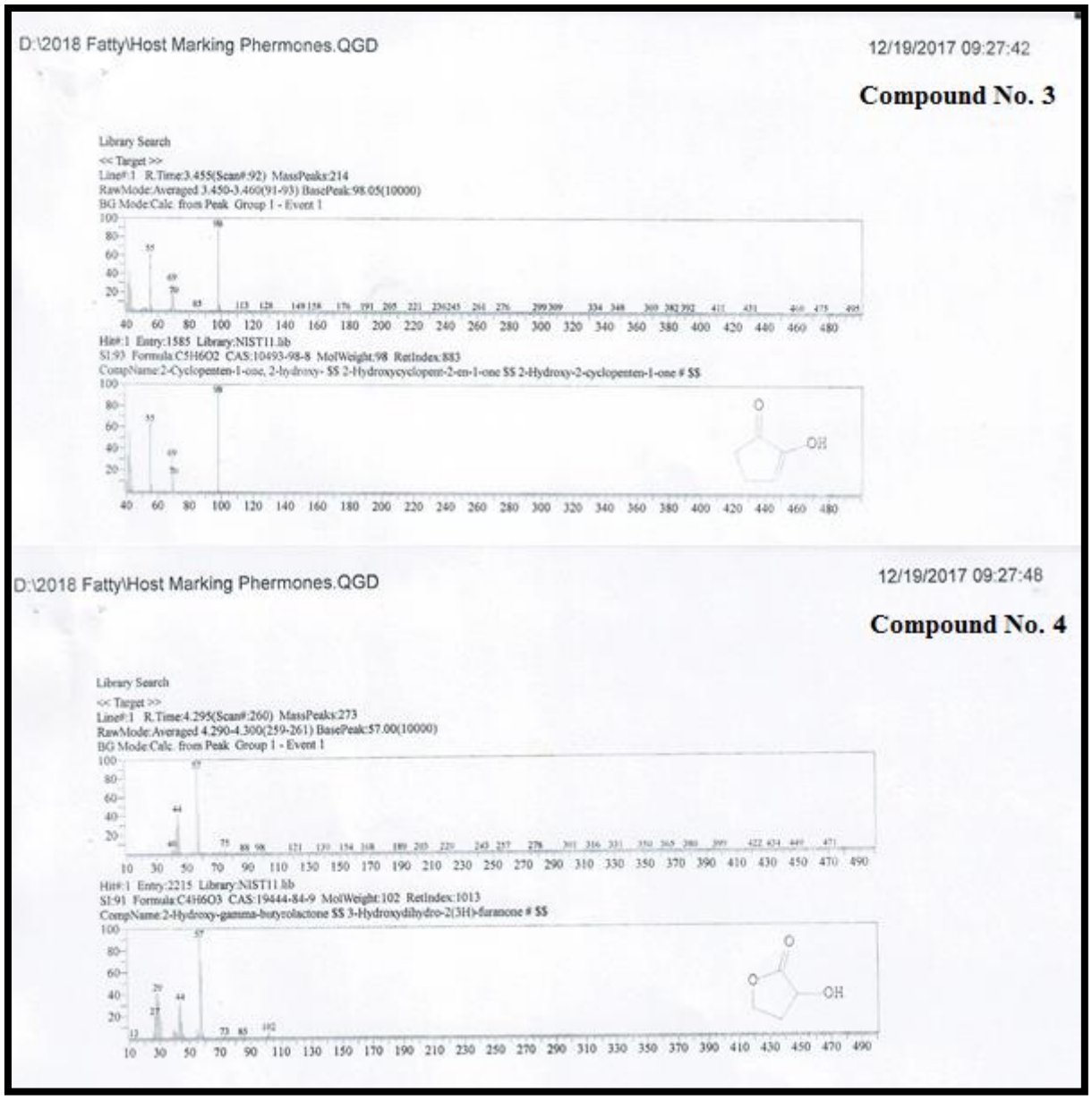
Appendix No. (21): Randomized Complete Block AOV Table for RESULTS Effect of Natural pheromone in *B. invadens* oviposition in Guava (Semi field Experiment -Season 2)

Source	DF	SS	MS	F	P
REPLICATI	3	1.800	0.600		
TREATMENT	4	700.700	175.175	179.67	0.0000
Error	12	11.700	0.975		
Total	19	714.200			

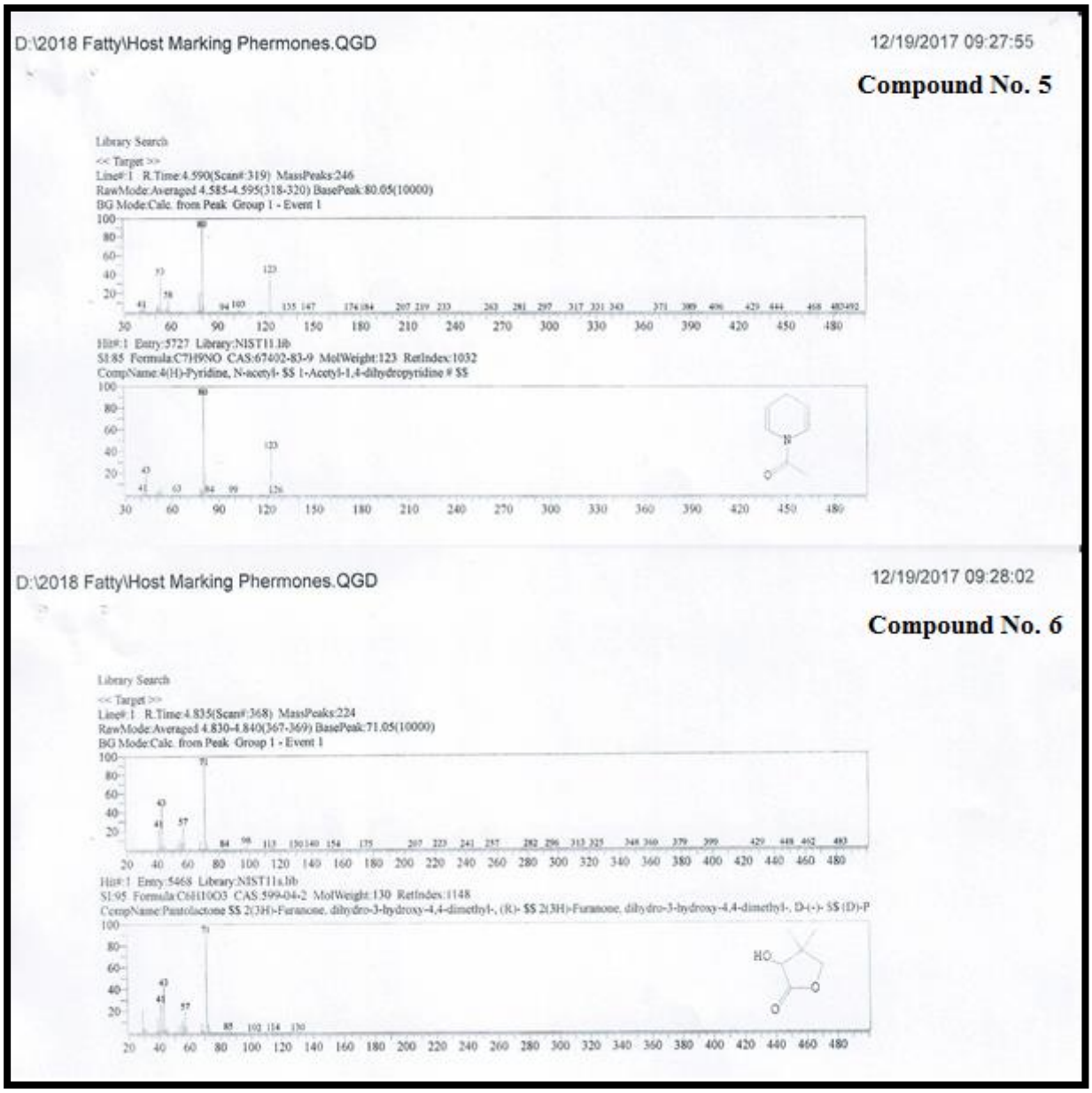
Grand Mean 6.7000 CV 14.74



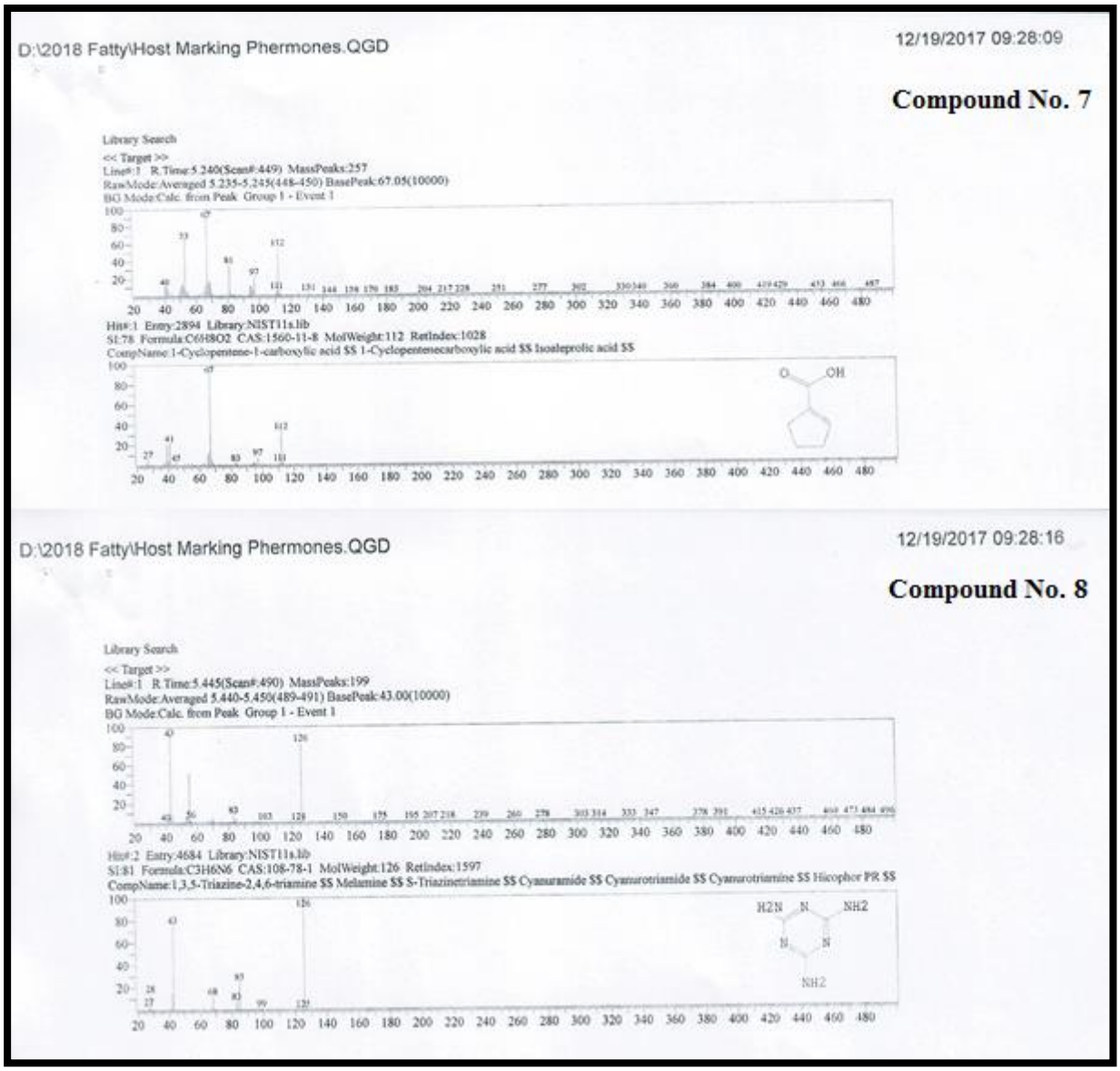
Appendix No.22 GC-MS compounds formulations



