

بسم الله الرحمن الرحيم



Sudan University of Science and Technology



College of Graduate Studies

**Effect of Some Plant Extracts on Mortality of Khapra beetle
(*Trogoderma granarium* Everts) (Coleoptera: Dermestidae).
اثر بعض المستخلصات النباتية على موت خنفساء الخابرا
(*Trogoderma granarium* Everts) (Coleoptera: Dermestidae).**

A thesis submitted in partial fulfillment of the requirements for the M. Sc. Degree in Plant Protection

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الآية

قال تعالى:

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(الَّذِي جَعَلَ لَكُمْ مِنَ الشَّجَرِ الْأَخْضَرِ نَارًا فَإِذَا أَنْتُمْ مِنْهُ تُوقَدُونَ)

صدق الله العظيم

(الآية 80 من سورة يس)

Dedication

To my mother, father and brothers

To my extended family

*To all my teachers and friends with
great regards and respect.*

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Table of Contents

Title	Page No.
الآية	I
Dedication	II
Acknowledgements	III
Table of Contents	IV
List of Tables.....	VII
List of Figures	VIII
List of Plates.....	IX
Abstract	X
البحث ملخص	XI
CHAPTER ONE.....	1
INTRODUCTION.....	1
CHAPTER TWO	3
LITERATURE REVIEW.....	3
2.1. Khapra beetle	3
2.1.1. Scientific classification	3
2.1.2. Distribution	3
2.1.3. Description	4
2.1.4. Biology.....	5
2.1.5. Economic importance	7
2.1.6. Damage	8
2.1.7. Signs of infestation	8
2.1.8. Host range	9
2.1.9. Control	9
2.1.9.1. Biological Control.....	9
2.1.9.2. Botanicals.....	10
2.1.9.3. Chemical Control.....	10
2.1.9.4. Physical control.....	11

2.1.9.4.1. Heat	11
2.1.9.4.2. Freezing.....	11
2.1.9.4.3. Controlled atmosphere.....	11
2.2. Wheat	13
2.2.1. Scientific classification	13
2.2.2 Distribution	13
2.2.3 Pest of wheat	13
2.3. Eucalyptus (Kaphor)	15
2.3.1. Scientific Classification	15
2.3.2.Distribution	15
2.3.3. Description.....	16
2.3.4. Medicinal uses.....	16
2.3.3. Allelopathy.....	16
2.4. Damas.....	18
2.4.1. Scientific Classification	18
2.4.2. Description	18
2.4.3. Geographical distribution.....	19
2.4.4. Uses.....	20
2.4.5. Chemical structural	20
CHAPTER THREE	21
MATERIALS AND METHODS	21
3.1. Insect culture	21
3.2. Plant materials.....	21
3.2.1. Collection of plant leaves.....	21
3.2.2. Preparation of plant extracts	21
3.2.2.1. Powder extract.....	21
3.2.2.2. Ethanolic extracts:.....	25
3.2.2.3. Aqueous extracts	25
3.3. Methods of treatment	27
3.3.1.Treatments with ethanolic and aqueous extracts:	27
3.3.2.Treatments with powder extract	27

7. Data analysis	28
CHAPTER FOUR.....	30
RESULTS.....	30
CHAPTER FIVE.....	42
DISCUSSION	42
CONCLUSION AND RECOMMENDATIONS.....	45
References	46
Appendices	58

List of Tables

Title	Page No.
Table 1. The effect of three different concentrations of two plants ethanolic extracts on insect mortality after 24h of treatment	30
Table 2. The effect of two different plants ethanolic extracts on mortality of <i>T. granarium</i> after 48h of treatment.....	32
Table 3. The effect of two different plants aqueous extracts on mortality of <i>T. granarium</i> after 24h of treatment.....	34
Table 4. The effect of two different plants aqueous extracts on mortality of <i>T. granarium</i> after 48h of treatment.....	36
Table 5. The effect of two different plants powder on mortality of <i>T. granarium</i> after one week of treatment.....	38
Table 6. The effect of two different plants powder on mortality of <i>T. granarium</i> after two weeks of treatment.....	40

