

Sudan University of Science and Technology
College of Graduate Studies and Scientific Research



**The Effect of Bee Glue Powder and Ethanolic Extracts of Neem
and Basil Mortality of the Asian Fruit Fly (*Bactrocera invadens*,
Drew, Tsuruta and White) (Diptera:Tephritidae)**

اثر بكرة صمغ النحل والمستخلص الايثانولي لنباتي النيم والريحان علي معدل موت ذبابة
الفاكهة الاسيوية

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الآية

قال تعالى:

بِأَيُّهَا سَلَا لِنَلْمَسِ عِوَضْلِهِمُ بِإِنَّ الَّذِينَ تَدْعُونَ مِنْ دُونِ اللَّهِ لَنْ
يُؤْتُوا لَهُمْ وَإِنْ يَسْأَلُوكُمْ الذُّبَابُ شَيْئًا فَاذْكُوهُ مِنْهُ
الطَّالِبُ وَالْمَطْلُوبُ (73)

صدق الله العظيم

سورة الحج الآية (73)

Dedication

To My:

*Mother soul, father, brothers, and
sisters*

Teachers

And every Friends.

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Abstract

Laboratory experiments were conducted at the Department of Plant protection, College of Agricultural Studies, Sudan University of Science and Technology (SUST) to evaluate the lethal effect Bee glue powder and ethanolic extracts of leaves Neem *Azadirachta indica* L. ,Basil *Ocimum basilicum*.

From each test four different concentrations were applied against adult stage of Asian fruit fly *Bactrocera invadens* Drew Tsuruta and White (Diptera:Tephritidae),as topical and as food bait and the result was obtained after 24,48 and 72hours.

In arandomized complete Block design replicated 4times for each material.

The four concentration of the three tested materials are potent to cause mortality to adult fruit flies and out compete the control.

The highest dose of each material obtained the highest mortality rate during the three assessment periods.The highest mortality for each compound was affained after 72 hours for both application techniques.The obtained results in this study open the door widely for scientist to in corporate these materials in food attractants that used to suppress the population of fruit flies.

ملخص البحث

اجريت تجارب معملية في قسم وقاية النبات، كلية الدراسات الزراعية، جامعة السودان للعلوم و التكنولوجيا. لتقييم الاثر القاتل لبدره صمغ النحل proplis والمستخلصات الإيثانولية لاوراق النيم علي الطور الكامل لذبابة الفاكهة الغازية الاسيوية

اربعة تركيزات مختلفة من كل مستخلص نباتي ومن بدره صمغ النحل بطريقتين هما المعاملة الموضعية والطعوم السامة

Bactrocera invadens Drew Tsuruta and White (Diptera: Tephritidae) .

اوضحت النتائج ان المواد المختبرة بطريقتي التطبيق لها المقدرة علي احداث الاثر السام في كل تراكيزها المختلفة وتفوقت علي الشاهد بفروق معنوية واضحة .

اثبتت النتائج ان التركيز الاعلي من المادة المختبرة 15%يزيد من نسبة تفوق الافة المستهدفة في كل المواد المختبرة بنسبة كبيرة كما ان زيادة فترة تعرض الافة للمادة المختبرة تؤدي لزيادة في نسبة الموت حيث ان نسبة النفوق بعد 72ساعة كانت هي الاعلي في كل المواد المختبرة ايضا .

هذه النتائج تعتبر اساس لدراسات قادمة تتضمن كيفية الاستفادة منها كمواد قاتلة للافات توضع في الجاذبات الغذائية لتقليل مخاطر تلوث الثمار وتساهم في المحافظة علي الاعداء الحيوية وسلامة البيئة .

CHAPTER ONE

INTRODUCTION

Fruit flies (Diptera, Tephritidae) are insect group of major economic significance. Several representatives are known to attack different types of commercial and wild fruits and vegetables, causing considerable damage to agricultural crops. They are among the most important pests worldwide because of their direct economic impact (i.e., female oviposition and larval feeding render fruit/vegetables unmarketable) and quarantine restrictions imposed by many countries to curtail their entry (Aluja and Mangan, 2008).

Globally, out of the 4,257 fly species comprising the family of Tephritidae, about 1,400 species are known to develop in fruits. Out of these, about 250 species already are, or may become, pests by inflicting severe damage to fruits of economic value (White and Elson-Harris, 1992; Thompson, 1998).

There are about 950 species and 150 genera of fruit flies (Tephritidae) known in Africa, most of which form a natural component of Africa's rich and varied biodiversity, in many cases attacking wild fruits and flowers. Most species which attack commercially grown fruit crops belong to just two genera, *Ceratitis* (94 species) and *Dacus* (177 species) (White, 2006). A few species belong to other genera such as the coffee fruit flies (*Trirhithrum spp.*) which are close relatives of *Ceratitis*, or to the genus *Bactrocera*, which are close relatives of *Dacus*.

Equatorial Africa is the original home to 915 fruit fly species from 148 genera, out of which 299 species develop in either wild or cultivated fruits. (White and Elson-Harris, 1992; Thompson, 1998). Most of them are highly polyphagous and their host ranges overlap to varying degrees. Though a great number of fruit species had already been reported to harbour fruit flies, numerous new host associations were recently found in Kenya. Reve fruit flies were added to the list

of the notorious national pests of the Sudan, in August 2008 (Ali *et.al*, 2008). Accordingly, understanding of host ranges, environmental reservoirs, and patterns of host utilization remains superficial, even for the most common African fruit flies of major economic importance.

Recently, *Bactrocera invadens* was recorded for the first time in Kenya (Lux *et. al.*, 2003a) and has subsequently been found in countries across tropical Africa. From recent records, it has spread over, at least, ten countries in central Africa and attacking important horticultural crops. Consequently, it is now recognized as highly invasive and possessing a wide range cultivated and wild of host plants. Pests has been recognized as an invading species belonging to an Asian species. (Drew *et. al.*, 2005).

Sudan has a great potentiality to produce good quality of fruits and vegetables and has very promising export market (Middle East & European countries). In recent years production have been seriously hampered, because of the persistent outbreaks of fruit flies which cause 80% infestation level in some areas.

For example, in the River Nile State fruit damage on mango was estimated 85-98 % (Gubara and Abu Elgasim, 2004).

The objectives of this study are:

According to severe losses caused by fruit flies a workshop was held at Khartoum where different aspects were presented and plans for future research was highlighted .

Thus this study is initiated in order to provide information regarding control of fruit flies.

CHAPTER TWO

2: LITERATURE REVIEW

2:1 True fruit flies (Diptera: Tephritidae):

2.1.2 Taxonomic status of fruit flies:

The identification and bionomics of harmful and beneficial fruit flies of economic importance has been monographed by White and Elson-Harris (1992).

Out of 4257 species, about 1400 Tephritid species are known to develop in fruits bearing organs, and 250 of them are reported to be or have potentials of being pests (Thompson, 1998).

2.1.3 Description:

Which species is quite large (~ 1 cm). It has two yellow thoracic lines and an orange-colored abdomen traced with a central T. Its wings are mostly transparent, with neither bands nor macula, but the anal stripe is often quite distinctive (plate 1). Several scutum color patterns may be present (Vayssières, *et. al.*, 2005).

Parts	Male	Female
Head	Vertical length 1.62 mm	As male
Antennae	Aristate	As male
Thorax	Yellow strip on side of thorax	As male
Wings	Mostly transparent, length 5.4–6.9 mm	As male
Legs	All femora yellow and all tibiae dark with hind tibiae conspicuously darker	As male
Abdomen	Oval, with extensive black markings Terga III-V with a distinct black mid-longitudinal line.	Oval, with extensive black markings Terga III-V with a distinct black mid-longitudinal line. Aculeus needle shaped, 1.6 mm



Plate A 1: Adult of fruit fly female



Plate A 2: Adult of fruit fly male

2.1.4 Distribution:

The family Tephritidae is represented in all the which content but the major pest genera have a limited natural distribution (Drew, 1989). Thus, *Anastrepha* spp. occur in South and Central America and the Caribbean. *Bactrocera* spp. are native to tropical Asia, Australia and South Pacific, *Ceratitis* and *Dacus* are native to tropical Africa (Drew, 1989). In a few cases, species have been accidentally introduced and have become established outside these natural ranges, mainly as a result of human activity (White and Elson-Harris, 1992).

In Sudan, fruit flies were reported at Khartoum State by Venkatraman and Elkhidir, (1965). Ali (1967) found fruit flies in the Northern region (Shendi, Hudeba), Khartoum, Kassala .Now it is 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 *B.invadens* has been recorded in eastern, western and central Sudan (Ahmed, 2001; Elhewaris, 2003, and Bashir, 2007).

2.1.5 Host Range:

Generally, fruit flies are polyphagous with host plants such as apple, guava, banana, date palm, okra, orange, papaya, peach, eggplant, tomato and cucurbits (Averill, 1996).

Venkatraman and Elkhidir (1965) reported fruit flies on egg-plant (*Solanum melongena*) and guava (*Pisidium* sp).

Ali (1967) reported that, nine species of fruit flies were found in Sudan, of which five are well known pests of economic crops in many parts in Africa. Schmutterer (1969) reported that the family Tephritidae was considered the fourth group of insect pest causing serious damage to fruit crops in Sudan. Among the fruit flies were *Dacus* species which highly infest cucurbits and

Ceratitis spp. which constitute the major pests of guava, citrus (orange, tangerine, and grape fruit), mango, chilis (peppers), egg-plant and coffee berries.

Siddig (1984) and Deng (1990) reported that the fruit fly *ceratitis capitata* is the major pest of guava. According to Beji (1996) the main species of fruit flies found in Kassala and Gash Delta are *Dacus* spp. (*Dacus ciliatus*, *Dacus cucurbitae* and *Dacus longistylus*) on water melon and melon, *Ceratitis capitata* and *Ceratitis cosyra* on guava and mango.

Among the fruit flies found in Sudan, *Ceratitis capitata* and *Ceratitis cosyra* are considered as devastating pests to fruit trees: mango, guava, and citrus all over the country especially at Shendi, Senga, Sennar beside a new species of the genus *Bactrocera* which was reported from Blue Nile areas as *Bactrocera invadens* (Drew *et. al.*, 2005).

2.1.6 Biology:

The fruit fly (*Bactrocera invadens* (Drew, Tsuruta and White) is a new fly species for which few scientific data are available. The females pierce the fruits using their ovipositors to lay their eggs in the pulp. Each female can lay on average 700 eggs depending on the host. The species is multivoltine (i.e. several generations / year) with an average life span of about 3 months (Ekesi *et. al.*, 2006). The mean generation time for *B. invadens* was found to be 30.7 days at $28 \pm 1^\circ$ C. However, generation time is largely dependent on temperature. In order to determine phenological events in the field for monitoring and eradication purposes, it is important to determine the temperature-development rate of the pest. The developmental rates of *B. invadens* were determined at five constant temperatures of 15°C, 20°C, 25°C, 30°C and 35°C and a photoperiod of L12:D12. The table below shows the mean total developmental time of the immature stages (egg to pupa) in days. (Rwomushana *et.al.*, 2008).