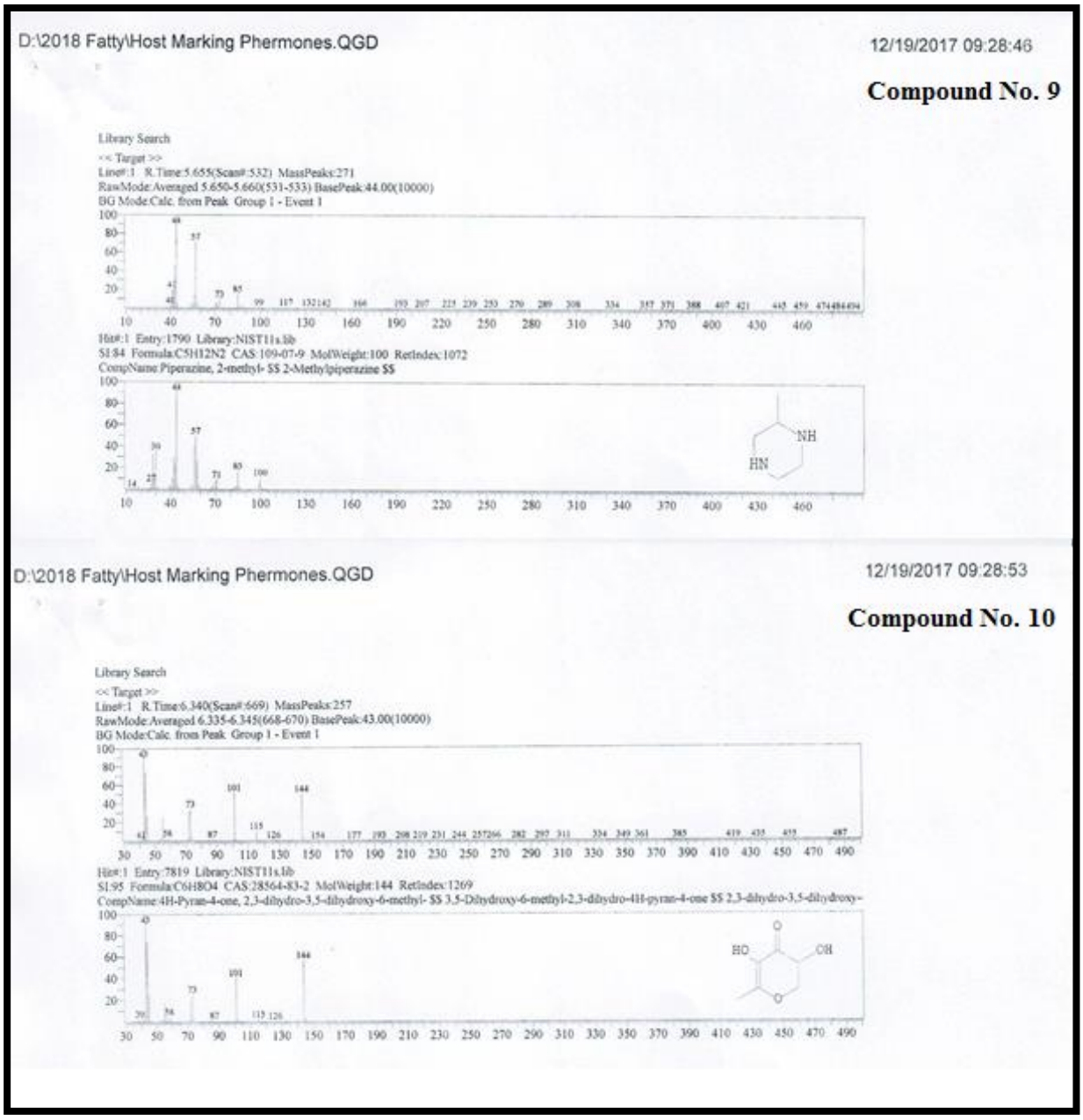
Appendix No.23 GC-MS compounds formulations



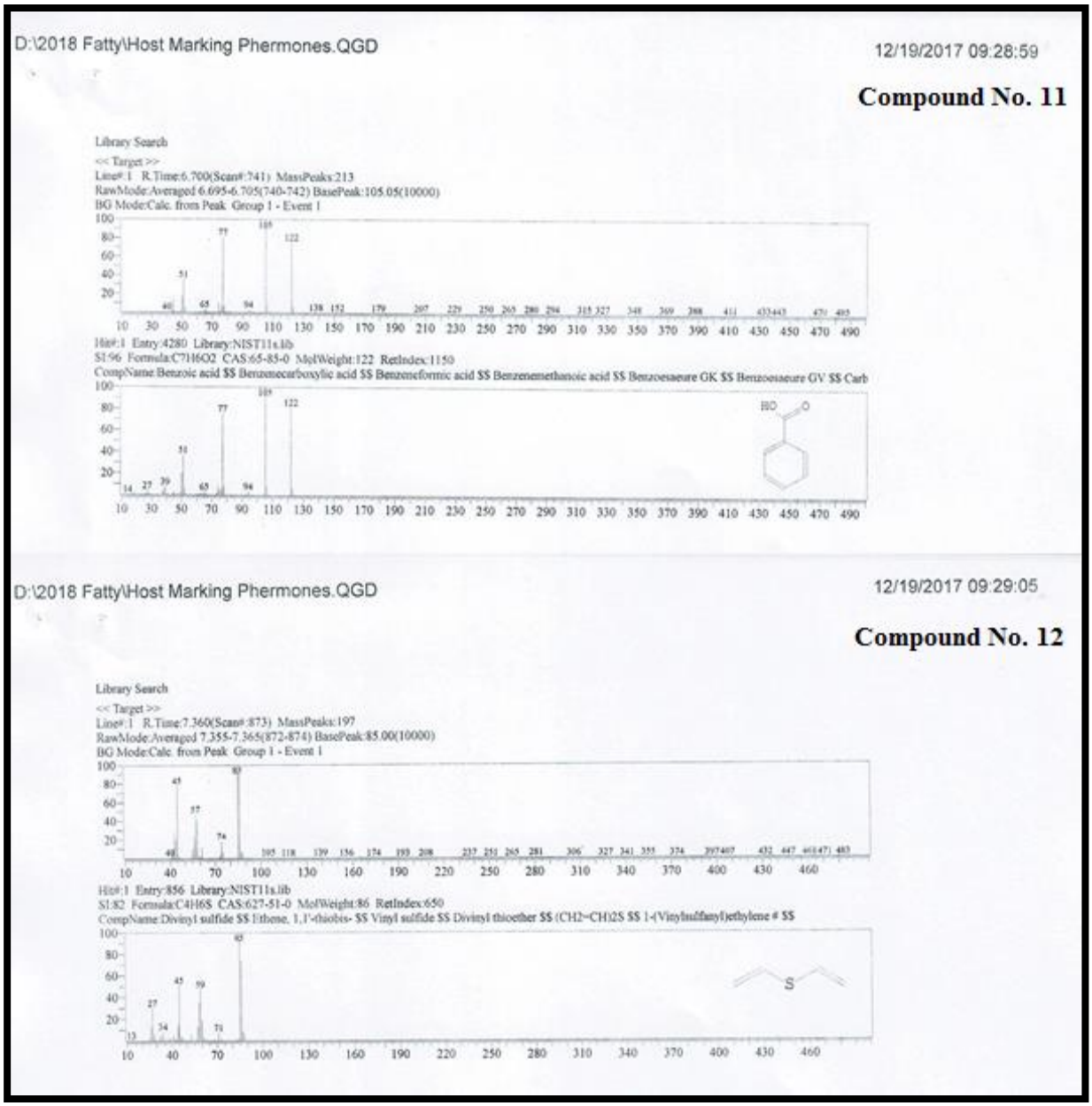
Appendix No.24 GC-MS compounds formulations



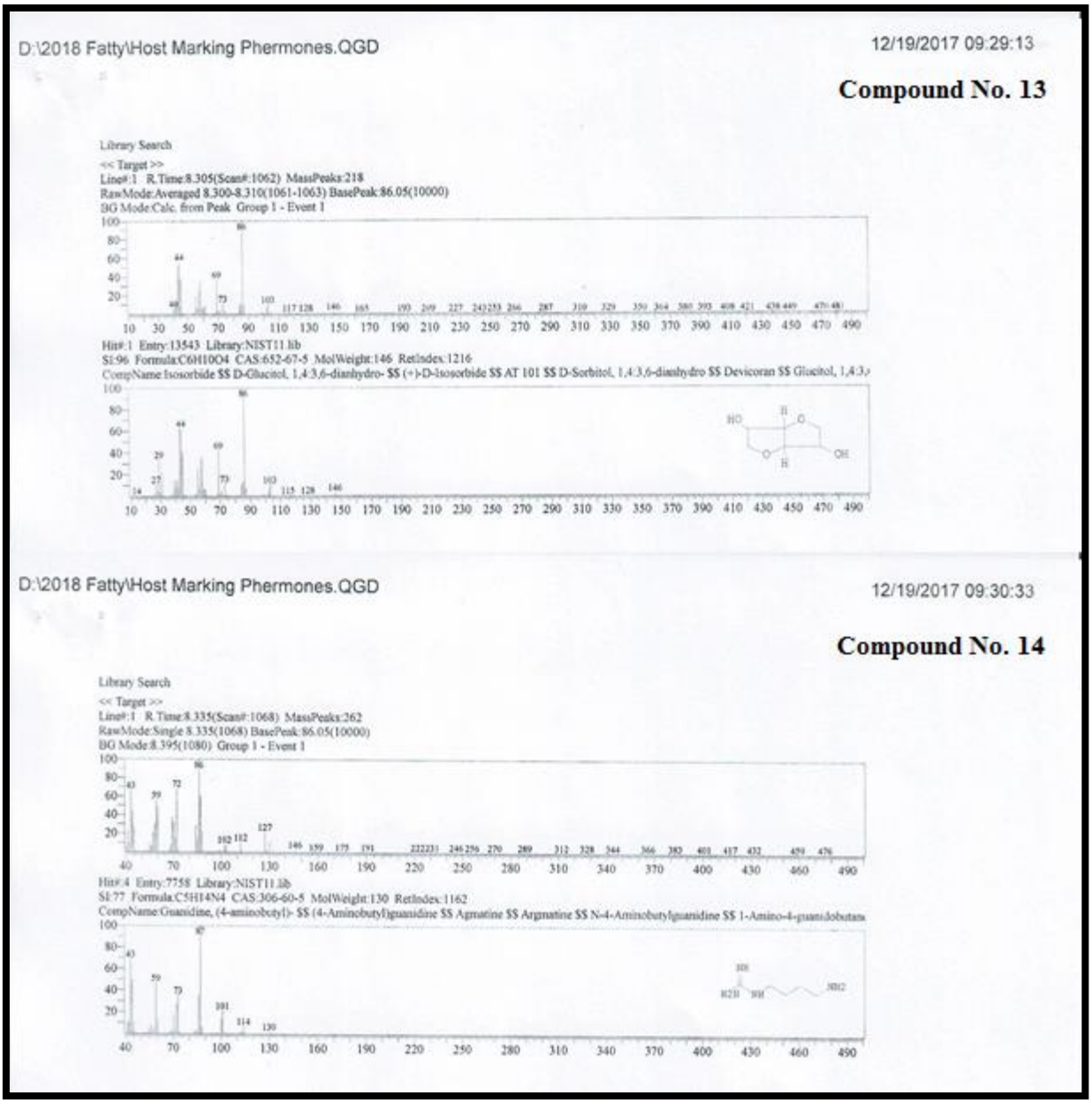
Appendix No.25 GC-MS compounds formulations