List of Figures

Title	Page No.
Figure 1. The effect of two different plants ethanolic extracts on mortality of <i>T. granarium</i> after 24h of treatment.....	31
Figure 2. The effect of two different plants ethanolic extracts on mortality of <i>T. granarium</i> after 48h of treatment.....	33
Figure 3. The effect of two different plants aqueous extracts on mortality of <i>T. granarium</i> after 24h of treatment.....	35
Figure 4. The effect of two different plants aqueous extracts on mortality of <i>T. granarium</i> after 48h of treatment.....	37
Figure 5. The effect of two different plants powder on mortality of <i>T. granarium</i> after one week of treatment	39
Figure 6. The effect of two different plants powder on mortality of <i>T. granarium</i> after two weeks of treatment.....	41

List of Plates

Title	Page No.
Plate 1: Adults of <i>Trogoderma granarium</i>	22
Plate 2: The larvae of <i>Trogoderma granarium</i>	22
Plate 3: Leaves of <i>Conocarpus lancifolius</i> plant.	23
Plate 4: Leaves of <i>Eucalyptus sp</i> plant.....	23
Plate 5: Powder of <i>Eucalyptus sp</i> plant.....	24
Plate 6: Powder of <i>Conocarpus lancifolius</i>	24
Plate 7: The Shaker apparatus	25
Plate 8: Ethanolic extract	26
Plate 9: Aqueous extract.....	26
Plate 10: The experimental design of ethanolic and aqueous extract.....	29
Plate 11: The experimental design of powder.....	29

Abstract

The experiment of the present study were conducted under laboratory condition at college of agricultural study, Sudan University of Science and Technology to evaluate the effect of two plants extracts Damas *Conocarpus lancifolius* and Kaphor *Eucalyptus* against the third (3rd) instar of the larvae of khapra beetle *Trogoderma granarium* (Everts) in wheat seeds.

Three formulations (powder, aqueous and ethanolic extracts) of leaves for each plant were used at three concentrations (10%, 20% and 30%) in this study. The results showed that all concentrations of all tested plants in various formulations gave significantly higher mortality percentage than control. The mortality were assessed after 24 hours and 48 hours of treatments for the test of ethanolic and aqueous extracts. However, for the test of powder extract, data for mortality were collected after one and two weeks of treatment. The experiments were conducted at three replications with Completely Randomized Design (CRD).

The highest concentration (30%) of Kaphor leaves for ethanolic and aqueous extracts, after 48 hours of exposure, gave the higher mortality percentages (63%), (47%) respectively compared with the other treatments. Whereas, the concentration (30%) of Damas ethanolic and aqueous extracts after 48 hour of exposure gave mortality percentages of 47%, and 37% respectively. For the leaves powder test, the highest concentration (30%) of Kaphor after two weeks of exposure, revealed higher mortality (40 %) compared with, the percentage (33%) achieved by the same concentration of Damas powder extract for the same period of exposure.

ملخص البحث

أجريت هذه الدراسة تحت ظروف المعمل بكلية الدراسات الزراعية (شمبات) لتقييم اثر نباتي (الدمس والكافور) ضد يرقات خنفساء الخابرا (العمر الثالث) على بذور القمح.

استخدمت ثلاث مستحضرات (بدره , مستخلص مائي , ومستخلص كحولي) بثلاث تركيزات (10% 20% 30%) لكل نبات. أوضحت النتائج المتحصل عليها أن كل التركيزات المختبرة من كل نبات بمختلف مستحضراتها أعطت نسب موت عاليه مقارنة بالشاهد. أخذت قراءات نسبة الموت ليرقات خنفساء الخابرا بعد 24ساعة و48 ساعة من المعاملة (بالنسبة للمستخلص المائي والكحولي) أما بالنسبة للبدره أخذت بعد أسبوع وأسبوعين من المعاملة. أجريت التجربة بثلاثة مكررات بإستخدام التصميم العشوائي الكامل (CRD).

أعطى أعلى تركيز من المستخلص الكحولي والمائي للكافور أعلى نسب موت (63% و 47%) على التوالي بعد 48 ساعة من المعاملة, في حين ان نفس التركيز من المستخلص الكحولي والمائي للدمس أعطى نسب موت (47% و 37%) على التوالي بعد 48 ساعة من المعاملة.

أيضا أعلى تركيز 30% من بدره الكافور أعطى نسبة موت 40% بعد أسبوعين من المعاملة في حين ان نفس التركيز من بدره الدمس أعطى نسبة موت فقط (33%) بعد أسبوعين من المعاملة.