Temperature °C	Mean (egg to pupa)time (days)
15	75.74
20	31.45
25	21.19
30	17.76

Females begin to lay eggs about 8 days after emergence from the puparium. Under optimum conditions, a female can lay more than 3000 eggs during her life time, but under field conditions approximately 1200 to 1500 eggs per female is considered to be the usual production. Ripe fruits are preferred for laying egg, but immature ones may be also attacked. Adult flies live for many months (Ronald, 2007).

2.1.6.1 Egg:

Female flies insert eggs under the skin of fruit in clusters of 10 to 50 about 1/25 to 1/8 inch below the fruit surface. The eggs are white, elongate, and elliptical. They hatch in 1-2 days, (Plate2).

2.1.6.2 Larvae:

Larvae are legless, and resemble an elongated cone. The mouth is at the pointed end of the body. The third instar is about 2/5 inch long. The entire larval stage lasts for 11-15 days, Plate (3).

2.1.6.3 Pupae

The puparium is yellowish-brown and seed-like. Emerging to adult stage takes about 10 days, Plate (4).

2.1.6.4 Adults:

The color of the fly is highly variable but mostly yellow with dark markings on the thorax and abdomen. Generally, the abdomen has two horizontal black stripes and a longitudinal median stripe extending from the base of the third segment to the apex of the abdomen, Plate (5). These markings may form a "T" shaped pattern, but the pattern varies considerably (Abdel Magid, 2010).



Plate 3: The Egg stage.



Plate 4: The larval stage.



Plate 5: The pupal stage.



Plate 6: The adult stage.

2.1.7 Damage and Economic Importance:

According to Lux *et. al.*, (2003b) 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 \$ (Lux *et. al.*, 2003b). In Sudan, mango is leading the horticultural exports. Although mango production is more than 600,000 t, only 6000 t is exported in the best cases. This is only 1% of the total production (Elgozuli, 2008).

Schmutterer (1969) reported that the symptoms vary from host fruit to another e.g. the infestation appears as dark spots in citrus and as black sunken areas in the lower half of the guava fruit.

2.1.8 Monitoring and Control of Fruit Flies:

2.1.8.1 Monitoring of Fruit Flies:

Attraction of fruit flies to their host fruit is accomplished by visual or olfactory stimuli or a combination of both. Considerable efforts for developing lure and kill methods for the control of fruit fly species have been exerted during last decades (Gunningham, 1989).

Sharp and Landolt (1984), reported that, mated females of papaya fruit fly use the fragrance of mature papaya fruits as guidance to find their host. i. In Mexico, *Toxotrypana curvicauda* respond to brown sugar and pineapple juice baited in McPhail trap (Castrejon *et.al.*, 2004).

In Europe, Mauritius and USA Nulure and Torula yeast; protein hydrolases derived from *Zea mays* are the most widely used food-bait for trapping fruit fly (Rosseler, 1989; Heath *et.al*, 1995; and Beije *et.al.*, 1997). According to many authors, ammonia is an essential substance used by females of fruit flies as food in order to develop eggs (Bateman and Morton, 1981; Mazor *et.al.*, 1987; Robacker, 1995 ; Robacker and Heath 1996, Epsky and Heath, 1998 ; and Hull and Cribb, 2001). Recently, ammonia-derived baits include AMPu (a blend of ammonium bicarbonate, methylamine hydrochloride and 1,4 diaminobutane [putrescine]) were found to be effective as Torula yeast (Robacker and Warfield, 1993; and Robacker, 1995).

The prospects of using ready-made protein hydrolase by poor fruit growers are limited because of scarceness and expenditure. Ammonia released by Human urine, chicken and duck feces (Bell *et.al.*, 1961) was found as a potential attractant for *Anastrepha* spp.(Hedström, 1988, and Piñero *et.al*, 2002). Aluja and Liedo (1986) reported that, the extracts of plant materials were evaluated for trapping.

Sidir Zizyphus spinachristi can attract *C. cosyra*. Rosseler (1989) mentioned that, ammonium acetate, 1, 4-diaminobutane (putrescine) and tri--methylamine (FA-3) are used as long-lasting dispensers for attracting fruit flies. Males of *C. cosyra* do not respond well to Trimed-lure (TML), cuelure or Methyl Eugenol (ME). However, they respond to Terpinyl Acetate and several terpinoids, while females respond to food baits as Nulure (Lux *et.al*, 2003b).

Males of *Ceratitis rosa* and *C. fasciventris* respond very well to TML where *B. invadens* is attracted to ME and respond to Nulure.

2.1.9 Control of Fruit Flies:

2.1.9.10 Mechanical Control:

One of the most effective mechanical control methods is bagging the fruits to exclude egg laying (Hill, 1983).

2.1.9.11 Cultural Control:

The principal cultural control method used for controlling this pest is field sanitation. Field sanitation directed towards the destruction of all unmarketable and infested fruits. Infested fruits should be buried 3 feet under soil surface with addition of sufficient lime to kill larvae. Harvesting of fruits weekly also reduces Food sources from which large populations may develop by keeping the quantity of ripe fruit on the trees to a minimum. Other procedures that reduce the amount of infield breeding of flies should be used (Heppner, 1985).

2.1.9.12 Legislative Control:

Vargas *et.al*, (1983) explained that, the economic importance of the fruit flies couldn't be evaluated entirely from the standpoint of the actual damage to the various crops affected. It must also be considered from the standpoint of quarantine.

Quarantine laws aimed at preventing the entry and establishment of flies in areas where they do not occur or have been established and are vigorously enforced. The United States (US) Government has strict laws regulating the movement of certain commodities to prevent the establishment of fruit flies into the continental US. The Japanese Government restricts the entry of commodities attacked by these pests into their country.

2.1.9.13 Biological Control:

An ovo-pupal parasite of Asian origin, *Fopius arisanus* (Hymenoptera: Braconidae) has been shown to be highly efficient in laboratory tests for controlling *B. invadens*, and was successfully used in the Pacific area (Vargas, 2004). This species is a favored candidate for biological pest control, and could be introduced into countries invaded by *B. invadens*. Recent research in Benin has shown that abundant weaver ants *Oecophylla longinoda* (Hymenoptera: Formicidae) considerably reduce the damage done by fruit flies in Mango

orchards information campaigns and integrated fruit production methods aim to encourage the introduction and protection of these useful weaver ants in West African orchards (Van Mele ,*et. al.*, 2007)

2.1.9.14 Chemical control:

The chemical or the insecticidal methods of control of fruit flies fall under three main categories: spray the adults with suitable insecticides, trapping of the adult flies by means of chemical attractant, and bait spray that in essence is an insecticide mixed with bait (Ali, 2007). The study by Deng (1990) who tested three insecticides to control the fruit fly, showed that Methidathion (Supracide) was the most effective compared to Carbaryl (Sevin) and Cypermethrin (polytrin).

2.1.9.15 Topical application:

Topical application has been used as a bioassay technique on large insects such as adult houseflies, *Musca domestica* (L.) (Dahm *et. al.*, 1961), and *Heliothis armigera* Hobner and *H. virescens* (F.) larvae (McCaffery *et. al.*, (1988). Various slidedip methods also have been used for determining resistance levels in both redaceous and phytophagous mites (Croft *et al.* 1976, Dennehy and Gennett 1984). However, residual assays that utilize a known concentration of insecticide applied to a surface such as leaves (Morse *et. al.*, 1986, Prabhaker *et. al.*, 1988), are thought to simulate pesticide uptake in the field better than topical application (Brattsten *et, al.* 1986). Other residual methods include vials (Campanhola and Plapp 1989, Schouest and Miller 1988), petri dishes (Hinck!e *et. al.*, 1985, Grafton-Cardwell *et.al.*, 1989) or glass plates (Hassan 1983). The availability of numerous techniques for estimating resistance levels in various insect species, each with varying degrees of .sensitivity (Busvine .1971, 1980), gives rise to the need of standardizing and employing the best method that truly represents the resistance pattern in the field among target pest populations.

2.1.9.16 Sterile Insect Technique (SIT):

The sterile insect technique (SIT), is a more ecologically acceptable control measure, but this approach is complicated and very expensive (Bateman, 1972). SIT may not work as a sole control strategy, particularly when the population density of the fruit flies is high and perhaps more importantly, when several species co-exist (Knipling, 1992). On the other hand, the use of the SIT may not be compatible with grower requirements, because sterile females will continue to oviposit and damage fruits, even if the eggs were not viable (Vargas. 2004). Furthermore, SIT for control of *C. cosyra*, *C. rosa*, *C. fasciventris*, and *C. anonae* is currently not possible because no appropriate methods for mass production of these species have been developed.

2:2 Botanical insecticides:

Higher plants are extremely abundant with biologically active secondary metabolites. Over 80% of all known Alkaloids, Terpenoids, and phenols and another secondary metabolite were produced by higher plants (Siddig , 1993). Many plant extracts or products have proven to be as potent as many conventional synthetic pesticides and are effective at very low concentrations. On the other hand botanical insecticides possess great advantage over synthetic pesticides in being more environmentally friendly, to be accepted by the majority of the farmers, governmental organizations and decision makers (Kelany, 2001). Stoll (2000) demonstrated that the use of plant extracts to control destructive insect in not new .Rotenone, Nicotine and Pyrethrin has been used for a considerable time in small scale subsistence and also commercial agriculture.

2.2.1 Neem Tree:

Neem is versatile tree, it is considered to be one of the most promising trees of the 21 century. It has great potential in the field of pest management,

environment protection and medicine. Also it has showing great promise as potential fertilizer (Abdalla, 2010).

2.2.2 Taxonomy:

Order: Rutales

Family: Meliaceae

Genus: *Azadirachta*

Species: *Azadirachta indica*

S.N: *Azadirachta indica A.juss*

E.N: Neem

A.N: نيم

(Vietmeyer, 1992, and Schmutterer, 2002)

2.2.3 Origin:

Neem is versatile tree of Indian and Burma origin where the ancient healers of that region knew it very well in health (ICIPE, 2002).

2.2.4 Morphology:

Neem is a fast growing tree that can reach a height of 15-20m, rarely to 35-40m. Its ever green but under severe drought it shed mostly or nearly all of it leaves. The branches are wide spread, the fairly dens crown is roundish or oval may reach diameter of 15-20m. In old tree standing specimen the trunk is relatively short straight and many reach a diameter of 1.2m. The bark is hard fissured or reddish-brown. The sap wood is grayish white and heart wood reddish when first exposed to the air becoming reddish after exposure. The root system consists of a strong tap root and well developed tateral roots. The alternate, pinnate leaves are medium (Ganguli, 2002).