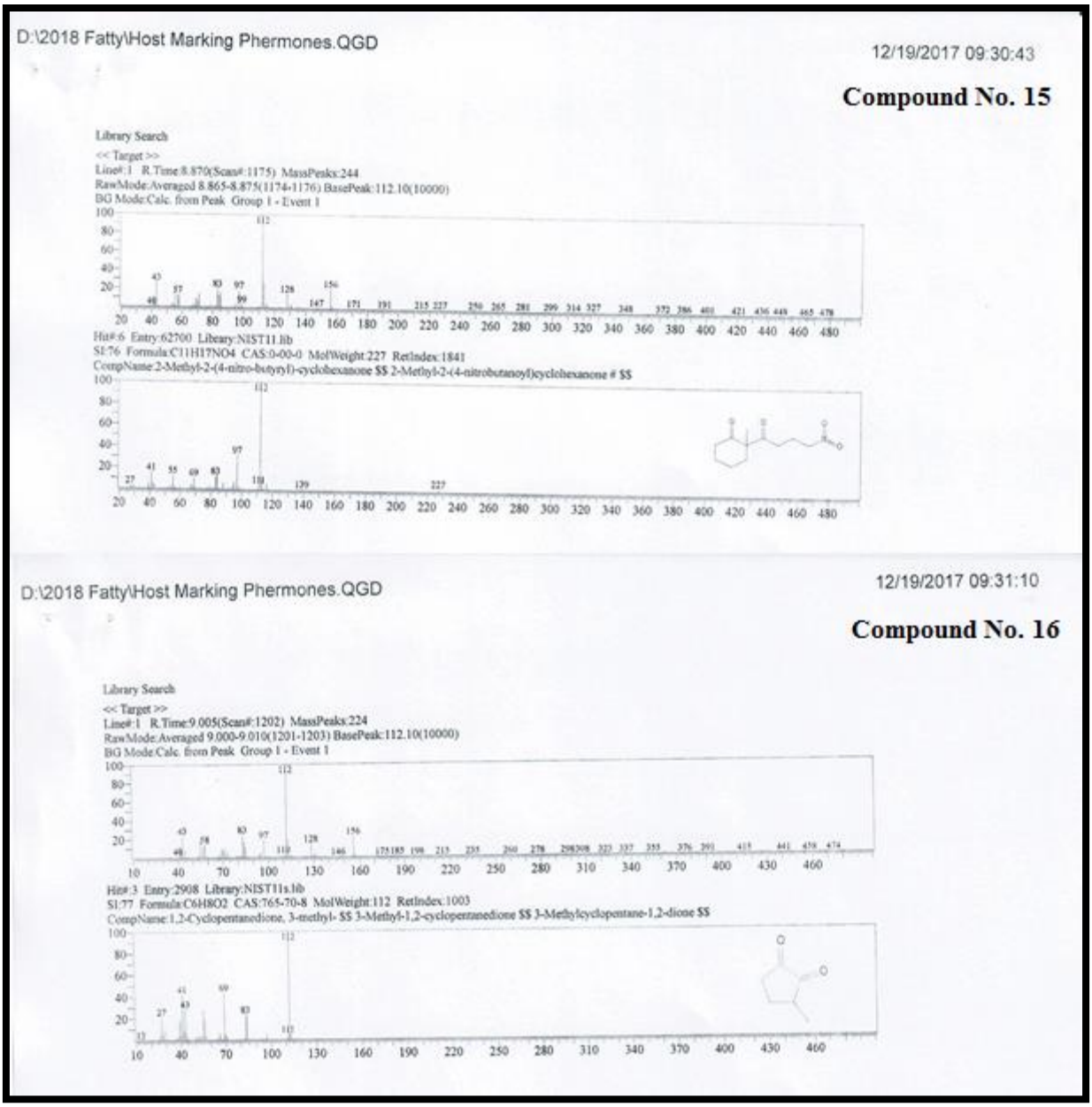
Appendix No.26 GC-MS compounds formulations



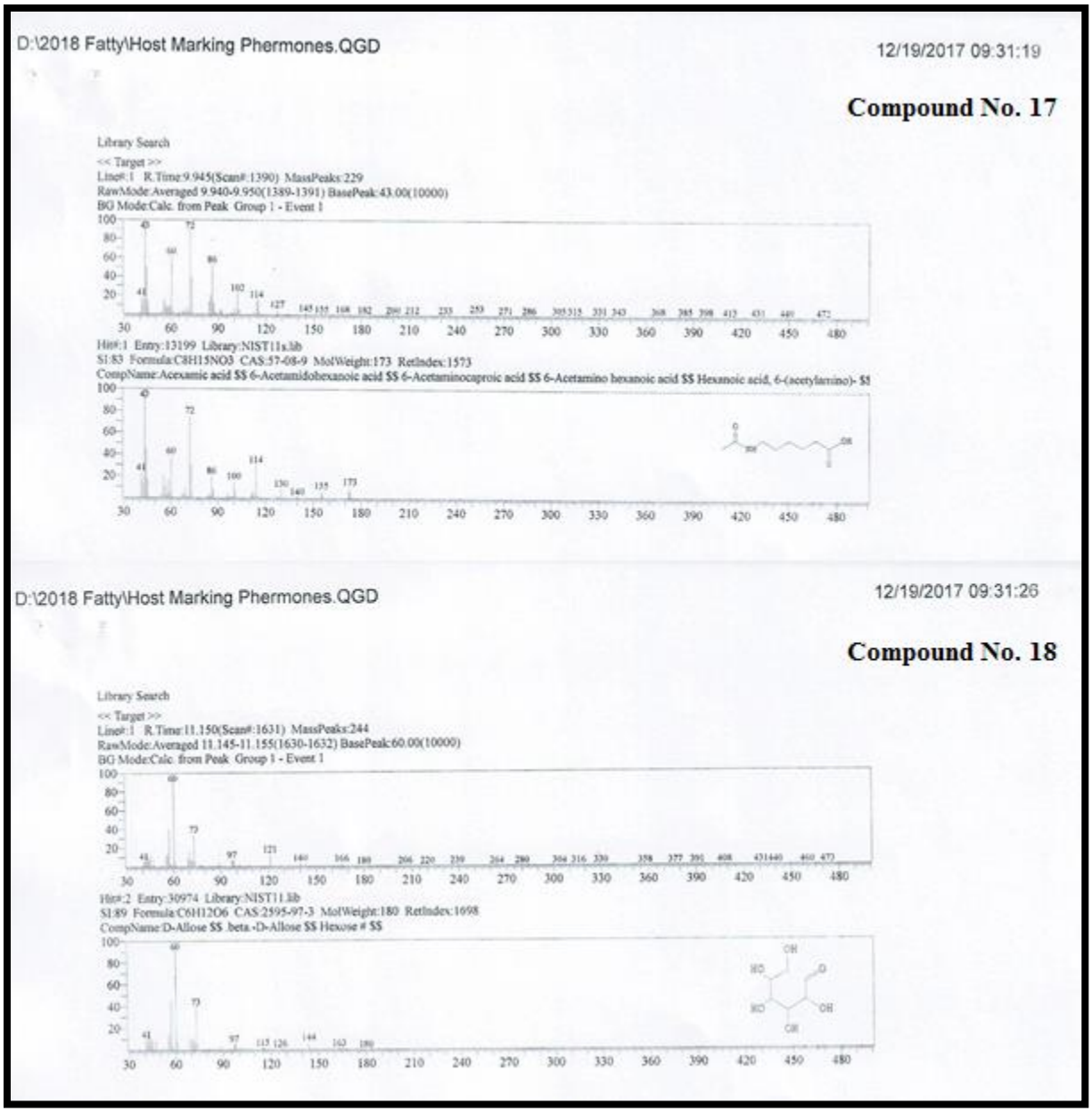
Appendix No.27 GC-MS compounds formulations



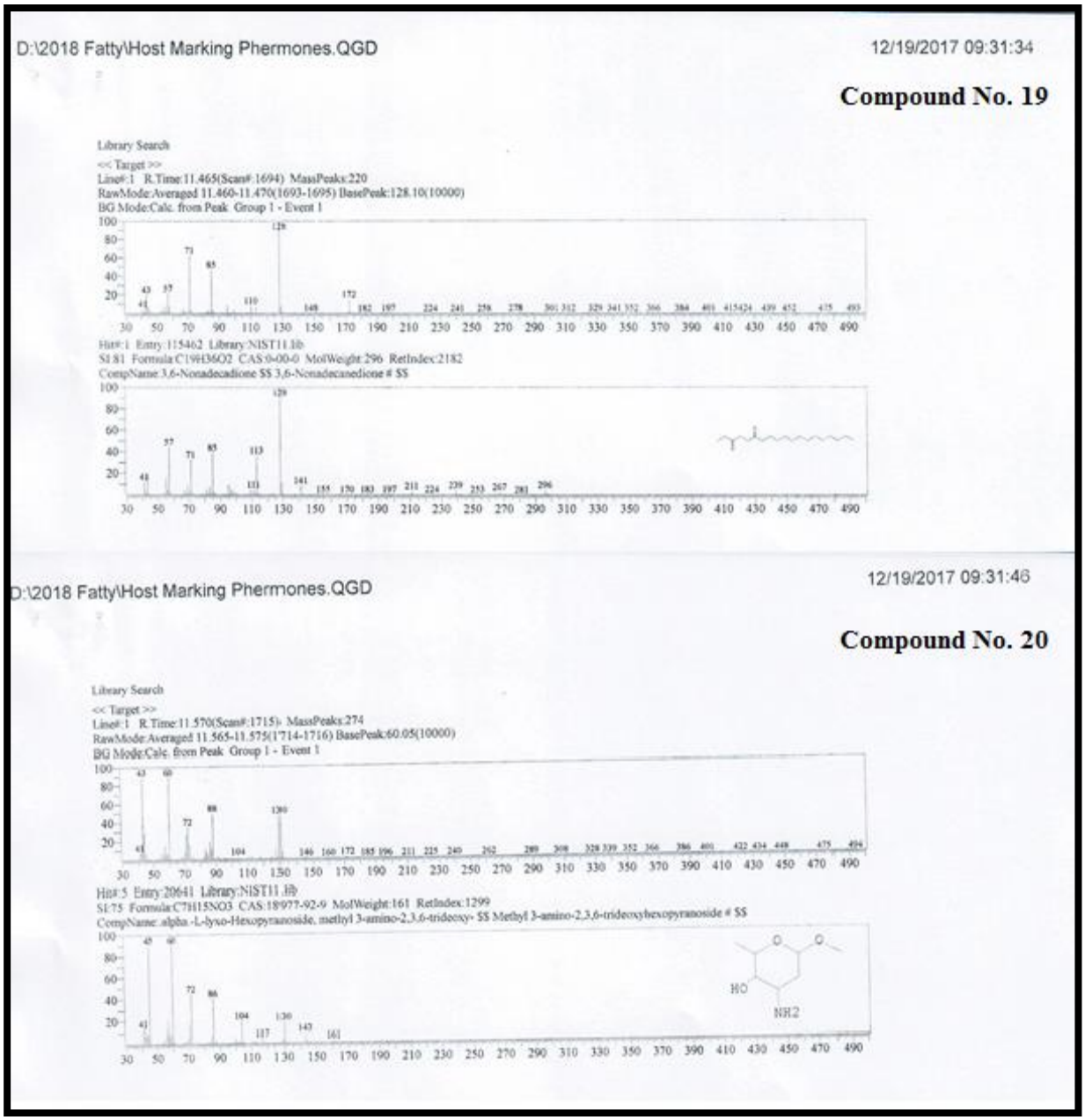
Appendix No.28 GC-MS compounds formulations