CHAPTER ONE

INTRODUCTION

Wheat is the second most important cereal crop after sorghum in Sudan. Wheat consumption is increasing progressively, as a sorghum substitute, during the last decades due to a shift in the food habits. Sudan imports about 1.1 million tons of wheat grain and flour to cover demands. Wheat is grown as a winter crop in almost all the irrigated schemes in Sudan. The average acreage of wheat in Sudan was 258,000 and 92,000 ha in 1990 and 2000 seasons, respectively (Kabbashi,*et al.*, 2006). The three great mills and the other 8 across the country they collectively produce 1.5 million tons / annum topped by an equal amount imported annually (Mckee, 2010).

The post harvest losses of wheat grains have been estimated to about 30%. In countries like Sudan, insects cause a lot of damage to stored grains and flour where the prevailing climates create conditions favorable to insect multiplication. (Lal, 1990).

The use of chemical insecticides has been a fundamental tool for pest control, but it has had serious consequences such as intoxication of people and animals, contamination of water, air, and soil, residues on food, high persistence in the environment, resistance in pests, and impact on beneficial insects, among other effects (Rodríguez *et al.*, 2003; Regnault-Roger *et al.*, 2004).

This has motivated the search for alternative pest controls without the negative effects of synthetic insecticides. Thus, botanical insecticides have become a more ecological and natural alternative for insect control (Rodríguez *et al.*, 2003). Secondary metabolites in plants function as a means for defense against possible damages by insects and are extracted directly from plants (Carrero, 1996). Many plants synthesize secondary metabolites, such as alkaloids, polyphenols, terpenoids, steroids, essential oils, lignans,

sugars and fatty acids, which have important biological properties against insect pests (Silva *et al.*, 2002; Regnault-Roger *et al.*, 2004). The effect of most plants that are used in pest control, more than being insecticidal are insect static (growth regulators) (Silva *et al.*, 2002), because they inhibit normal development of insects. Some plants inhibit feeding in several ways: repelling feeding, and by suppressing or deterring it; others inhibit growth, egg production and development (Metcalf and Metcalf, 1992; Rodríguez *et al.*, 2003).

There are many plant species which can be used as a source of successful potential insecticides such as: *Azadirachta indica* (A. Juss) (neem), *Sesamum indicum* (sesame), *Calotropis procera* (usher), *Allium sativum* (garlic) and *Ocimum basilicum* (rehan).

Sudan with its diverse geographical region is rich in endogenous and exotic plant which may offer a promising reservoir of nationally occurring toxicants that can be used as effective components in pest control program.

Eucalyptus and damas (buttonwood) tree are traditionally known to resist damage by termites and do not harbor many insect.

The main objective of this study was to investigate the insecticidal activity of leave (powder, aqueous, and ethanolic extracts) of kafoor and damas on mortality of *Trogoderma granarium* (Everts).

The specific objectives of the research were:

- To evaluate the effect of two plants ethanolic extracts on mortality of *T. granarium*.
- To evaluate the effect of two plants aqueous extracts on mortality of *T. granarium*.
- To evaluate the effect of two plants powder extracts on mortality of *T. granarium*.

CHAPTER TWO

LITERATURE REVIEW

2.1. Khapra beetle

2.1.1. Scientific classification

Kingdom : Animalia

Phylum : Arthropoda

Class : Insecta

Order : Coleoptera

Family : Dermestidae

Genus : Trogoderma

Species : granarium

SN : *T. granarum* (Everts)

The khapra has been diagnosed in 1898.

The khapra beetle is one of the most important stored product pests worldwide. Its economic importance lies not only in the serious qualitative and quantitative losses it can cause to a wide variety of stored commodities, but also to its capability to withstand starvation for years and to live on food with very low moisture content, its habit of hiding in cracks and crevices, and its ability to survive the adverse environment as a diapausing larva and its resistance to many commonly used pesticides.

However, the most important feature of this feared pest is that it is a very important quarantine pest, causing export restrictions to countries where it is established (Myers, *et al.*, 2012).

2.1.2. Distribution

Khapra beetle is apparently native to India (Hinton, 1945). It was distributed in India, Ceylon, Malaya, Europe, China, Japan, Korea, Philippines, Australia, and Madagascar, and had become established in most of those countries

(Hinton,1945). It was discovered in stored guinea corn in Nigeria in 1948, and may have been present in stored groundnuts as early as 1944 (Howe, 1952; Pasek, 1998).