2.2.5 Distribution:

Neem is widely distributed throughout South East Asia and West Africa and part of Central America (Stoll, 2000). Neem is introduced to Sudan in the 20 century. The first one were planted at shambat in 1916, today trees are spread in town and villages along the Blue and White Nile, irrigated areas of Central Sudan, Kordofan and Darfur (Schmutterer, 1969).

2.2.6 Ecology:

The Neem trees is famous for its drought resistance, normally it thrives in areas with sub-arid to sub humid conditions with an annual rainfall between 400 and 1200 mm. it can also grow in regions with an annual rainfall 400MM. but in such cases it depends largely on the ground water levels. Neem can grow in many different types of soil, but it seems to develop best on well drained, deep sandy soils. It is a tropical and subtropical tree, and exists at annual means temperatures and does not tolerate (Ganguli, 2002).

2.2.7 Chemical Compounds of Neem tree:

Various compounds were isolated from different parts of neem tree using different chemicals. Most of the known active compounds belong to the group of titer penoids (Schmutterer, 1990). Azadirachtin and Solanin are the most important constituents of Neem seed kernel composition, other active compounds in the seed kernel are Salanin, Salanol, Acetate, Nimbin and Deactly nimbidin (Jacobson, 1989).

2.2.8 Uses of Neem in pest and disease control:

Neem is deemed very effective in the treatment of scabies although only preliminary scientific proof exists which still has to be corroborated and is recommended for those who are sensitive to Permethrin. A known insecticide which might be irritants and also the scabies mite has yet to become resistant to Neem, so in persistent cases Neem has been shown to be very effective, there is

also anecdotal evidence of its effectiveness. In treating infestations of head lice in humans, it is also very good for treating worms (soak the branches and leaves in lukewarm water and drink it). In the traditional medicine Neem trees originated on the Indian subcontinent. The Neem twig is nature's tooth brush to over 500 million people daily in India alone. Herbal medicine is the oldest form of therapy practiced to be mankind and much of the oldest medicinal use of plants seems to have been based on highly developed 'dowsing instinct' (Grigs, 1981). Siddig (1993) reported from Sudan that Neem seed water extracts at 1Kg/1Liter of water repelled foliage pest of potato including *B. tabaci*, *Aphis gossypii* and *J. lybica* and yield increased to 5 ton/ ha. Mohammed (2002) reported that Neem seed showed good performance against *A. gossypii*, *B. tabaci*, and *J. lybica* on Okra. Dawood (2001) reported that Neem water extracts at 1Kg/liter water reduced the number of onion thrips at 63.5% under the field condition.

2.3 Rehan, Holy basil (*Ocimum basilicum* L).

Sweet basil is native to western and tropical Asia .It belongs to the family Lamiaceae.By the 16th century it was also cultivated in England.

Today,it is widespread in Europe,Asia,Africa and the western Hemisphere as condiment for medicinal purposes and as scent,Sixty different varieties of this plant have been identified in the tropics.Holy basil is found throughout India and Nepal up to elevations of 1800 m in the Himalayas and on the Andaman Nicobar Islands.It is regarded by the Hindus as their most sacred plant (Stoll, 2000).

Synthetic insecticides are expensive for subsistence farmers and they may pose potential risks owing to the lack of adequate technical knowledge related to their safe use.One alternative to Synthetic insecticides is insecticidal plants; African farmers are traditionally familiar with them (Thiam and Ducommuun, 1993).Oils extracted from plants have been extensively used in tropical

countries for crop protection (Singh, *et al.*, 1978; Dabire, 1993); Rajapakse and Van Emlen, 1997). The tropical flora is a major source of plant-based insecticides (Aranason, *et al.*, 1989). Aromatic species, particularly those in the family Labiatae or (Lamiaceae) are among the most widely used plants in insect pest control (Morton, 1981., Lambert, *et al.*, 1985; Lawrence, 1988; Shaaya, *et al.*, 1997).

Among the most common species of *Ocimum* (Labiatae) in West Africa are *Ocimum basilicum* L. (Sweet basil) and *Ocimum canum* Sims. (White basil) (Berhaut, 1975).

Essential oils are volatile secondary metabolites that plants produce for their own needs other than nutrition i.e. protectant or attractant. In general, they are complex mixtures of organic compounds that give characteristic odour and flavour to the plants. The essential oil composition of *O. basilicum* varies depending on the environment and the chemo type (Brophy and Jogia, 1986). It has an essential oil content of 0.2%, with 32 chemical components, including linalool (22.3%), methyl Eugenol (24.7%) and (E)-methyl chavicol (23.6%). Pino *et al.* (1994) found that samples from Cuba have an essential oil content of 1.9-2.5% and 30 chemical components, including methyl chavicol (66.8%), 1,8-cineol (5.4%) and linalool (5%), whereas samples from Burkina Faso (Belanger, *et al.*, 1995) have an essential oil content of 0.7-1.8% with 25 chemical components, predominantly 1,8-cineol (60.2%), α -terpinol (6.5%) and B-pinene (5.7%).

Ocimum basilicum and *Ocimum gratissimum* were cultivated in order to determine the essential oil content and its chemical composition at different harvesting dates after planting. In fresh *O. basilicum* oil content was 0.26% at three and four months after establishment and it drastically decreased to 0.14% at five months after planting; Estragole was the main constituent (81-83%) of *O. basilicum* oil at all harvesting dates (Sanda *et al.*, 2001).

2.3.1 Insect Control Properties of *Ocimum spp.*

Dalziel in (1937) reported that *Ocimum vividae* Wild., has been named the mosquito plant and it was used to repel mosquito in West Africa. *O.vividae* any disease, was used a mosquito repellent. The herbivorous animals refused to eat *O.canumsims*, a perennial which had not suffered from any disease .

The sacred tubi-plant *O.santum* according to Guenther, (1961), has been used in India, beside,, medicinal and antibiotic effects, as and insect repellent.

Malaka (1972) stated that a preparation of the leaves of *O.basilicum* L. has been used among many methods to protect yams before planting against termites.

Shah and patel (1976) found that *O.sanctum* contained methyl eugenol and had been used to attract the mango fruit fly, *Dacus correctus* Bezz.and observation on the population counts revealed that all the fruit flies attracted to tulsi plant were male only.

Deshpande and Tiphis (1977) obtained by TLC eight fractions from the essential oil of *Ocimum basilicum* .They tested the activity of each fraction against stored grains insect pests, namely *Tribolium castaneum* ,*Sitophilus oryzae*,*Stagobium paniceum* and *Bruchidiuus chinensis*. From bioassay tests methylcinnamate and methylcharicol were found to be the components mainly responsible for the insecticidal activity.

Rajendran and Copalan (1978) found that, *Opium sanctum* showed no clear juvenile– hormone like activity. The same authors, Rajendran and Copalan (1979) reported that, the extracts of four plant species ,including *Ocimum sanctum* Linn., caused mortality in *Dysedercus cinquulatus* Fabr., *Spodopter liturales* Fabr and *Pericalla ricini*. They concluded that, these plants have not only juvenile–hormone mimicking substance but also insecticidal properties. Bowers and Nishida (1980) isolated two compounds with highly potent juvenile

hormone activity from the oil of sweet basil, *Ocimum basilicum* L. They named them Juvocimene 1 and Juvocimene 2.

Juvocimene 2 induces the formation of nymphal adults intermediates in the milk weed bug at treatment levels as low as 10 pg. Juvocimene 1 is approximately 10 times less active than Juvocimene 2. Both plant derived hormones are several orders of magnitude more active than the natural juvenile hormone. They suggested that the sweet basil may have developed an additional and far more sophisticated chemical defense against insect morphogenetic development.

Pandey, *et. al* (1983) investigated the efficacy of certain plant extracts against brinjal *Aphis gossypii* Glov. At different concentrations (0.1, 0.5 and 1.0%) and depending on concentration, an extract of mature seed of *O.basilicum* L.was shown to give 45.40-56.22 %mortality.

Mansour, *et .al.*, (1986) investigated the effect of essential oils isolated from 4 species of the family Labiatae on adult females of *Tetranychus cinnabrinus* in the laboratory .They showed that concentrations of the acetone solutions of the oils from 0.1 to 2% cause mortality and induce repellency within 48 hours of introducing adult females, and consequently egg-laying was found to be reduced, and seven day old residues still had some activity. The most effective oils (their EC50 in brackets) were *Lavandula angustifolia*, *L.latifolia* (EC50 0.094), *L.angustifolia* (0.1%), *Ocimum basilicum* (EC50 1.4%), *Salvia fruticosa* (EC50 1.4%) and rosemanry (EC50 2.2%).

2.4 Propolis (bee glue):

Propolis is a wax –like resinous substance collected by honey bees from tree buds or other botanical sources and used as cement to seal cracks and open spaces in the hive, its colour varies from green to brown and reddish, depending on its botanical source.

Honey bees use propolis to seal gap inside the hive that smaller than 3/16 or 1/4 (5mm or 6mm) while they leave themselves a bee space approximately 9.5mm or 38 larger spaces being filled with wax conib (Burdock, 1998).

2.4.1 propolis is now thought to:

- 1- Reinforce the structural stability of the hive
- 2- Reduce vibration
- 3- Make the hive more defensible by sealing alternate entrances
- 4- Bees may also use it to prevent infection with disease and parasites in the hive.

2.4.2 Composition:

The composition of propolis varies from hive to hive, district and from season to season. Occasionally, bees gather calking compounds of human manufacture. Even propolis samples taken from a single colony can vary, making controlled clinical tcsts virtually impossible (Banskota *et al*, 2001 and Bankova, 2005).

The source of propolis varies with the latitude. In termperate regions bees collect resins from trees, mostly poplars and to a lesser extent conifer the biological role of propolis in trees is seal wounds and defend against bacteria, fangi and insects. In tropical regions, bees gather propolis from flowers, especially clusia, that have adapted propolis and tropicals are different. poplar propolis is rich in flavanoids . Clusia propolis contains polyprenylated benzophenones . Typical propolis has approximately 50 constituents, primarily resins and vegetable balsams (50%) waxes (30%), essential oils (10%) and pollea (5%) . Propolis is sticky at and above room temperature . At lower temperature it becomes hard and very brittle (Burdock,1998).