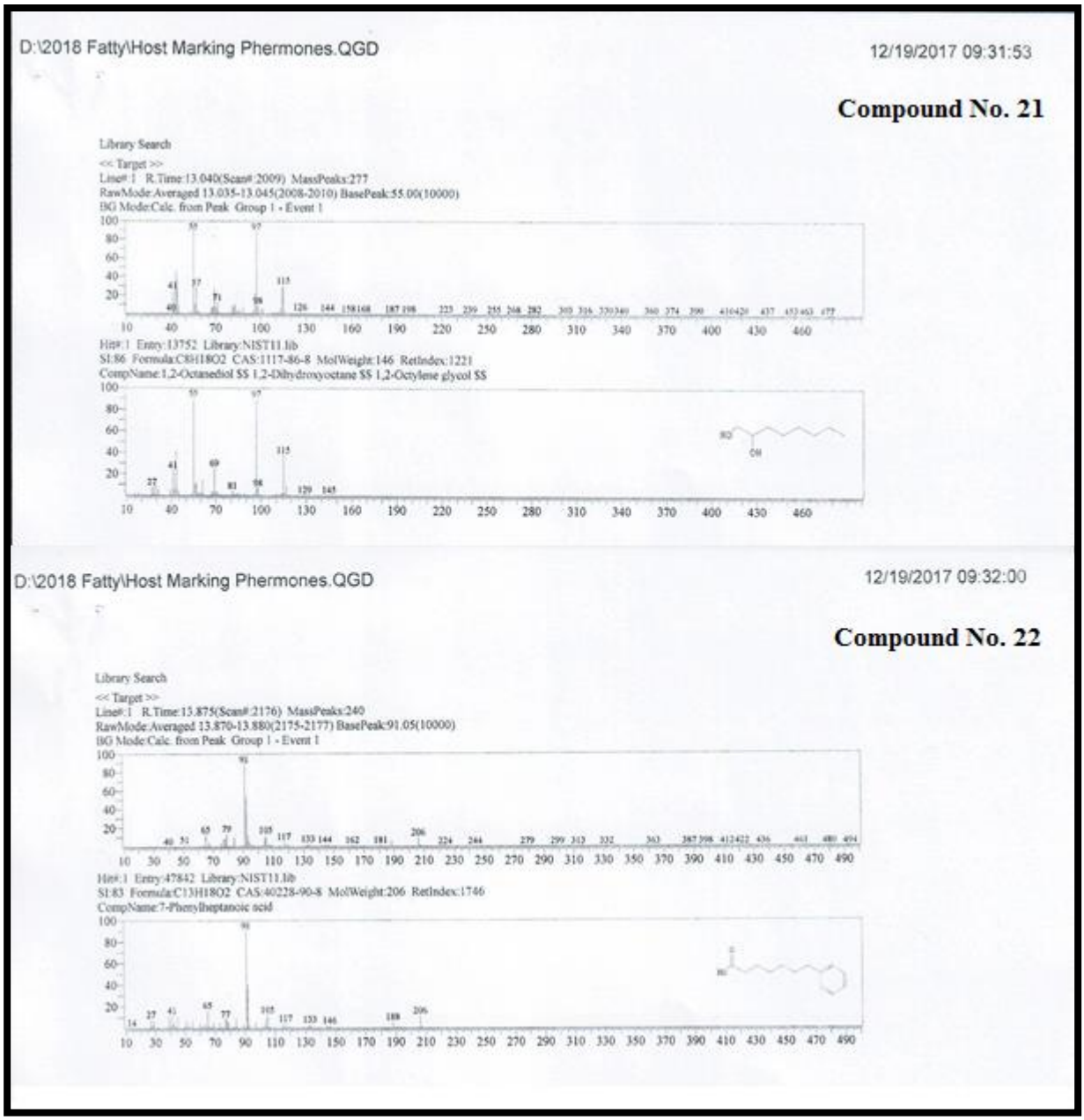
Appendix No.29 GC-MS compounds formulations



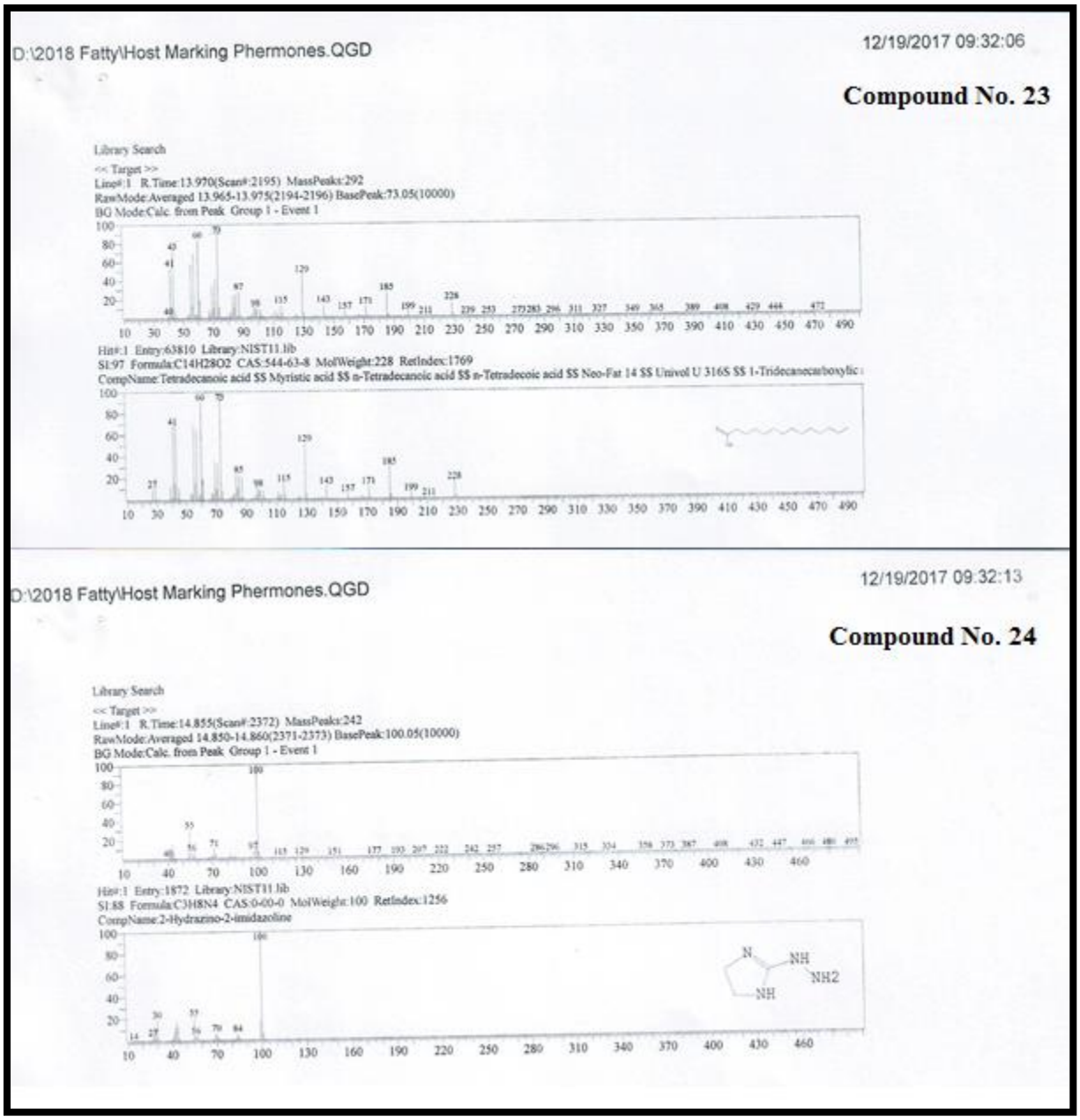
Appendix No.30 GC-MS compounds formulations