Africa: Algeria, Burkina Faso, Egypt, Kenya, Libya, Mali, Mauritania, Morocco, Niger, Nigeria ,Senegal, Sierra Leone (intercepted only), Somalia, South Africa, Sudan, Tanzania, Tunisia, Zambia, Zimbabwe Viljoen (1990).

In North America, khapra beetle was found in Mexico but failed to become established. In the United States, khapra beetle has been detected in Arizona, California, New Mexico, and Texas; it was eradicated from those sites. The beetle was detected at a Connecticut residence in 2006; control activities were conducted, and monitoring was scheduled for 2007 (EPPO, 2006).

2.1.3. Description

Adults: The adults are oblong-oval beetles, approximately 1.6 to 3.0 mm long and 0.9 to 1.7 mm wide. Males are brown to black with indistinct reddish brown markings on their elytra. Females are slightly larger than males and lighter in color. The head is small and deflexed with short 11-segmented antennae. The antennae have a club of three to five segments, which fit into a groove in the side of the pronotum. The adults are covered with hairs (Beal 1960 and Faber 1971).

Larva: total length of the first-instars larva is 1.6-1.8 mm, a little more than half of which consists of a long tail, made up of a number of hairs borne on the last abdominal segment. Body width is 0.25-0.3 mm, and colour uniformly yellowish-white, except for the head and body hairs which are brown. The head bears a short antenna of three segments. A characteristic feature of the larva is the presence of two kinds of body hairs: simple hairs, in which the shaft bears many small, stiff, upwardly directed processes; and barbed hairs, in which the shaft is constricted at regular intervals, and in which the apex consists of a barbed head. This brown or yellowish-brown

head is as long as the combined lengths of four of the preceding segments. Simple hairs are scattered over the dorsal surface of the head and body segments. The tail consists of two groups of long simple hairs, borne on the 9th abdominal segment. Barbed hairs are found in pairs of tufts, borne on certain abdominal tergites. As the larva increases in size, the colour changes progressively from the pale yellowish-white of the first-instar larva to a golden or reddish-brown. The density of the body hairs increases but these hairs and the tail become much shorter in proportion to the length and breadth of the larval body, and in the 4th instar the hairs give the appearance of four dark transverse bands. The mature larva is approximately 6 mm in length and 1.5 mm in breadth (OEPP/EPPO,1981).

Pupa: At the last ecdysis, the larval skin splits, but the pupa remains within this skin for the whole of its life. The pupa is of the excrete type; male smaller than female, average lengths being 3.5 mm and 5 mm, respectively with whitish colour (OEPP/EPPO,1981 and APHIS, 1984).

Eggs: Initially milky-white, later pale-yellowish; typically cylindrical, 0.7 mm long and 0.25 mm broad; one end rounded, the other more pointed and bearing a number of spine-like projections, broader at the base and tapering distally (OEPP/EPPO,1981).The eggs Laid loosely and singly in the host material (APHIS, 1984).

2.1.4. Biology

The adults are short-lived, mated females living 4-7 days, unmated females 20-30 days and males 7-12 days. Mating occurs about five days after emergence, and egg laying begins almost immediately at 40°C. Egg laying may begin at one to three days at cooler temperatures, but no eggs are produced at 20°C. Eggs hatch in three to 14 days after the female lays an average of 50 to 90 eggs that are loosely scattered in the host material.

Complete development from egg to adult can take 26 to 220 days, depending upon temperature. Optimum temperature for development is 35°C. If the temperature falls below 25°C for a period of time or if larvae are very crowded, they may enter diapause. They can survive temperatures below -8°C. In diapause, the larvae can molt but are inactive and may remain in this condition for many years (Anonymous 1981).

The eggs hatch in 3-14 days (OEPP/EPPO,1981). Optimum conditions for development are 33-37°C, 45-75% r.h. (Howe, 1958). Khapra beetle is able to survive short periods at 60°C, and at -15°C for several hours. The upper limits are considered to be in the vicinity of 46°C. At 70% r.h., minimum temperature of development is about 22°C. On hatching the larvae are about 1mm long. There are five molts in the development of the larvae, and the cast skin is shed following each molt (Morschel, 1972).