2.4.3 Physical characteristics:

The colour of propolis ranges from yellow to dark brown depending on the origin of the resins. But, even transparent propolis has been reported. At 25 to 45 °C propolis is a soft, pliable and very sticky substance. At less than 15 °C, and particularly when frozen or at near freezing it becomes hard and brittle. It remains brittle after such treatment even at higher temperature. Above 45 °C, it becomes increasingly sticky and gummy. Typically, propolis becomes liquid at 60 to 70 °C but for some samples the melting point may be as high as 100 °C. The most common solvents used for commercial extraction are ethanol (ethylalcohol) ether, glycol and water for chemical analysis a large variety of solvents may be used in order to extract the various fractions many of the bactericidal components are soluble in water or alcohol (Arvouet *et. al* ,1993).

2.4.4 Chemical characteristics:

The composition of propolis varies with its geographic and plant source, as well as with the collection season (Banskota, *et al*, 2001 and Bankova, 2005). The alcohol extract of propolis is called propolis wax or tincture, with the insoluble residue known as propolis resin (Burdock, 1998) propolis contains 50% resin and vegetable balsam 30% wax and aromatic oils, 5% pollen, and 5% other substance including minerals such as magnesium, nickel, iron, calcium, and zinc (Burdock, 1998 and Castaldo and Capasso, 2002).

Propolis contains flavonoids such as quercetin, pinocembrin galangin, and pinobanksin, as well as hydroquinone, caffeic acid esters (Burdock, 1998 and Castaldo and Capasso, 2002).

A number of other compounds have been identified in propolis from specific geographic source (Popova *et. al.* , 2005).

2.4.5 Biomedical research:

Propolis is the focus of a large number of research projects. (Sforcin and Bankova 2011).Some preliminary research findings (published in the biomedical literature), together with their limitations, are described below.

2.4.6 As an antimicrobial:

Preliminary scientific studies show some types of propolis have *in vitro* antibacterial (Orsi,*et.al*,2005) and antifungal(Cafarchia,*et.al*,1999) activity with active constituents including flavonoids like galangin (Cushnie and Lamb 2005)and hydroxycinnamic acids like caffeic acid. (Qiao and Chen 1991) In the absence of any *in vivo* or clinical studies however, it is not clear if this antimicrobial activity has any therapeutic relevance.

2.4.7 Acaricidal effect of bee propolis extracts:

A number of researchers have reported insecticidal effect of bee propolis. Solvent extracts of propolis samples from Brazil and Bulgaria exhibited leishmanicidal activity against different species of *Leishmania* (Gerzia *et. al.*, 2007). In Nigeria, Osipitan *et. al.*, (2010) tested propolis ethanolic extracts against the larger grain borer, *Prostephanus truncates* (Horn) in maize grains. A reduction of the borer population in maize was observed. Interestingly, pesticides commonly used in agriculture were detected in honey and propolis samples (Lucia *et al*, 2011) in Uruguay.

Recently bee propolis extracts have been reported to have acaricidal effect on red spider mites (*Tetranychus spp.*), which attack tomatoes, (Kareru and Wamaitha, 2012, unpublished work).

Compounds present in propolis can provide potential alternative in the place of currently used insect pest control agents because they constitute a rich source of bioactive chemicals and may act in many way on various types of pest complex.

They also have no or little harmful effects on non target organisms such as pollinators, natural enemies and are biodegradable.

Both ethanolic and ethyl acetate extracts of bee propolis acted on red spider mites in a concentration and time dependent manner. The activity of ethanolic extracts at concentrations of 75 and 100 mg/ml was not significantly different with that of the positive control used.

Ethanolic and ethyl acetate extracts acted on tomato red spider mites in a concentration and time dependent manner, and had no significant differences in activity.

Bee propolis extracts could thus be used as a safe insecticide in the control of red spider mites. However, further research are needed to be done on its potential on other life stages of red spider mites and other common tomato pests. The insecticidal activity was thought to be due to bioactive phytochemicals of plant origin ingested by the bees during pollination.

2:5 Insecticide:

2.5.1 Cyper methrin:

Cypermethrin is a pyrethroid insecticide. It was first synthesized in 1974 (WHO,1989). Cypermethrin is a synthetic chemical similar to the pyrethrins in pyrethrum extract which comes from the chrysanthemum plant. Pyrethroids, including cypermethrin were designed to be effective longer than pyrethrins (WHO 1989).

2.5.2 Mode of action:

Cypermethrin kills insects that eat or come into contact with it (Tomlin ,1994) and works by quickly affecting the insect's central nervous system.

2.5.3 Environmental effects:

Cypermethrin is a broad-spectrum insecticide, which means it kills beneficial insects and animals as well as the targeted insects (Pascual and peris,1992). Fish are particularly susceptible to cypermethrin(Stephenson,1982). Resistance to cypermethrin has developed quickly in insects exposed frequently and can render it ineffective(Martinez-Cabrillo,1991).

CHAPTER THREE

3:MATIRISLS AND METHODS

The tests were conducted under laboratory condition at the College of Agricultural Studies -Shambat, Sudan University of Science and Technology during January- April 2015 to study the effect of ethanolic extracts of leaves Neem,Basil and Bee glue powder of (*B.invadens Drew*, Tsrusta and White) .

The materials and methods used in the present study (Plates 6-15) are mentioned below:-

3- 1-Equipment:

- 1-plastic cages
- 2-Brush
- 3-Hand sprayer
- 4-Sensetive balance
- 5-Thirmometer
- 6- spoon
- 7-Soxhlet extractor
- 8-Rotary evaporator
- 9- Masks
- 10-Collection sample
- 11-Micropipette
- 12-Petri-dishes
- 13-Marker pen
- 14-Pencil
- 15-Gloves
- 16-Masks
- 17-Scalpel
- 18-Camera

3-2-Materials:

1-Neem leaves

2- Rehan leaves

3- propolis powder

4- Ethanol 99.7%

9- Distilled water

10-Cotton

11-Yeast

5- Soap

6- UHU

7-Muslin cloths

8- Sand



Plate 7: Rearing plastic containers



Plate 8: Glass cage for adult rearing of fruit fly.



Plate 9: Soxhlet for extraction Apparatus



Plate 10: Rotary Evaporator



Plate 11. a- Tools 10. b- Small Water Sprayer (for soil moistening)



Plate 12: Thermo-Hygrometer.



Plate 13: Infested guava

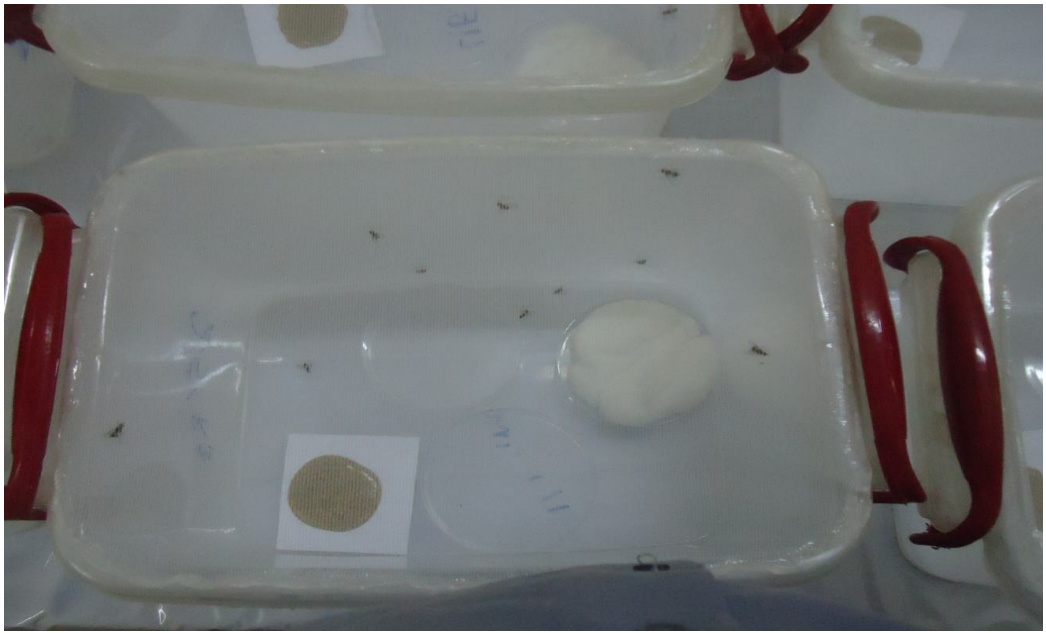


Plate 14: Fruit flies in plastic cage after treatments.



Plate 15: Some of the equipments used in The Study

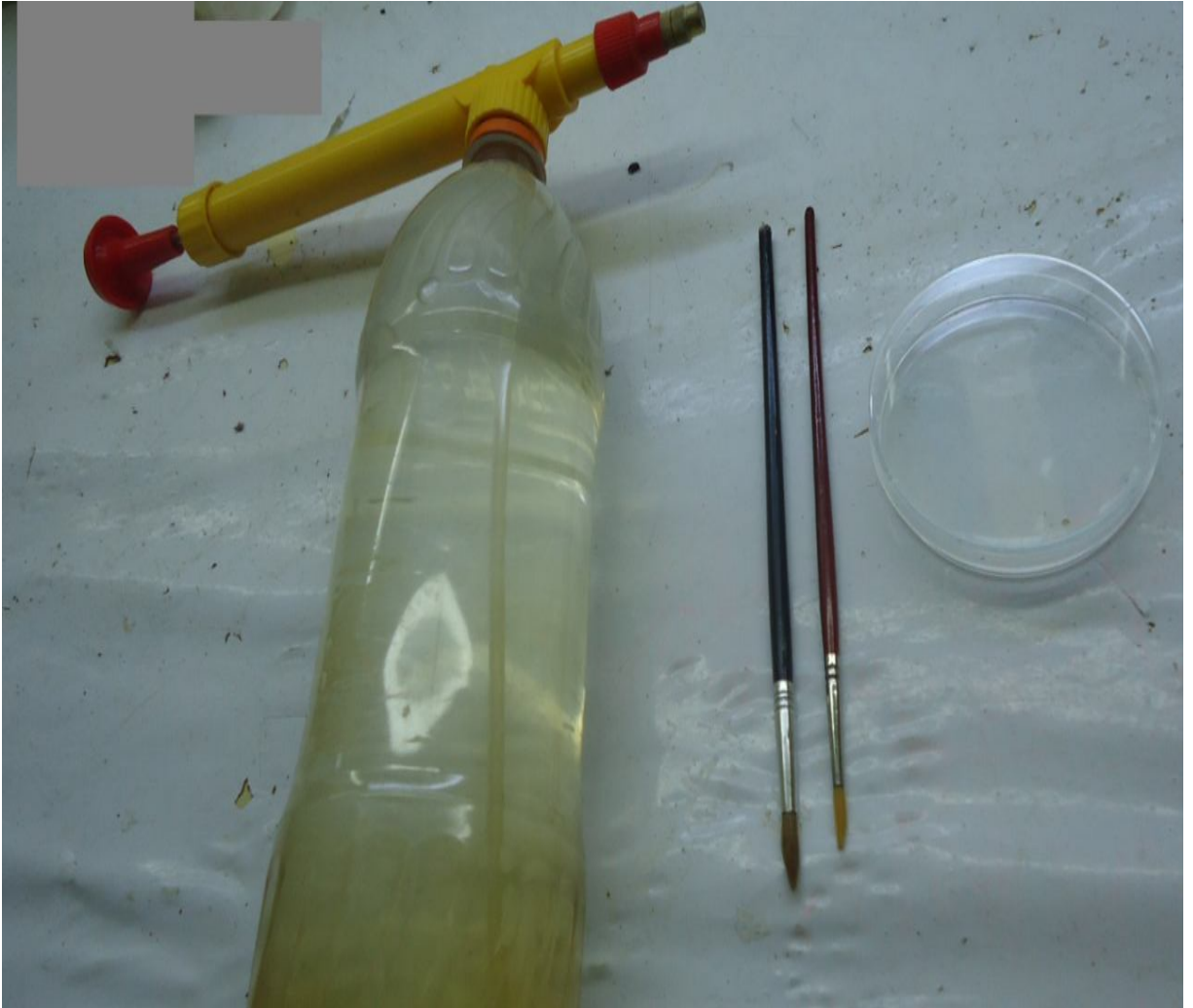


Plate 16: Hand spray, Brushes and Petri-dishes

3:3 Rearing methods:

3:3:1 Fruit fly rearing:

Guava infested fruits were collected from in Khartoum, Sudan and brought to the Entomology laboratory at the College of Agriculture, Sudan University of Science and Technology. Collected fruits were placed on a layer of sand (2-3 cm) in plastic containers 31x20x19 cm and 25x17x16 cm in size and screened with light cloth netting material for ventilation. Sand (2-3cm) was kept moistened with water using hand sprayer. The rearing of the newly emerged adults continued for 19-21 days. Newly emerged adults were transferred to glass cages (30x30x30cm) where they fed on a media containing sugar and yeast at a ratio 3:1. A small piece of cotton was soaked with water and placed in Petri dish as a source of water for the flies. Temperature in the laboratory was between 27 ± 2 °C and relative humidity (RH) was between 30 ± 5 %. Five pairs of newly emerged adults were transferred to other plastic containers to be used for the experiments .

3:4 Ethanolic extract of leaves Neem and Basil:

Leaves of neem and basil were collected from the field of shambat area and dried under shaded area.

The Bee glue powder collected from the company of Albaraka product of honey bee. Both Neem and Basil leaves were crushed very fine powder using an electronic blender. The extraction was conducted at the Chemistry laboratory, College of Agricultural Studies, Sudan University of Science and Technology.

3.4.1 Preparation of ethanolic extraction of leaves of Neem and Basil:

To prepare ethanolic extract of leaves of neem or basil ,60 gm powder of each material was dissolved in 500 ml ethanol 99.7% and remained in a Soxhlet apparatus for 6 hours. The ethanolic solvent of Neem and Basil was removed

using Rotary evaporator .The obtained materials of each were weighed and carefully stored to carry out the experiment.

3.4.2 Preparation of Bee Glue:

The bee glue was provided by Albaraka company for honey bee products .

To prepare a solution of bee glue 5 gm powder of bee glue dissolved in 0.5 ml liquid soap and 500 ml water . The obtained material was weighed and carefully stored to carry out the experiment.

3:5 Bioassay procedure:

Stock solution from ethanolic extract of leaves of Neem and Basil were prepared separately based on preliminary tests, the concentrations to be tested were 1%, 5 %, 10% and 15%. Consequently further dilutions were made to prepare the desired concentrations.

Stock solution from solution of bee glue were prepared separately based on preliminary tests, the concentrations to be tested were 1%, 5 %, 10% and 15%. Consequently further dilutions were made to prepare the desired concentrations.

3:6 Topical application:

Five pairs of *Bactrocera invades* were used for each treatment and each treatment was replicated three times. Similarly five pairs were used as control where only distilled water mixed with 0.1% soap was topically applied.

The five pairs of fruit flies (*Bactrocera invadens*) used in this study were placed into a refrigerator about two minutes before the treatment, to make them inactive and easily handled during the treatment. A micro pipette was used to apply one micron of each concentration on the meso thorax of the adult.

Treated insects were kept in plastic cages (18x22x29cm) and fed with mixed of sugar and yeast at a ratio 3:1. Small piece of cotton wicks was soaked with water

and placed in Petri dish as a source of water for the flies. The dead adults were recorded after 24, 48 and 72 hours of exposure.

3:7 Food bait:

Fifteen pairs of *B. invadens* were used in this experiment. Fifteen ml of ethanolic extracts of each of neem and basil were added to 60 ml of guava juice and diluted to 50% same amount was applied to the powder of bee glue.

Different concentration was made from each solution and cotton was soaked with a mixture of each concentration was provided to adult flies. The insects in control were provided with guava juice only soaked in cotton wicks.

Observation was made after 24,48,72 hours of exposure.

3:8 Experiment Design:

Both experiments were carried out using Randomized Complete Block Design (RCBD).

3:9 Statistical analysis:

The obtained data was statistically analyzed by computer software MSTATC according to analysis of variance (ANOVA); Duncan's Multiple Range Test was used for mean separation.

CHAPTER FOUR

4: RESULTS

4:1 Effect of different concentration of ethanolic extract of leaves of neem on mortality rate of Fruit Fly *B. invadens* after 24,48,72 hours(Food bait application):

Generally, all concentrations of ethanolic extract of leaves of neem significantly better than the control and less on efficiency than Cyper methrin.

According to concentrations of leaves extract of neem for the different three assessment times, it is clear that, higher concentration gave higher mortality.

For each concentration as indicated in Table-1- the mortality increased within the time elapse. The mortality rates recorded 23.3, 50, 66.7, 86.7% for the concentrations 1,5,10 and 15% respectively after 72 hours.

4:2 Effect of different concentration of ethanolic extract of leaves of neem on mortality rate of Fruit Fly *B. invadens* after 24,48,72 hours(Topical application):

As indicated in Table .2. Highly significant differences were observed between various treatments.

All treatments gave better mortality than the control. Regarding neem concentrations, it is clear that, and the highest dose gave the highest mortality for the three assessment periods 24, 48, 72 hours after application.

From the present study, it is very obvious that the mortality of adult flies of *B. invadens* increased according to time interview, the concentration 15% gave 36.7, 50.7 and 53.3 % mortality for 24,48,72 hours respectively (Table.2.) and same for the remained concentration of ethanolic extract of leaves of neem.

Table 1: Effect of ethanolic extracts of leaves of Neem on mortality rate of *B.invadens* after 24,48,72 hrs (Food Bait application).

Treatments and concentration		Mortality (%)		
		Exposure time (hrs.)		
		24 hrs	48 hrs	72 hrs
Neem	1%	3.3(1.5) d	16.7 (4.1) d	23.3 (4.8) d
	5%	16.7 (4.1) c	36.7 (5.6) c	50 (7.0) c
	10%	36.7 (6.0) b	50 (7.0) bc	66.7 (8.2) bc
	15%	53.3 (7.3) b	66.7 (8.2) b	86.7 (9.3)ab
Cyper methrin		100.0(10)a	100.0(10)a	100.0(10)a
Control	0 %	0 (0.7)d	0 (0.7)e	0 (0.7)e
	CV %	14.4	14	11.0
	SE	0.6	0.5	0.4
	LSD	1.7	1.5	1.3

Means followed by the same letter are not significant different at (P< 0.05)

Data in parentheses transformed using square root transformation ($\sqrt{X + 0.5}$) before analysis.

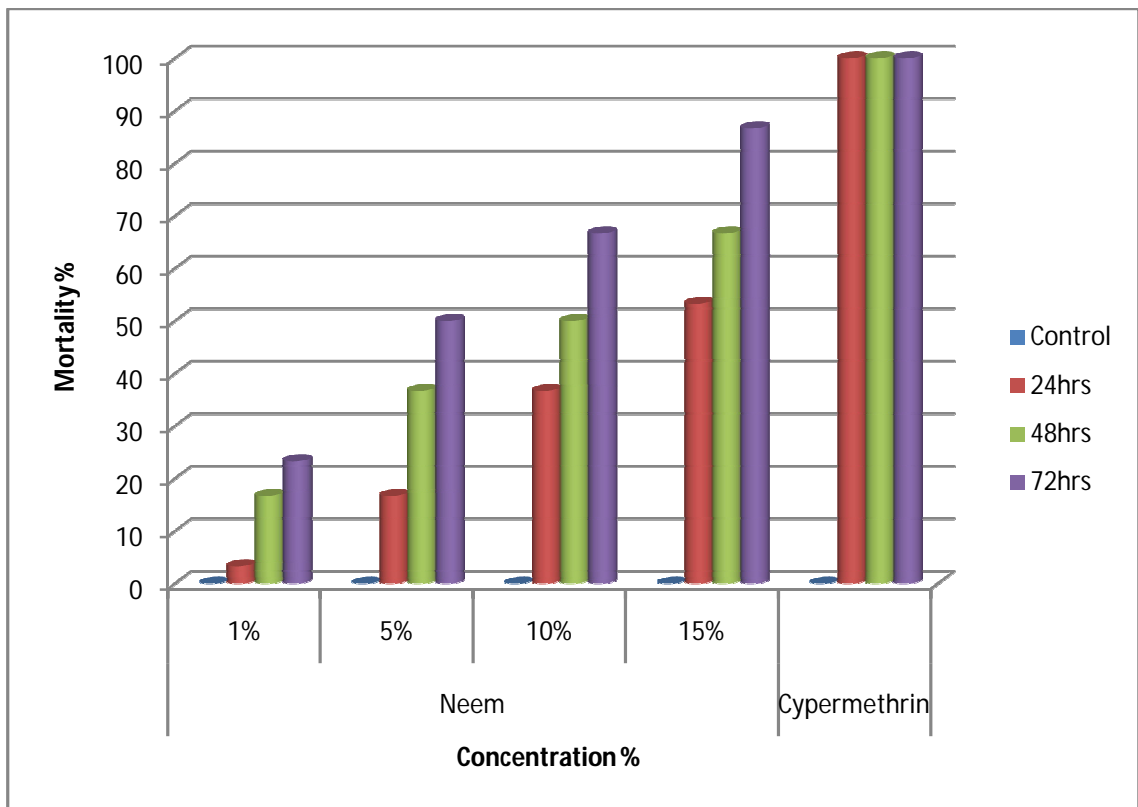


Figure 1: Effect of ethanolic extracts of leaves of Neem on mortality rate of *B. invadens* after 24,48,72 hrs (Food Bait application).