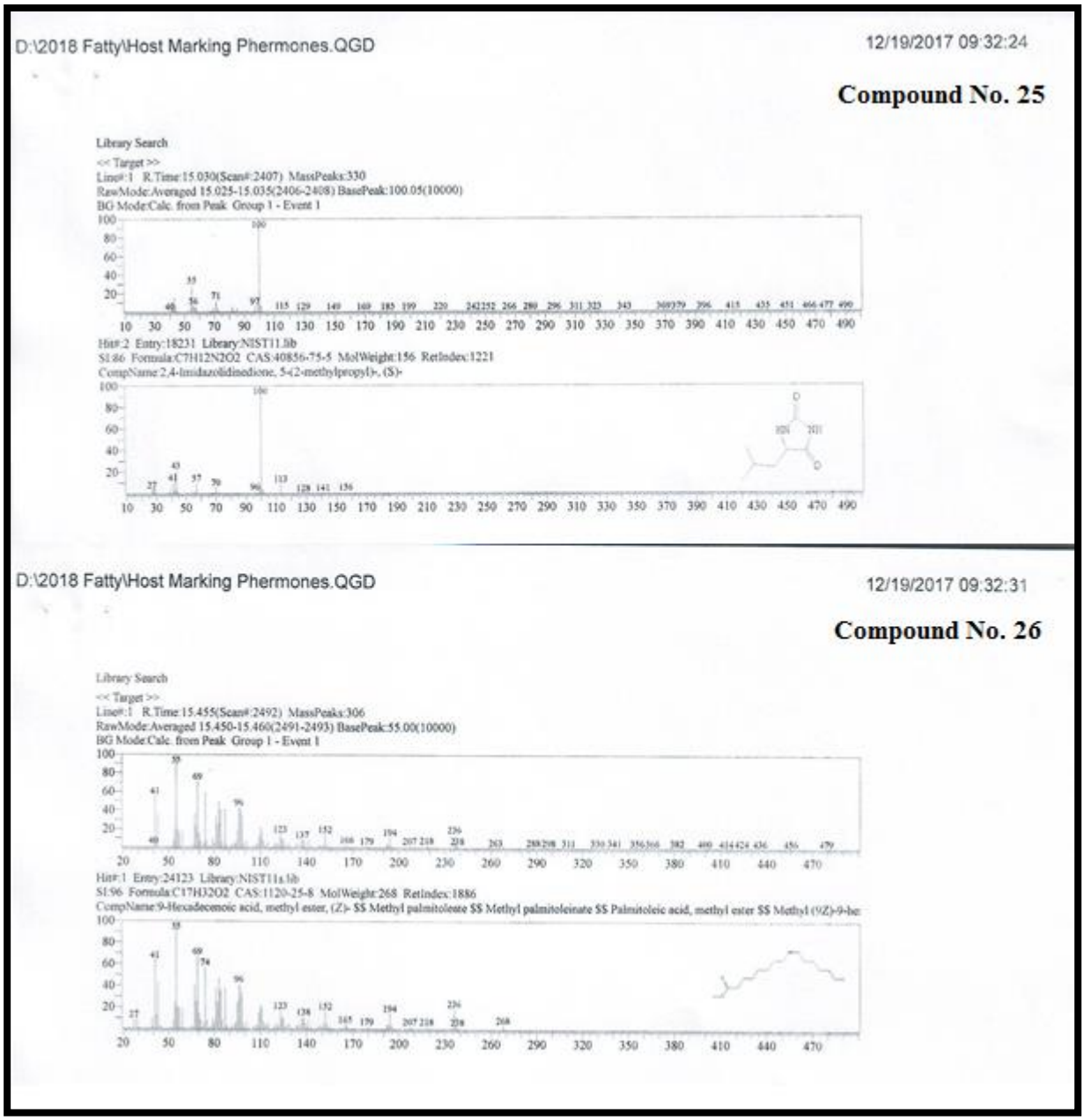
Appendix No.31 GC-MS compounds formulations



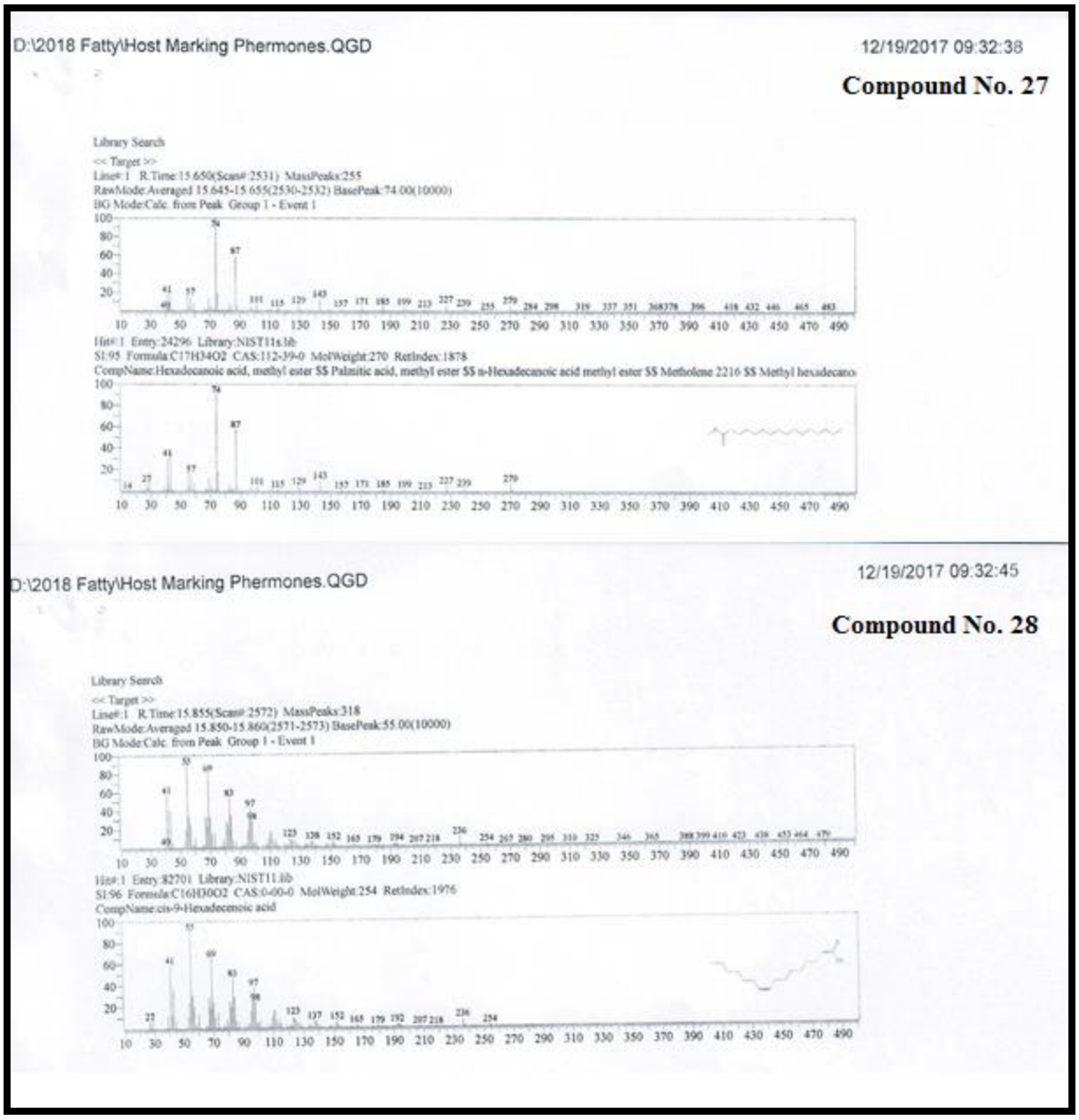
Appendix No.32 GC-MS compounds formulations



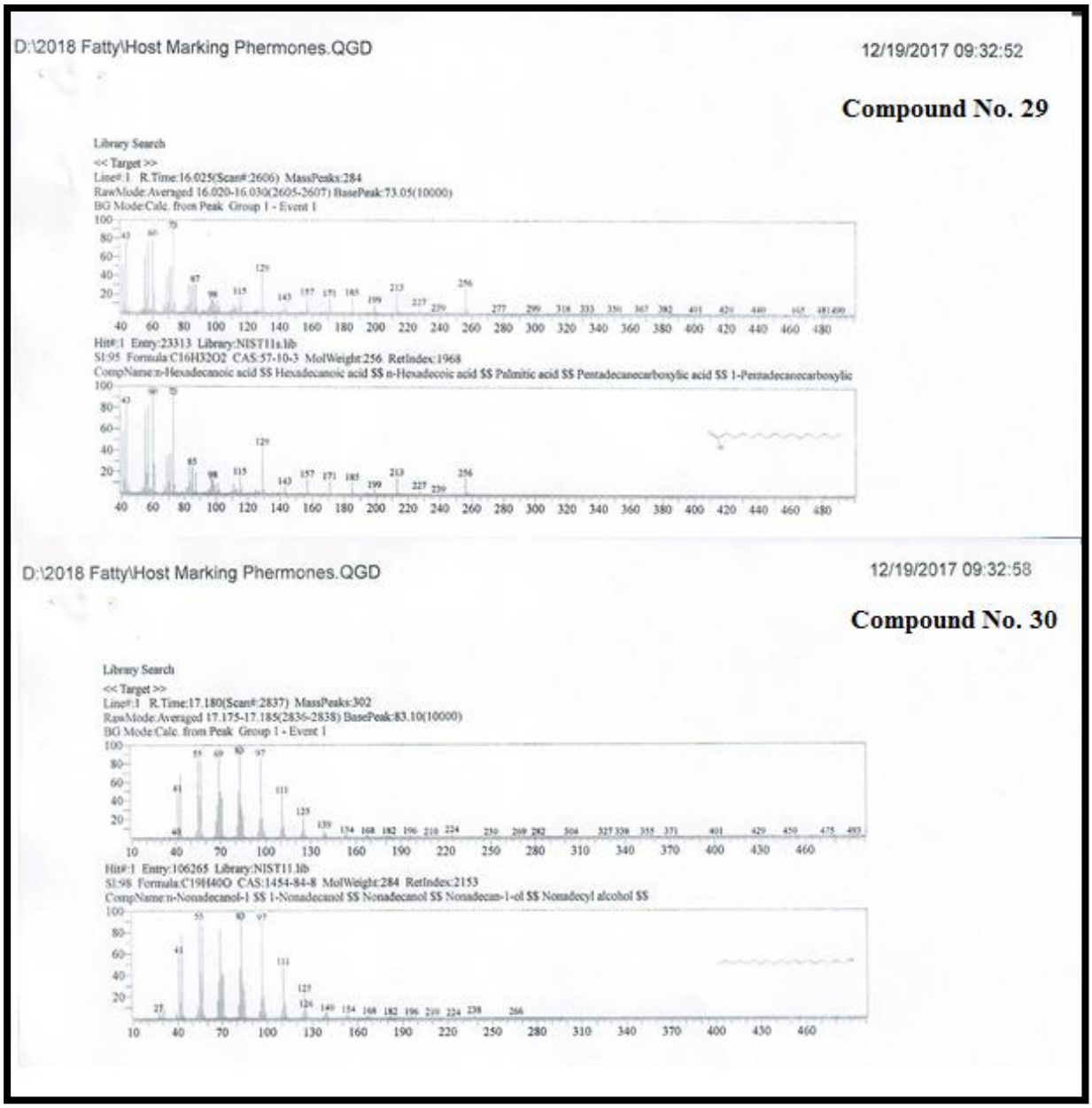
Appendix No.33 GC-MS compounds formulations