Complete development takes place within the range 21 to over 40°C. The life cycle from egg to adult takes an average of 220 days at 21°C, 39-45 days at 30°C and 75% RH and 26 days at 35°C, the optimum. Development can take place at a relative humidity as low as 2%, at which the life cycle is prolonged. OEPP/EPPO (1981). The rate of increase of populations at 33-37°C is about 12.5 times per month: this compares with 20 times at 32-35°C (minimum RH 30%) for *Rhyzopertha dominica* and 25 times at 27-31°C (minimum RH 50%) for *Sitophilus oryzae*, the principal competitors of Khapra beetle as pests of whole grain. In the zone where Khapra beetle is indigenous, where mean temperatures are consistently above 25°C, the larvae develop rapidly into the pupal stage, e.g. in 15 days at 35°C (OEPP/EPPO,1981).The pupa whitish colour (APHIS, 1984). Hadway (1956) reported that, pupal stage lasted in 3-6 days for males and 3.8 days for females. In favorable temperatures, eggs, pupae, and adults each took about a week for development. While the larval stage may survive a month to several years under diapause condition (Burgess, 1962).

2.1.5. Economic importance

Khapra beetle, *T. granarium* is a serious pest of cereal grains and oilseeds, and many countries, including the USA, Australia, China, Kenya, Uganda and Tanzania, have specific quarantine regulations against possible importation. Massive populations of the insect may develop and grain stocks can be almost completely destroyed. Infestations of *T. granarium* are well known in large-scale stores but there appear to be no documented cases of infestations in farms. Losses due to *T. granarium*, sometimes in conjunction with other storage pests, have been reported in the literature and are summarized as follows: losses in wheat grain stored in PVC bins after 90 days were 23.06% due to *T. granarium*, *Tribolium castaneum*, *Sitophilus oryzae* and *Rhyzopertha dominica* compared with 1.73% in fumigated bins (Singh, *et al.*, 1994).

In a grain silo survey in Iraq between 1977 and 1978, *T. granarium* was present in more than 50% of samples. Infestation levels ranged up to 685 insects/kg grain. The mean percentage of infested grains ranged from 2.5 to 5.7% according to the origin of the wheat. The percentage wheat loss ranged from 3.1 to 6.6 (Al-Saffour and Kansouh, 1979).

In Punjab, India, populations of *T. granarium* varied from 121 to 415 per 500g of wheat in a state survey in 1971-72. The pest damaged 9-14.5% of the grain resulting in 1.04-3.02% weight loss (Bains, *et al.*, 1976).

In laboratory tests, feeding losses caused by infestations of *T. granarium* on wheat grain were estimated. The percentage infestation 30 days after the release of 10 pairs of adults into tubes containing 20g of grain was 9.4 and 15% at 30 and 36°C (optimum temp.), respectively. The percentage net loss in weight was 1.1 and 2.6, and percentage loss in viability was 12 and 24 at the different temperatures (Prasad *et al.*, 1977).

Analysis of wheat grain samples containing 5 to 100% *T. granarium*-infested grains showed that levels of protein, gluten, crude fat, ash, reducing and non-

reducing sugars, and sedimentation value decreased with increased numbers of damaged grains. Values for alcoholic stability and free fatty acids increased. The proportion of seeds that germinated varied from nil when all kernels were damaged to 95% for no damage. Damage caused a loss; in weight averaging 16.36% (Girish *et al.*, 1975). *T. granarium* has also been shown to decrease the mineral content of maize (Jood *et al.*, 1992).

2.1.6. Damage

In India, khapra beetle infests about 5% of stored grains, but can affect an entire bin or lot under heavy infestation (Pasek, 1998). Khapra beetle is a dirty feeder because it damages more grain kernels than it consumes. The larvae contaminate grain with body parts and setae. Severe infestations can make grain unpalatable or unmarketable. Heavy infestations of larvae can reduce the host to frays (USDA, 1982).

Feeding activity reduces plant material to crude fat, sugar, starch, protein, and nitrogen. Feeding also results in an increase in moisture, crude fiber, and total protein content (Mason, 2002).

The body of the immature stage has barbed hairs that can contaminate grain. Exposure to grain contaminated with hairs can lead to dermal and gastric health hazards. The hairs can cause skin irritation in people handling heavily infested grain (Mason, 2002; Barak, 1995).