Table 2: Effect of different concentration of ethanolic extracts of leaves of Neem on mortality rate of B.invadens after 24,48,72 hrs (Topical application) .

Treatments and concentration		Mortality (%)		
		Exposure time (hrs.)		
		24 hrs	48 hrs	72hrs
Neem	1%	0 (0.7)e	6.7(2.4)d	16.7(4.1)d
	5%	6.7(2.4)d	16.7(4.1)c	20(4.4)cd
	10%	16.7(4.1)c	23.3(4.8)c	30 (5.5)c
	15%	36.7(5.8)b	50.7 (7.1)b	53.3 (7.3)b
Cyper methrin		100.0(10)a	100.0(10)a	100.0(10)a
Control	0 %	0 (0.7)e	0 (0.7)e	0 (0.7)e
	CV %	17.7	15.7	13.2
	SE	0.4	0.43	0.4
	LSD	1.2	1.353	1.2

Means followed by the same letter are not significant different at (P< 0.05)

Data in parentheses transformed using square root transformation ($\sqrt{X + 0.5}$) before analysis.

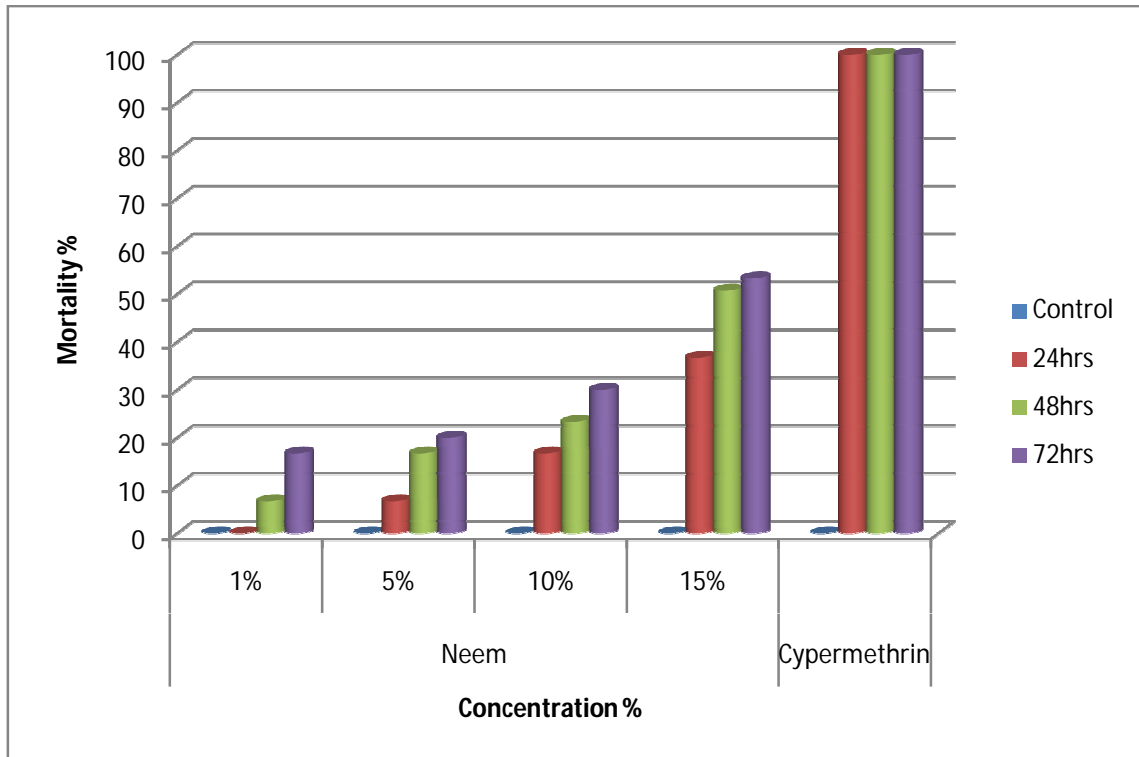


Figure 2: Effect of different concentration of ethanolic extracts of leaves of Neem on mortality rate of *B. invadens* after 24,48,72 hrs (Topical application) .

4:3 Effect of different concentrations of ethanolic extracts of leaves of Basil after 24,48 and 72 hours on mortality rate of B.invadens(Food Bait application).

Table .3.Shows the mortality rate of B.invadens fed and different concentrations of ethanolic extract of leaves of Basil after 24,48 and 72 hours .

Significant difference was observed between treatments where all treatments gave higher mortality than the control .

Present study assessment that,the highest concentration 15% of ethanolic extract caused the highest mortality rate throughout the three assessment periods (36.7,50 and 63.3%) for 24,48 and 72 hours respectively .

The results also indicated that the mortality rate increased according to the elapse of time of feeding.For all concentrations the peak mortality rate 16.7,26.7,43.3 and 63.3% at 72 hours for 1,5,10 and 15 % of ethanolic extract of leaves of Basil respectively.

Table 3. Effect of different concentration of ethanolic extracts of leaves of Basil on mortality rate of B.invadens after 24, 48, 72 hrs (Food Bait application).

Treatments and concentration		Mortality (%)		
		Exposure time (hrs.)		
		24 hrs	48 hrs	72 hrs
Basil	1%	3.3 (1.5) d	6.7 (2.4) d	16.7(4.1)e
	5%	13.3 (3.6) c	23.3(4.8) c	26.7 (5.2)d
	10%	23.3(4.8) bc	36.7 (5.8)c	43.3 (6.6)c
	15%	36.7(6.0) b	50 (7.1)b	63.3 (7.9)b
Cyper methrin		100.0(10)a	100.0(10)a	100.0(10)a
Control	0 %	0 (0.7)d	0 (0.7)e	0 (0.7)f
	CV %	16.7	14.2	9.04
	SE	0.5	0.4	0.3
	LSD	1.4	1.3	0.9

Means followed by the same letter are not significant different at (P< 0.05)

Data in parentheses transformed using square root transformation ($\sqrt{X + 0.5}$) before analysis.

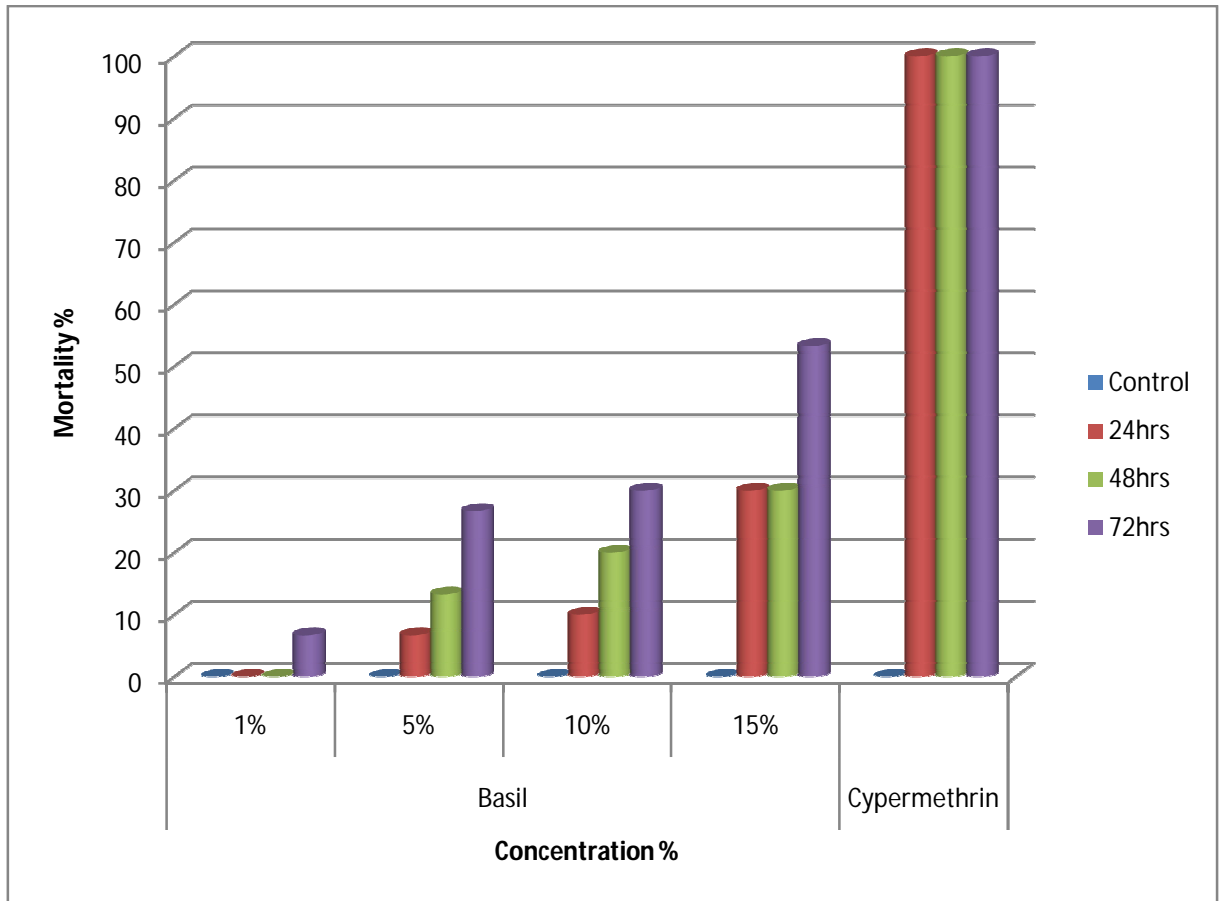


Figure 3: Effect of different concentration of ethanolic extracts of leaves of Basil on mortality rate of *B.invadens* after 24, 48, 72 hrs (Food Bait application).

4:4 Effect of different concentrations of ethanolic extracts of leaves of Basil after 24,48 and 72 hours on mortality rate of B.invadens(Topical application).

The result of this trial revealed significant difference between treatments for all assessment periods.

Inspite of the first dose after 24 hours of exposure all doses in all assessment times cause mortality to adult flies of B. invadens .

It is very clear that,the increase of the dose flare up the mortality rate and within the elapse of time ,the mortality increased (Table .4.).The highest dose 15% caused more than 50% mortality rate after 72 hours.

Table 4. Effect of different concentration of ethanolic extracts of leaves of Basil on mortality rate of B.invadens after 24, 48, 72 hrs (Topical application).