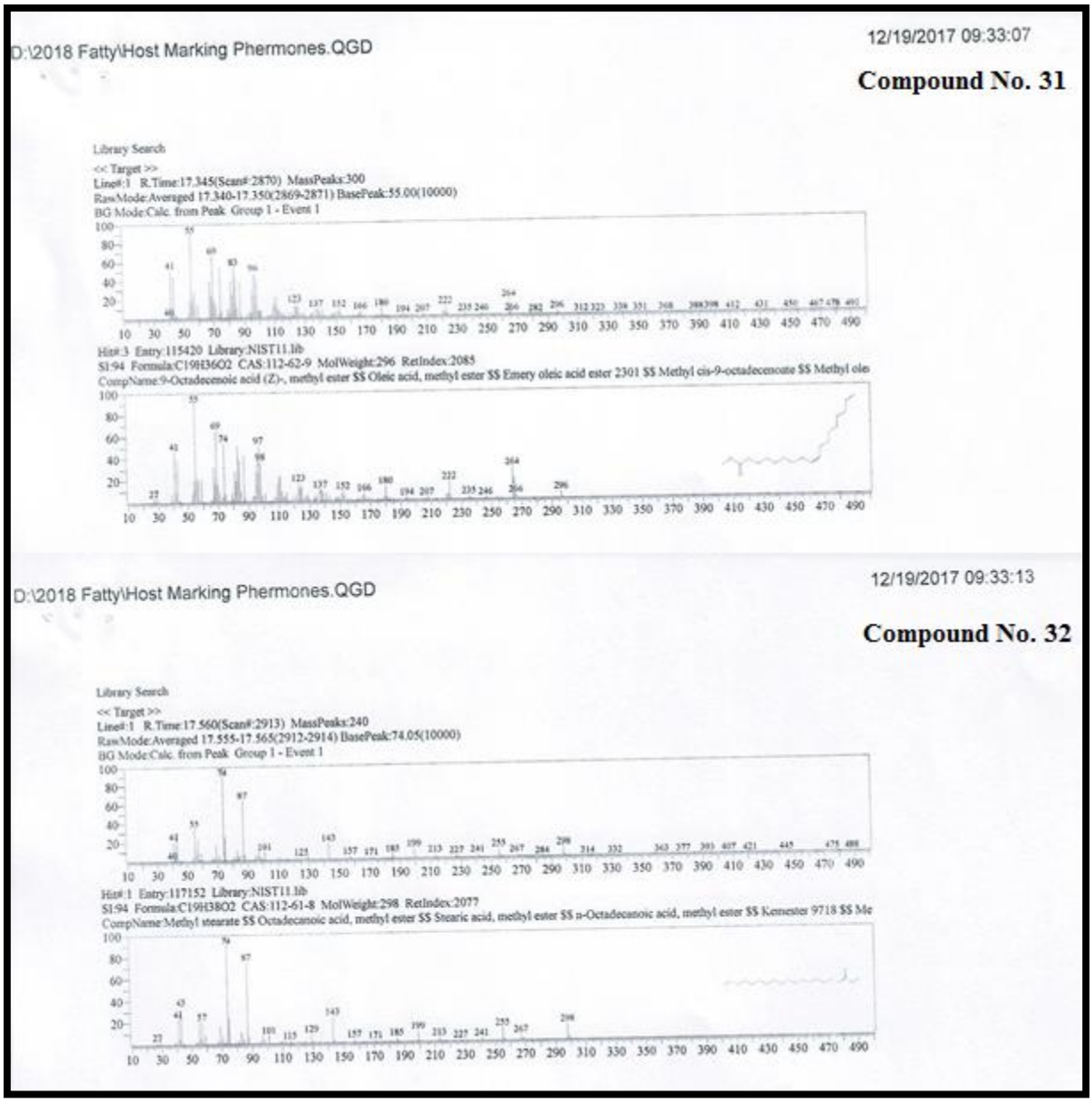
Appendix No.34 GC-MS compounds formulations



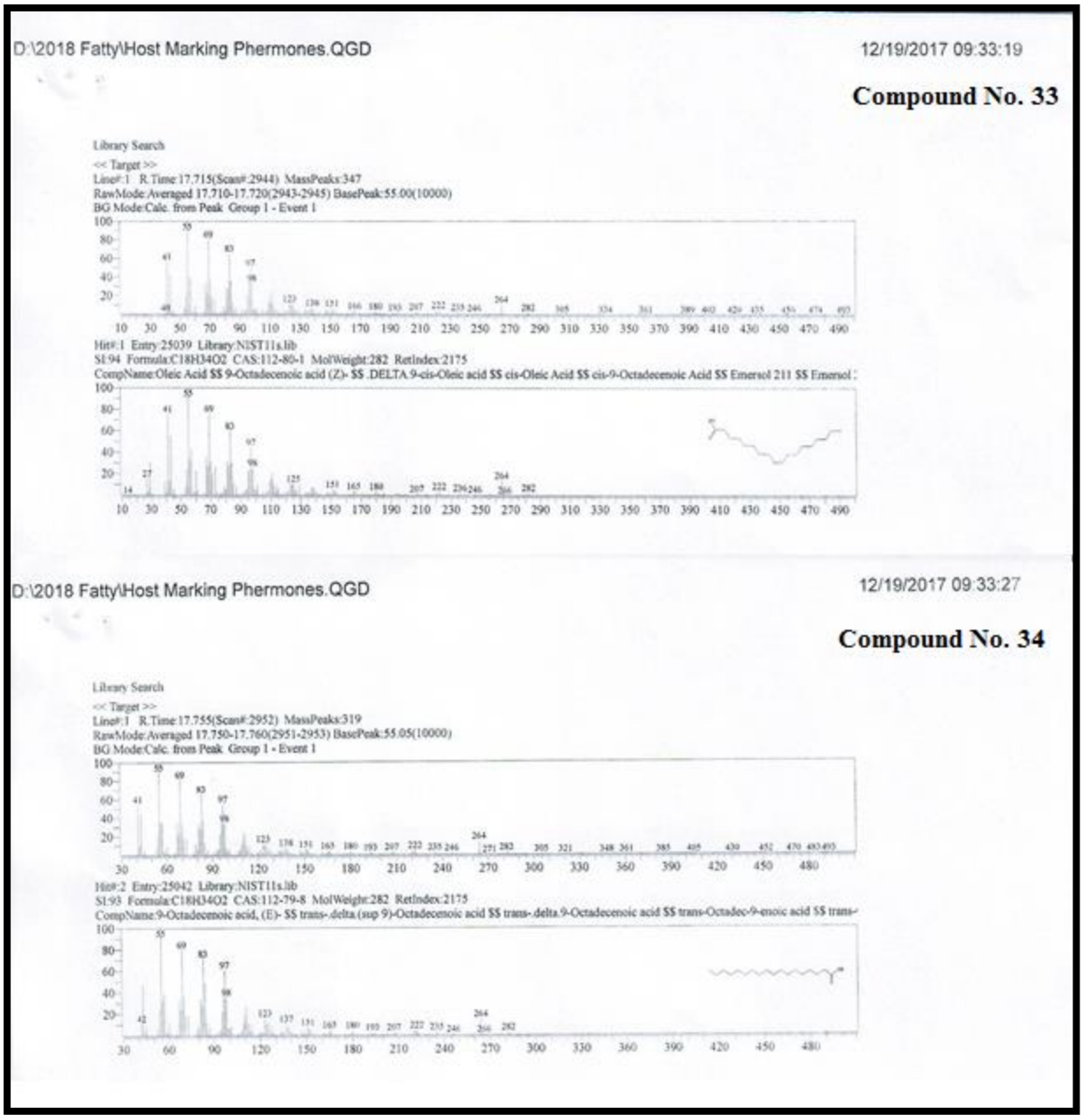
Appendix No.35 GC-MS compounds formulations



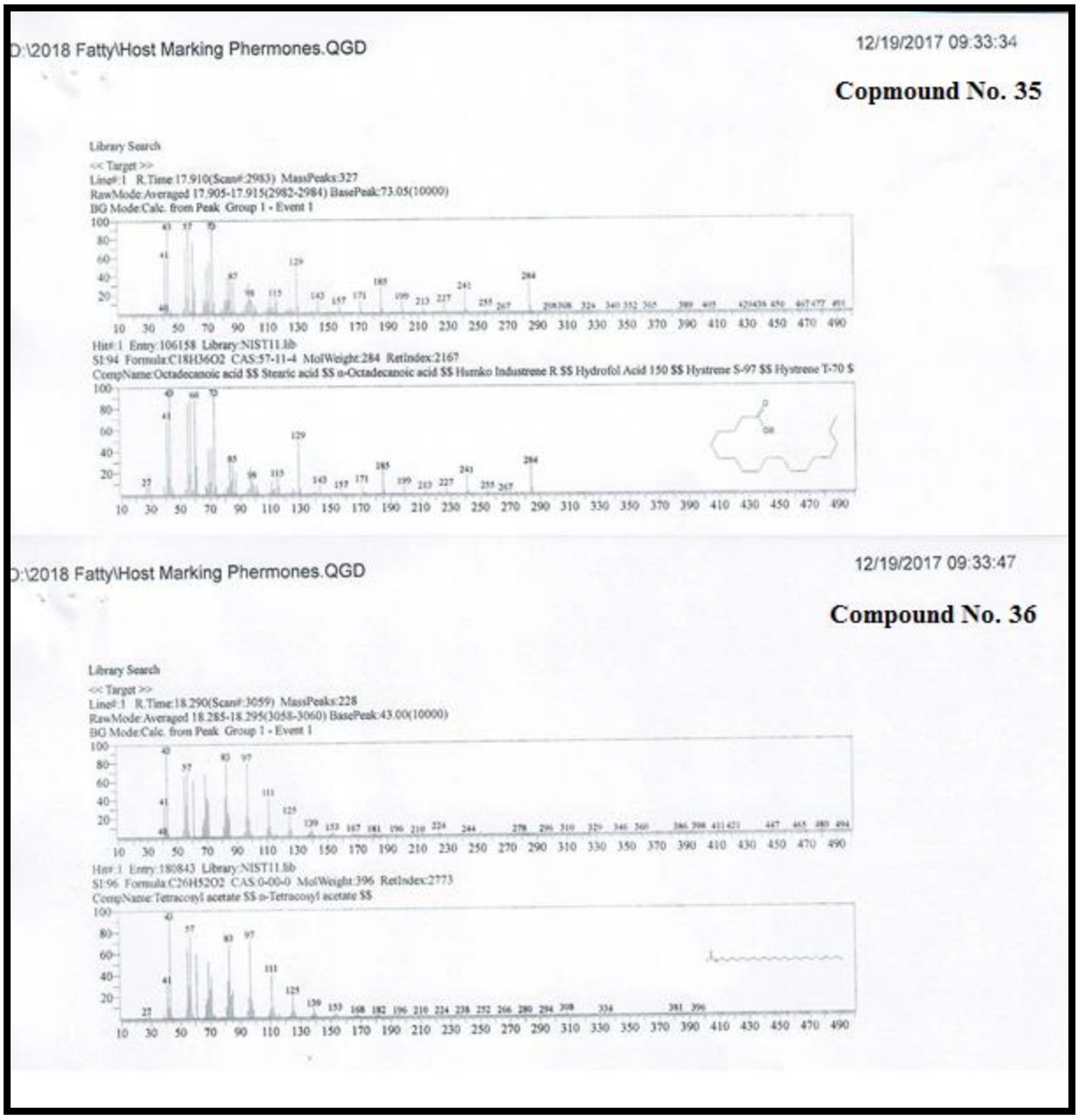
Appendix No.36 GC-MS compounds formulations