Treatment and concentration		Mortality (%)		
		Exposure time (hrs.)		
		24 hrs	48 hrs	72 hrs
Basil	1%	0 (0.7)e	3.3(1.5)cd	13.3(3.6)d
	5%	6.7(2.4)d	10(2.8)c	16.7(4.1)d
	10%	20(4.5)c	23.3(4.8)b	36.7 (5.8)c
	15%	36.7(5.8)b	36.7(6.1)b	56.7(7.6)b
Cyper methrin		100.0(10)a	100.0(10)a	100.0(10)a
Control	0 %	0 (0.7)e	0 (0.7)d	0 (0.7)e
	CV %	15.6	32.9	12.2
	SE	0.4	0.6	0.4
	LSD	1.1	1.8	1.1

Means followed by the same letter are not significant different at (P< 0.05)

Data in parentheses transformed using square root transformation ($\sqrt{X + 0.5}$) before analysis.

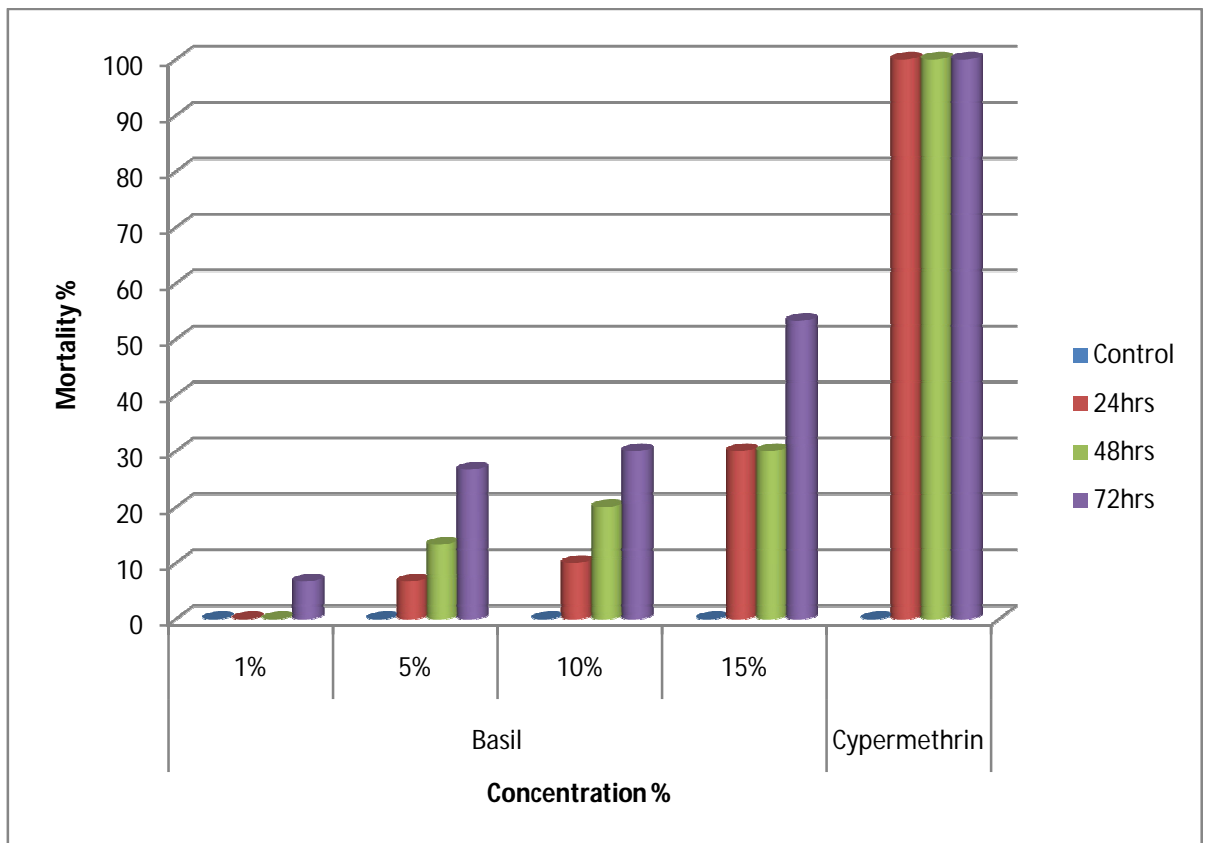


Figure 4: Effect of different concentration of ethanolic extracts of leaves of Basil on mortality rate of *B.invadens* after 24, 48, 72 hrs (Topical application).

4:5 Effect of different concentration of Propolis after 24,48 and 72hours on the mortality of B. invadens (Food Bait application).

According to data shown in Table.5.significant difference was observed between different treatments on reducing the number of adult fruit flies .

Regarding the different concentration of propolis it is clear that ,the mortality of adult flies increased due to the increase of concentration for each assessment period .

It is very obvious that,the mortality rate for specific dose is increased with the pass of time .

The highest dose caused 50,73.3 and 93.3 % of mortality rate after 24,48 and 72 hours respectively.

Table 5. Effect of different concentrations of Propolis on mortality rate of *B.invadens* after 24, 48, 72 hrs (Food Bait application).

Treatment and concentration		Mortality (%)		
		Exposure time (hrs.)		
		24 hrs	48 hrs	72 hrs
Bee glue	1%	0(0.7)d	10(2.8)e	13.3(3.6)c
	5%	13.3 (3.6)c	16.7(3.9)c	20(4.4)c
	10%	43.3(6.6)b	50(7.1)b	66.7(8.2)b
	15%	50(7.1)b	73.3(8.6)ab	93.3 (9.7)a
Cyper methrin		100.0(10)a	100.0(10)a	100.0(10)a
Control	0 %	0 (0.7)d	0 (0.7)d	0 (0.7)d
	CV %	9.4	18.3	9.7
	SE	0.3	0.6	0.3
	LSD	0.8	1.8	1.1

Means followed by the same letter are not significant different at (P< 0.05)

Data in parentheses transformed using square root transformation ($\sqrt{X + 0.5}$) before analysis.

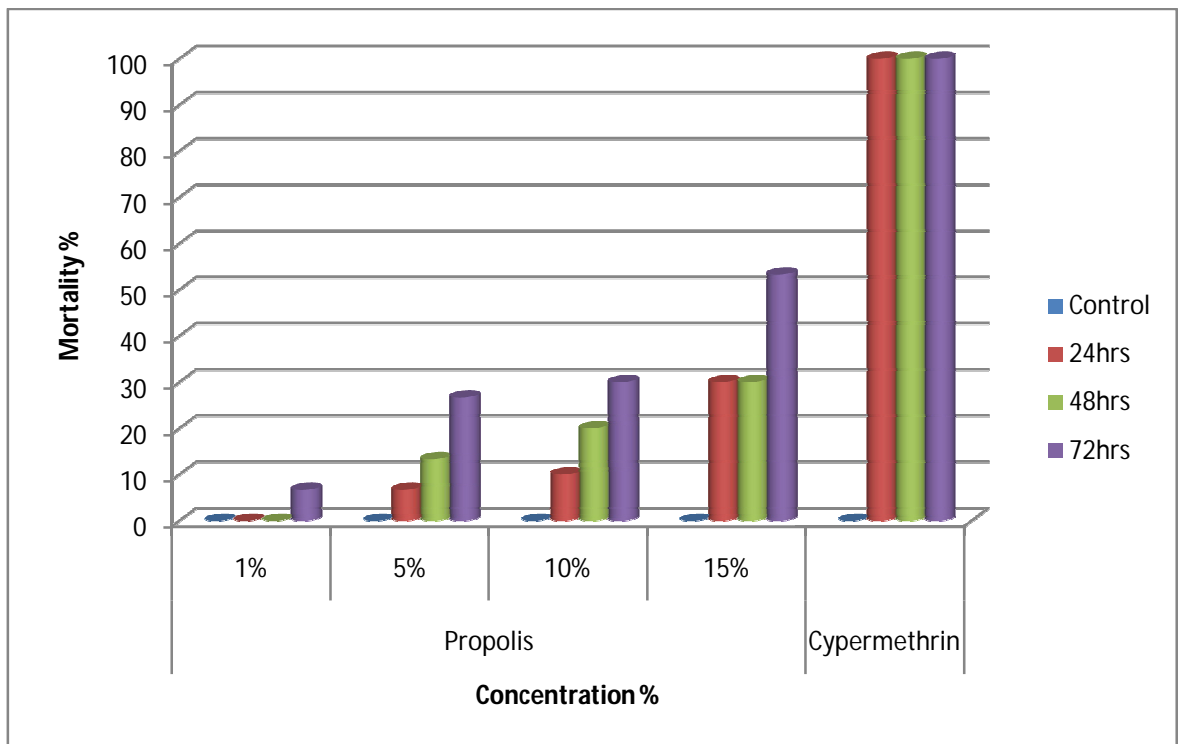


Figure 5: Effect of different concentrations of Propolis on mortality rate of *B. invadens* after 24, 48, 72 hrs (Food Bait application).

4:6 Effect of different concentration of Propolis after 24,48 and 72hours on the mortality rate of B. invadens. (Topical application)

The effect of Topical application four concentrations of Bee glue on the mortality rate of B.invadens is presented .Table.6.

Generally ,significant difference was observed between all treatments.

All treatments obtained highest mortality than the control .

The mortality rate of adult of B.invadens is increased with the increase of dose of propolis and also the mortality increase d within the time elapse.

The highest mortality rate of B.invadens (53.3%) was obtained whit the highest concentration of propolis (15%) after 72 hours.

Table 6: Effect of different concentrations of Propolis on mortality rate of *B.invadens* after 24, 48, 72 hrs (Topical application).

Treatments and concentrations		Mortality (%)		
		Exposure time (hrs.)		
	Exposure time (hrs.)	24 hrs	48 hrs	72 hrs
Bee glue	1%	0 (0.7)d	0 (0.7)d	6.7 (2.4)d
	5%	6.7 (2.4)cd	13.3 (3.6)c	26.7 (5.2) c
	10%	10 (2.8)c	20 (4.4)c	30(5.5) c
	15%	30 (5.2) b	30 (5.5)b	53.3(7.3)b
Cyper methrin		100.0(10)a	100.0(10)a	100.0(10)a
Control	0 %	0 (0.7)d	0 (0.7)d	0 (0.7)e
	CV %	27.9	13.5	14.7
	SE	0.6	0.3	0.4
	LSD	1.8	1	1.4

Means followed by the same letter are not significant different at (P< 0.05)

Data in parentheses transformed using square root transformation ($\sqrt{X + 0.5}$) before analysis.

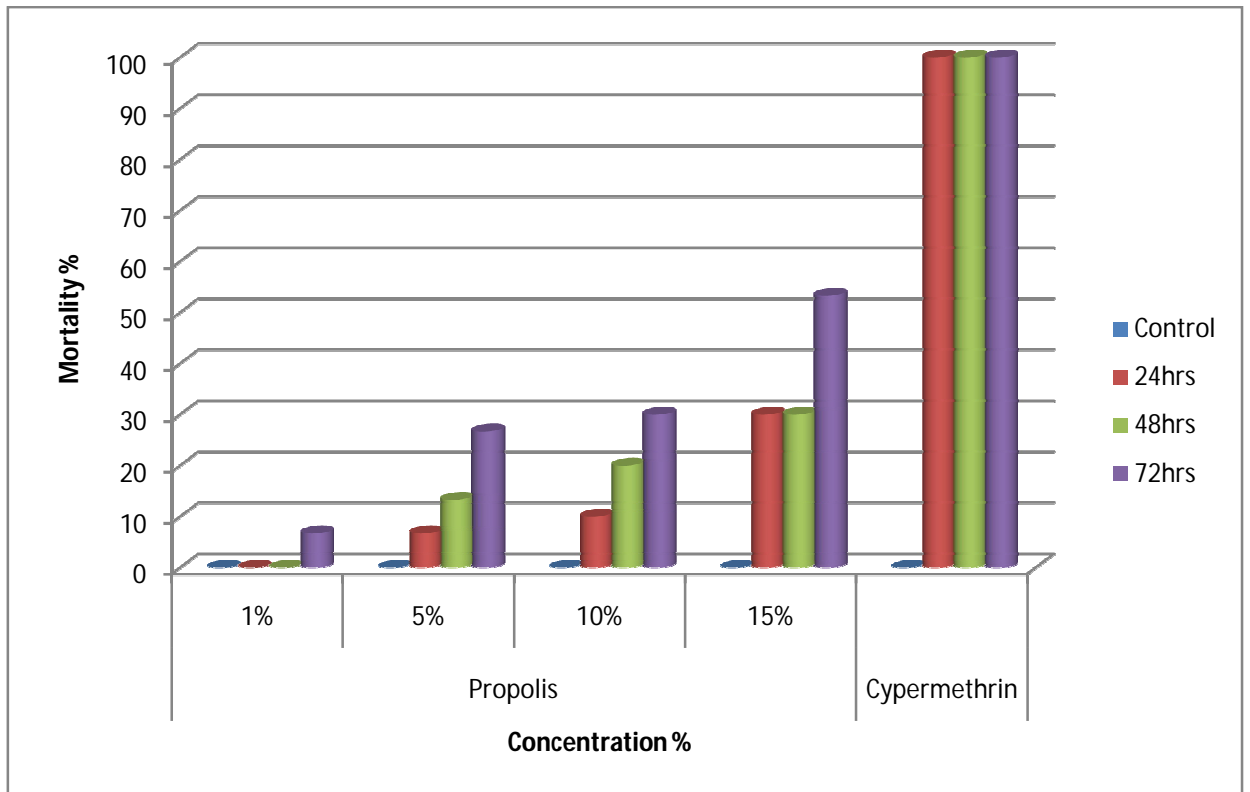


Figure 6: Effect of different concentrations of Propolis on mortality rate of *B. invadens* after 24, 48, 72 hrs (Topical application).

CHAPTER FIVE

5: DISCUSSION

Horticultural production in most African countries is limited by many biotic constraints. Biotic factors include, among others, heavy fruit fly infestations, which constitute enormous threat to fruit and vegetable production throughout the world. In the tropics, the problem is further aggravated by the prevailing warm weather condition, which interferes with the fruiting patterns, resulting in overlapping fruit infestation all the year (Mohamed, 2004).

This study is aimed to evaluate the lethal effect of ethanolic extracts of leaves of Neem *Azadirachta indica A.juss* and Basil *Ocimum basilicum L* and powder of Bee glue(Propolis) against the adult Fruit fly *B. invadens*.

Both application methods cause mortality to adult *B. invadens* .As indicated in the results all tested products are potent to cause mortality to *B. invadens* and the mortality percentage increased with the increase of the dose and elapse of time.

Highest mortality rate (86.7)obtained by ethanolic extract of neem at 15% after 72 hours of food baiting .Also highest mortality (93.3) was obtained by powder of Propolis at the highest concentration (15%) after 72 hours of food baiting.

Higher plants are extremely abundant with biologically active secondary metabolites .Over 80% of all known Alkaloids, Terpenoids, Phenols and other secondary metabolite were produced by higher plants (Siddig, 1993). Many plant extracts or products have proven to be as potent as many conventional synthetic pesticides and are effective at very low concentrations. On the other hand botanical insecticides possess great advantages over synthetic pesticides in being more environmentally friendly and accepted by the majority of the farmers, governmental organizations and decision makers (Kelany, 2001). Stoll (2000) demonstrated that the use of plant extracts to control destructive insects

is not new. Rotenone, Nicotine and Pyrethrin has been used for a considerable time in small scale subsistence and also commercial agriculture.

Dawood (2001) reported that Neem water extracts at 1Kg/liter water reduced the number of onion thrips at 63.5% under the field condition.

Malaka (1972) stated that, preparation of the leaves of *O.basilicum* L. has been used among many methods to protect yams before planting against termites. Shah and patel, 1976 found that *O.sanctum* contained methyl eugenol and had been used to attract the mango fruit fly, *Dacus correctus* Bezz.and observation on the population counts revealed that all the fruit flies attracted to tulsi plant were male only. (Deshpande and Tiphis, 1977) obtained by TLC eight fractions from the essential oil of *Ocimum basilicum* .They tested the activity of each fraction against stored grains insect pests, namely *Tribolium castaneum*, *Sitophilus oryzae*, *Stagobium paniceum* and *Bruchidiuus chinensis* .From bioassay tests methylcinnamate and methylcharicol were found to be the components mainly responsible for the insecticidal activity.

Solvent extracts of propolis samples from Brazil and exhibited insecticidal activity against the larger grain borer, *Prostephanus truncates* (Horn) in maize grains. A reduction of the borer population in maize was observed. Interestingly, pesticides commonly used in agriculture were detected in honey and propolis samples (Lucia *et al*,2011) in Uruguay,recently bee propolis extracts have been reported to have acaricidal effect on red spider mites (*Tetranychus spp.*), which attack tomatoes, (Kareru and Wamaitha, 2012, unpublished work).

5:1 Conclusions:

All tested concentrations of Bee glue powder and ethanolic extracts of leaves of neem and basil gave significantly higher mortality percentage than the control after 24, 48, and 72hrs of exposure. It can also be noted that all the in the concentrations of the extracts resulted in a significantly higher increase of mortality percentage.

The mortality caused by the highest concentration of the and ethanolic extracts of leaves of neem and basil used in this study 15% was significantly lower than the mortality caused by the basil leaves thanol extract after 72hrs of exposure by using both topical and feeding methods. Also the highest concentration of the neem leave ethanolic extract (15%) gave significantly higher mortality percentages than the basil leave ethanol extract after 72hrs of exposure by using food bait.

The mortality caused by the highest concentration of the proplis powder used in this study (15%) was significantly lower than the mortality caused by the basil and neem leave thanol extract after 72hrs of exposure by using both topical and feeding methods. Also the highest concentration of the proplis powder (15%) gave significantly higher mortality percentages than the basil leaves ethanol extract after 72hrs of exposure by using feeding method, but the result seem of result obtained on the treatment of neem.

The results indicated that the neem , basil leaves ethanolic extracts and bee glue powder obtained high rate of mortality on fruit fly *Bactrocera invadens* than the control and stander. This rate of mortality found increasing with increase of time.

5:2 Recommendations:

Ethanollic extract of leaves of Neem and Basil powder of Propolis contain.

Compounds can be used against adult fruit flies in order to reduce depending on synthetic insecticides. Following recommendation are of importance:

- 1- Evaluate doses higher than tested one might give promising results.
- 2- Incorporate these botanicals in trials of food attractants as alternative to insecticides to safe environment, fruits and natural enemies.
- 3- Basil plant contains methyl eugenol so strenuous efforts should be conducted to produce it locally in order to reduce the cost of its importation.
- 4- Benefit from bee production to reduce fruit fly example(bee glue) .
- 5- More studies specially basil plant because contain methyl eugenol that attract to male of fruit fly.
- 6- More studies are highly encouraged for confirmation .

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APPENDICES

Appendix 1

Table 1: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	187.927	37.585	41.102	0.0000
Within	12	10.973	0.914		
Total	17	198.900			

Appendix 2

Table 2: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	161.224	32.245	46.921	0.0000
Within	12	8.247	0.687		
Total	17	169.471			

Appendix 3

Table 3: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	178.478	35.696	65.967	0.0000
Within	12	6.493	0.541		
Total	17	184.97			

Appendix 4

Table 4: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	191.009	38.202	78.587	0.0000
Within	12	5.833	0.486		
Total	17	196.843			

Appendix 5

Table 5: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	166.764	33.353	57.671	0.0000
Within	12	6.940	0.578		
Total	17	173.704			

Appendix 6

Table 6: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	149.216	29.843	60.493	0.0000
Within	12	5.920	0.493		
Total	17	155.136			

Appendix 7

Table 7: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	212.016	42.403	209.112	0.0000
Within	12	2.433	0.203		
Total	17	214.449			

Appendix 8

Table 8: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	195.289	39.058	38.397	0.0000
Within	12	12.207	1.017		
Total	17	207.496			

Appendix 9

Table 9: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	211.427	42.285	121.007	0.0000
Within	12	4.193	0.349		
Total	17	215.620			

Appendix 10**Table 10: Analysis of variance table (One way ANOVA table):**

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	191.009	38.202	78.587	0.0000
Within	12	5.833	0.486		
Total	17	196.843			

Appendix 11**Table 11: Analysis of variance table (One way ANOVA table):**

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	166.764	33.353	57.671	0.0000
Within	12	6.940	0.578		
Total	17	173.704			

Appendix 12**Table 12: Analysis of variance table (One way ANOVA table):**

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	149.216	29.843	60.493	0.0000
Within	12	5.920	0.493		
Total	17	155.136			

Appendix 13**Table 13: Analysis of variance table (One way ANOVA table):**

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	191.811	38.362	97.807	0.0000
Within	12	4.707	0.392		
Total	17	196.518			

Appendix 14**Table 14: Analysis of variance table (One way ANOVA table):**

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	176.623	35.325	33.031	0.0000
Within	12	12.833	1.069		
Total	17	189.456			

Appendix 15**Table 15: Analysis of variance table (One way ANOVA table):**

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	158.711	31.742	76.385	0.0000
Within	12	4.987	0.416		
Total	17	163.698			

Appendix 16**Table 16: Analysis of variance table (One way ANOVA table):**

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	187.178	37.436	36.542	0.0000
Within	12	12.293	1.024		
Total	17	199.471			

Appendix 17**Table 17: Analysis of variance table (One way ANOVA table):**

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	180.538	36.108	114.425	0.0000
Within	12	3.787	0.316		
Total	17	184.324			

Appendix 18**Table 18: Analysis of variance table (One way ANOVA table):**

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	167.809	33.562	57.810	0.0000
Within	12	6.967	0.581		
Total	17	174.776			