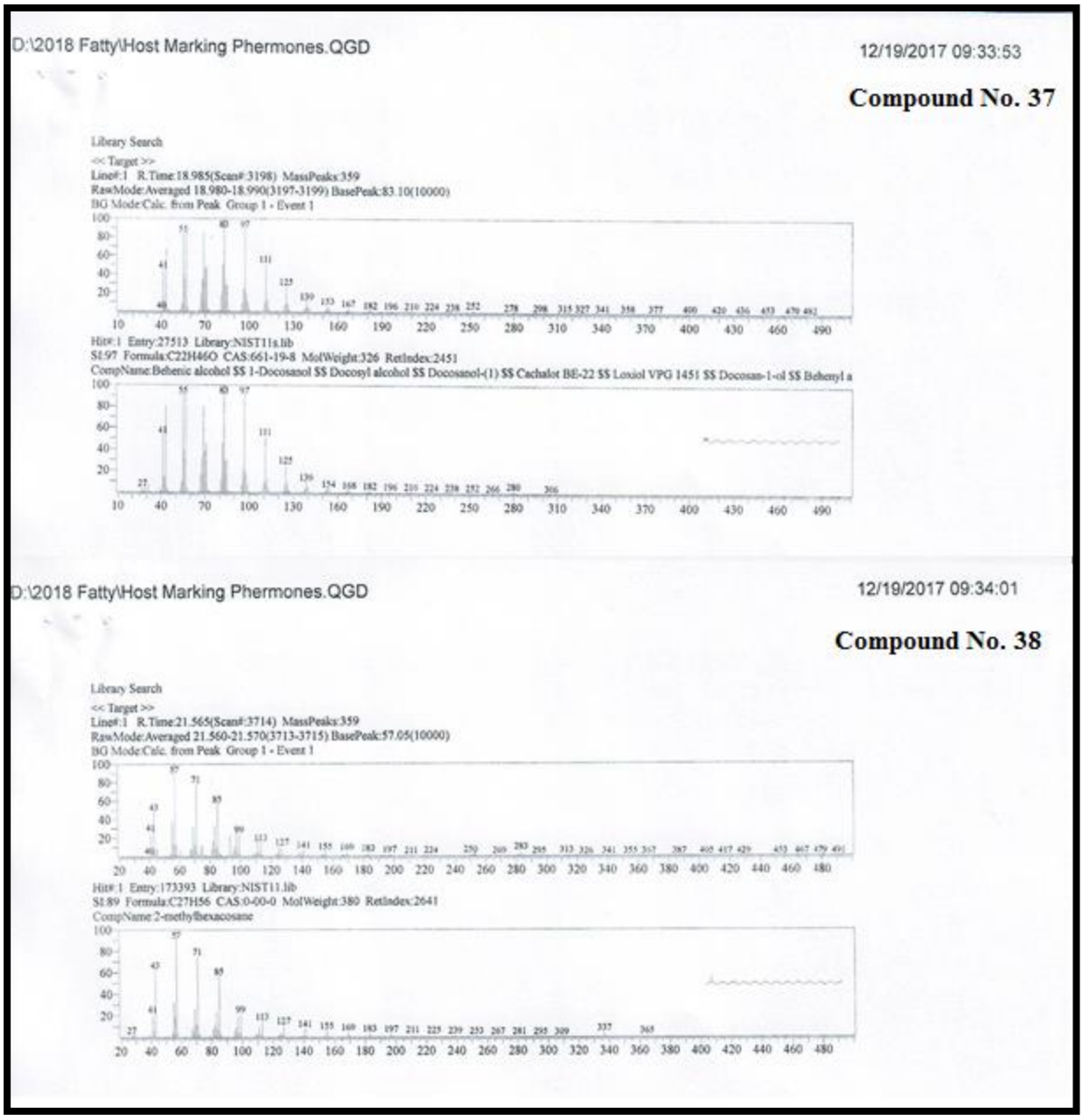
Appendix No.37 GC-MS compounds formulations



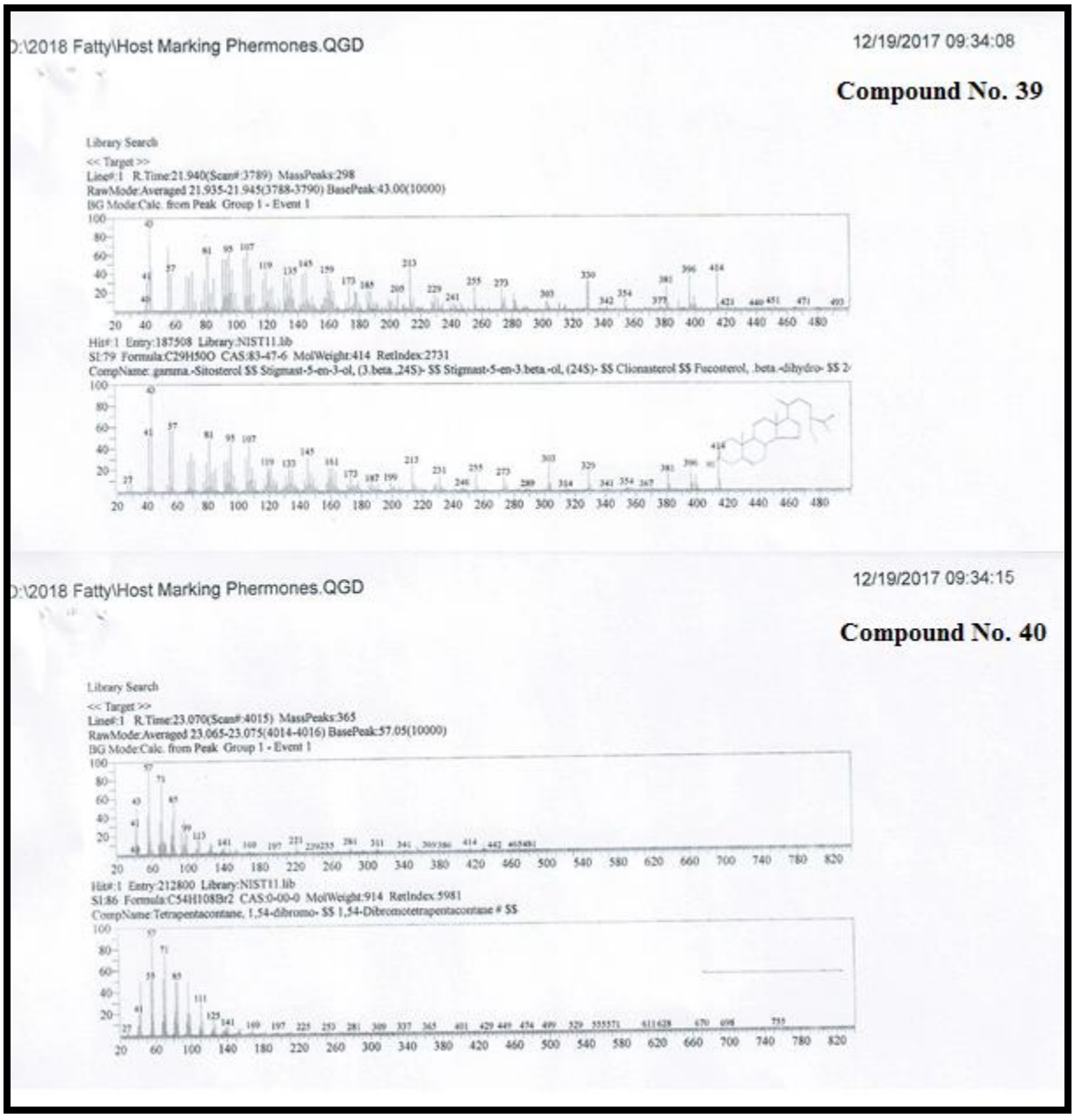
Appendix No.38 GC-MS compounds formulations



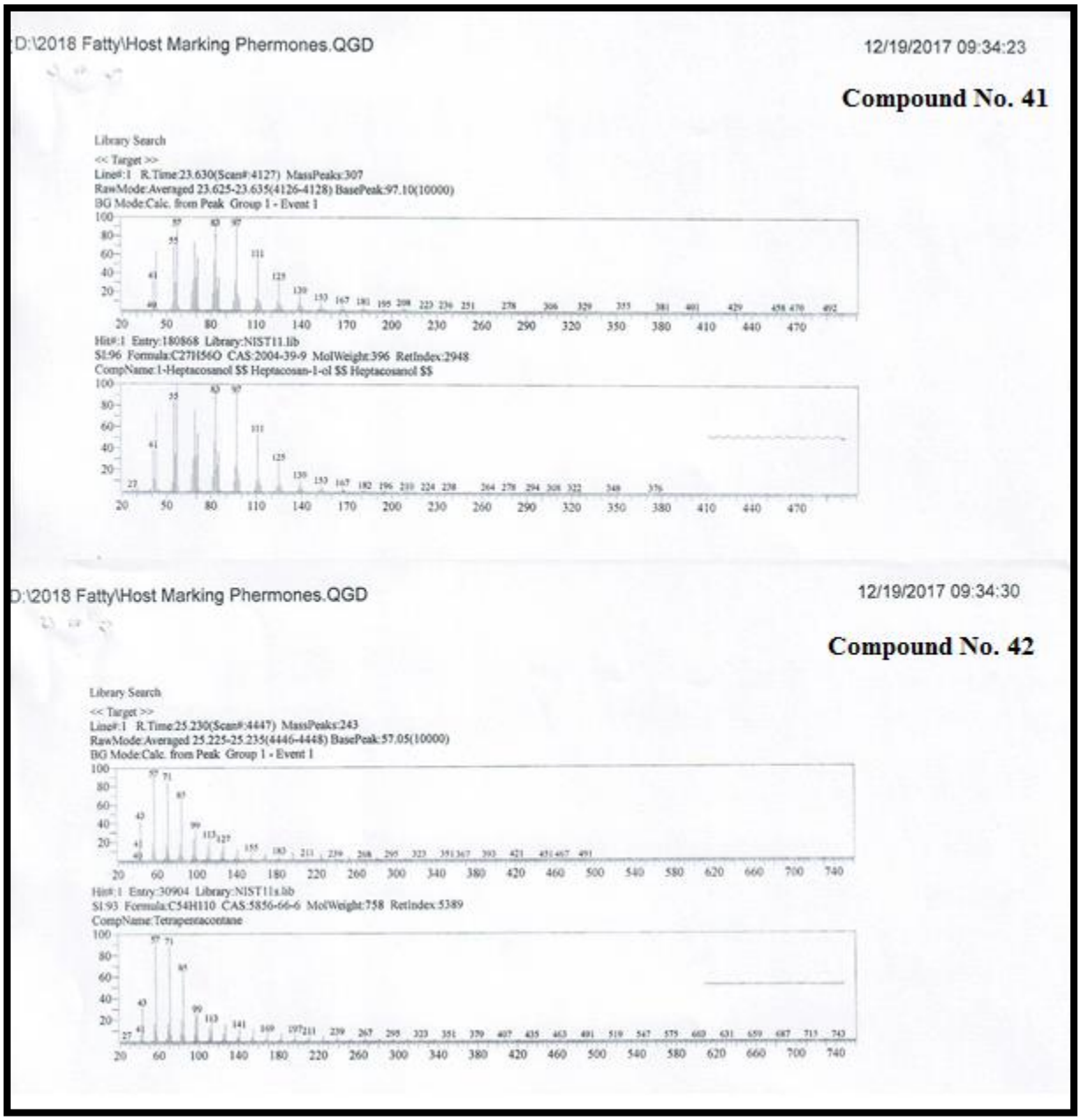
Appendix No.39 GC-MS compounds formulations



Appendix No.40 GC-MS compounds formulations



Appendix No.41 GC-MS compounds formulations



Appendix No.42 GC-MS compounds formulations

Appendix No. 43:Locally Manufactured Lynfield Traps

Appendix No. 44:Locally Manufactured Lynfield Traps Catchability

(22.6.2016 - 13.7.2016)

Site / Treatments	Methyl Eugenol + Malathion								
		1	2	3	4	5	Total	Mean	CTD
Elkadaro	1st week	61	38	44	64	52	259	52	7
	2nd week	46	29	52	63	39	229	46	7
	3rd week	656	526	583	230	204	2199	440	63
Elfaki Hashim	1st week	39	16	5	18	20	98	20	3
	2nd week	23	31	31	20	12	117	23	3
	3rd week	27	35	29	59	26	176	35	5

Appendix No. 45:Locally Manufactured Lynfield Traps Catchability Anova Table (Elkadaro Site)

Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	4	55113	13778		
TREATMENT	2	509693	254847	16.94	0.0013
Error	8	120331	15041		
Total	14	685138			

Grand Mean 179.13 CV 68.46

Appendix No. 46:Locally Manufactured Lynfield Traps Catchability ANOVA Table (Elfaki Hashim Site)

Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	4	355.60	88.900		
TREATMENT	2	661.73	330.867	2.09	0.1855

Error	8	1263.60	157.950		
Total	14	2280.93			

Grand Mean 26.067 CV 48.21

Appendix No. 47 :Locally Manufactured Lynfield Traps Catchability

(6.2.2017 - 5.3.2017)

Site / Treatments	Methyl Eugenol + Malathion								
		1	2	3	4	5	Total	Mean	CTD
Elkadaro	1st week	40	27	60	38	45	210	42	6
	2nd week	8	3	7	8	24	50	10	1
	3rd week	2	17	0	9	7	35	7	1
	4th week	2	2	5	8	3	20	4	-
Elkhojalab	1st week	110	16	21	37	31	215	43	6
	2nd week	33	8	15	11	33	100	20	3
	3rd week	12	20	30	9	4	75	15	2
	4th week	4	9	5	15	27	60	12	2

Appendix No. 48 :Locally Manufactured Lynfield Traps Catchability Anova Table (Elkadaro Site)

Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	4	163.50	40.87		
TREATMENT	3	4683.75	1561.25	21.28	0.0000
Error	12	880.50	73.37		
Total	19	5727.75			

Grand Mean 15.750 CV 54.39

Appendix No. 49 :Locally Manufactured Lynfield Traps Catchability

Anova Table (Elkhojalab Site)

Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	4	1710.0	427.500		
TREATMENT	3	2965.0	988.333	2.14	0.1479
Error	12	5532.0	461.000		
Total	19	10207.0			

Grand Mean 22.500 CV 95.43

Appendix No. 50 : Evaluation of Different Traps Efficacy
(Al-Halfaia Site :16.5.2017 - 6.6.2017)

Treatments / Replicates	1	2	3	4	Total	Mean	CTD
ME + Cyper	66	71	53	46	236	59	8
ME + Malation	140	120	200	144	604	151	22
Sticky Trap + ME	1200	940	740	320	3200	800	144
Food Trap + Cyper	209	205	156	270	840	210	39
Food Trap +Malation	414	530	417	551	1912	478	68

Appendix No. 51 : Evaluation of Different Traps Efficacy Anova Table

(Al-Halfaia Site :16.5.2017 - 6.6.2017)

Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	3	57980	19327		
TREATMENT	4	1448901	362225	11.38	0.0005
Error	12	381942	31829		
Total	19	1888823			

Grand Mean 339.60 CV 52.53

Appendix No. 52 : Evaluation of Two Types of Locally Manufactured Traps Efficacy (Al-kadaroo Site (27.9.2017 - 18.10.2017)

Site / Treatments	Methyl Eugenol + Malathion								
Trap1		1	2	3	4	5	Total	Mean	CTD
	1st week	85	67	89	77	56	374	75	11
	2nd week	25	33	43	17	24	142	28	4
	3rd week	36	45	53	29	43	206	41	6
	4th week	28	32	49	64	46	219	44	6
Trap2	1st week	723	979	550	1219	845	4316	863	123
	2nd week	496	845	1389	877	1612	5219	1044	149
	3rd week	1108	1056	2146	2057	1043	7410	1482	212
	4th week	2378	3154	1265	986	512	8295	1659	237

Appendix No. 53 : Evaluation of Two Types of Locally Manufactured Traps Efficacy (Al-kadaroo Site (27.9.2017 - 18.10.2017)

A- Week 1. Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	4	123749	30937		
TREATMENT	1	1553936	1553936	46.02	0.0025
Error	4	135061	33765		
Total	9	1812746			

Grand Mean 469.00 CV 39.18

B- Week 2. Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	4	410308	102577		
TREATMENT	1	2577593	2577593	25.81	0.0071
Error	4	399530	99882		
Total	9	3387431			

Grand Mean 536.10 CV 58.95

C- Week 3. Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	4	643319	160830		
TREATMENT	1	5189762	5189762	32.30	0.0047
Error	4	642607	160652		
Total	9	6475688			

Grand Mean 761.60 CV 52.63

D- Week 4. Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	4	2291126	572782		
TREATMENT	1	6522178	6522178	10.94	0.0297
Error	4	2385462	596366		
Total	9	1.119E+07			

Grand Mean 851.40 CV 90.70

Appendix No. 54 : Species monitoring using different types of pheromones in three sites:

(7. 3. 2018)

Site 1:

Treatment / Replicates	1	2	3	4	Total	Mean	CTD
ME	123	126	171	-	420	140	20
C	0	0	0	-	0	0	0
T	0	0	0	0	0	0	0
TA	38	77	49	-	164	55	8

Site 1: Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	3	7566.5	2522.17		
TREATMENT	3	29508.0	9836.00	7.61	0.0077
Error	9	11629.5	1292.17		
Total	15	48704.0			

Grand Mean 36.500 CV 98.48

Site 2:

Treatment / Replicates	1	2	3	4	Total	Mean	CTD

ME	2071	1466	2520	-	6057	2019	288
C	0	0	0	-	0	0	0
T	0	0	0	0	0	0	0
TA	38	39	56	59	192	48	7

Site 2: Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	3	897416	299139		
TREATMENT	3	6740403	2246801	7.43	0.0083
Error	9	2719735	302193		
Total	15	1.035E+07			

Grand Mean 390.56 CV 140.75

Site 3:

Treatment / Replicates	1	2	3	4	Total	Mean	CTD
ME	659	1182	391	878	3110	778	111
C	2	1	0	2	5	1	-
T	0	0	0	0	0	0	0
TA	23	40	38	-	101	34	5

Site 3: Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	3	84090	28030		
TREATMENT	3	1774166	591389	20.95	0.0002
Error	9	254080	28231		
Total	15	2112336			

Grand Mean 201.00 CV 83.59

Appendix No. 55 : Species monitoring using different types of pheromones in three sites: (13.3.2018)

Site 1:

Treatment / Replicates	1	2	3	4	Total	Mean	CTD
ME	142	114	152	220	628	157	22
C	0	0	0	-	0	0	0
T	0	0	0	0	0	0	0
TA	43	36	28	-	107	36	5

Site 1: Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	3	617.2	205.7		
TREATMENT	3	67694.2	22564.7	31.16	0.0000
Error	9	6517.6	724.2		
Total	15	74828.9			

Grand Mean 45.938 CV 58.58

Site2:

Treatment / Replicates	1	2	3	4	Total	Mean	CTD
ME	1155	1765	992	862	4772	1193	170
C	0	0	0	0	0	0	0
T	0	0	0	0	0	0	0
TA	30	18	20	-	68	23	3

Site2: Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	3	122315	40772		
TREATMENT	3	4233615	1411205	35.60	0.0000
Error	9	356742	39638		
Total	15	4712672			

Grand Mean 302.63 CV 65.79

Site3:

Treatment / Replicates	1	2	3	4	Total	Mean	CTD
ME	230	371	320	199	1120	280	40
C	0	1	-	-	1	-	-
T	0	0	0	0	0	0	0
TA	23	5	-	-	28	14	2

Site3: Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	3	4522	1507.4		
TREATMENT	3	231284	77094.6	46.95	0.0000
Error	9	14779	1642.1		
Total	15	250584			

Appendix No. 56 : Species monitoring using different types of pheromones in three sites: 20.3.2018

Site 1:

Treatment / Replicates	1	2	3	4	Total	Mean	CTD
ME	161	207	152	-	520	173	25
C	2	0	0	-	2	-	-
T	0	0	0	0	0	0	0
TA	50	51	20	-	121	40	6

Site 1: Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	3	9538.7	3179.6		
TREATMENT	3	45420.7	15140.2	8.22	0.0060
Error	9	16579.1	1842.1		
Total	15	71538.4			

Grand Mean 40.188 CV 106.80

Site 2:

Treatment / Replicates	1	2	3	4	Total	Mean	CTD
ME	1077	1164	624	770	3635	909	130
C	0	0	0	0	0	0	0
T	0	0	0	-	0	0	0
TA	20	50	12	-	82	27	4

Site 2: Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	3	55145	18382		
TREATMENT	3	2441482	813827	52.31	0.0000
Error	9	140013	15557		
Total	15	2636639			

Grand Mean 232.31 CV 53.69

Site 3:

Treatment / Replicates	1	2	3	4	Total	Mean	CTD
ME	521	246	284	499	1550	388	55
C	2	0	0	3	5	1	-
T	0	0	0	0	0	0	0
TA	9	9	26	-	44	15	2

Site 3: Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	3	14238	4746		
TREATMENT	3	441315	147105	28.10	0.0001
Error	9	47112	5235		
Total	15	502665			

Grand Mean 99.938 CV 72.40

Appendix No. 57 : Species monitoring using different types of pheromones in three sites:

(27.3.2018)

Site 1:

Treatment / Replicates	1	2	3	4	Total	Mean	CTD
ME	335	-	140	184	659	220	31
C	0	0	0	-	0	0	0
T	0	0	0	0	0	0	0
TA	54	0	24	-	78	26	4

Site 1: Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	3	19070	6356.7		
TREATMENT	3	76143	25381.1	5.71	0.0181
Error	9	40012	4445.7		
Total	15	135225			

Grand Mean 46.063 CV 144.75

Site 2:

Treatment / Replicates	1	2	3	4	Total	Mean	CTD
ME	663	515	630	865	2673	668	95
C	0	0	0	0	0	0	0
T	0	0	0	0	0	0	0
TA	17	7	19	15	58	15	2

Site 2: Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P
REPLICATI	3	16474	5491		
TREATMENT	3	1320926	440309	83.79	0.0000
Error	9	47296	5255		
Total	15	1384695			

Grand Mean 170.69 CV 42.47

Site 3:

Treatment / Replicates	1	2	3	4	Total	Mean	CTD
ME	362	148	211	350	1071	268	38
C	0	0	0	0	0	0	0
T	0	0	0	0	0	0	0
TA	1	21	1	-	23	8	1

Site 3: Randomized Complete Block AOV Table for RESULTS

Source	DF	SS	MS	F	P

REPLICATI	3	7141	2380.4		
TREATMENT	3	212090	70696.8	24.12	0.0001
Error	9	26378	2930.9		
Total	15	245610			

Grand Mean 68.375 CV 79.18