If swallowed, consumers can experience ulcerative colitis. This is particularly distressing for young children, who develop vomiting and diarrhea, and refuse food (Anon., 2005).

2.1.7. Signs of infestation

The obvious signs of a khapra beetle infestation are the larvae and cast skins. However, the larvae look very similar to those of other relatively unimportant *Trogoderma* species, as well as some carpet beetles. Larvae and adults are best identified by microscopic examination. Detection methods include

examination of cracks and crevices and inspecting behind paneling on walls and under timbers, tanks, shelves, etc. Larvae are most likely to be seen just before dusk, since they tend to be more active at that time (Anonymous, 1981).

2.1.8. Host range

Khapra beetle prefers grain and cereal products, particularly wheat, barley, oats, rye, maize, rice, flour, malt, and noodles. Given a choice of rye, wheat, and oats, khapra beetle prefers wheat. This pest will feed on almost any dried plant or animal matter, including dog food, dried orange pulp, bread, and dried coconuts (Hinton, 1945; Szito, 2005).

Khapra beetle can feed on products with as little as 2% moisture content, and can develop on animal matter such as dead mice, dried blood, and dried insects (Pasek, 1998).

In addition, there are grocery commodities that can serve as hosts for the insect include bread, dried coconuts, corn meal, crackers, white and whole wheat flour, hominy grits, baby cereals, pearl barley, and wheat germ (Szito, 2005).

2.1.9. Control

2.1.9.1. Biological Control

Infestations by dermestids are usually controlled by treatments with insecticides. However, insecticides may cause hazards to man and the environment. Especially in the storage of small subsistence farmers in the tropics the use of insecticides may be dangerous and their costs prohibitive. Hence, there is a need for the development of alternative methods such as biological control, an efficient component in integrated pest management. Al-Kirshi *et al.*, (1997) considers the potential of the larval parasitoid *Laelius*

pedatus (Say) to control the Khapra beetle in cereals. The parasitoid wasp has desirable characteristics to control Khapra beetle. However, the advantages of biological control for Khapra beetle in the high valued grain industries of Australia would be limited.

2.1.9.2. Botanicals

The most extensive research on control methods against the khapra beetle that has been carried out during the last 10 years deals with the use of plant extracts (essential oils, botanical powders etc.).

In India, the use of deoiled neem (*Azadirachta indica*) seed powder mixed into wheat seemed to be an effective and cheap method to control the pest in stored wheat (Singh and Kataria, 1986).

In Sudan, it was found that treating wheat with powdered neem seeds reduced the damage and the effect increased as the dosage increased. However, Garlic fumigation reduced the rate of increase in population of *T. granarium* larvae over prolonged storage (15-60 days) (Siddig,1980 and Abdalla,2003).

2.1.9.3. Chemical Control

The most effective treatment is fumigation with methyl bromide(CH_3Br). The control of species required high concentration of methylbromide because different developmental stages and physiological states (diapause) exhibit different sensitivities. Replacement of methyl bromide with phosphine, carbon dioxide, carbonyl sulphide, sulfuryl fluoride or other fumigants and their combination are being investigated. Surface treatment is not reliable because of the unique ability of larvae of spending long period hiding in cracks and crevices in facultative diapauses (inactive state). Khapra beetle shows signs of tolerance or resistance to phosphine and malathion (Smith, 1964).

2.1.9.4. Physical control

2.1.9.4.1. Heat

Heat treatment has proved to be very effective. The treatment involves a 30 minutes exposure at 60°C. It was resulted in 100% kill of all stages of the khapra beetle (Anon, 2005). Mortality of larvae being at 42.5°C complete mortality however, required 8 days exposure at that temperature (Anon, 2004). Diapausing larvae are more resistant to high temperature than non-diapausing larvae. It has been reported that some natural mortality of larvae occur in stores due to warming caused by activities of the khapra beetle itself. In storage facilities trapping proved to be a useful surveillance tool using pheromone and larval traps. Treatment with fast electrons, using a linear accelerator could provide an efficient method of controlling khapra beetle in stores (Anon, 2005).

2.1.9.4.2. Freezing

The use of low or freezing temperatures does not seem to be a feasible alternative method for the control of the khapra beetle. Although, populations of *T. granarium* have been shown to decline at 20 C and 70% R.H. (Burges, 2008). It is well documented that *T. granarium* is one of the most cold-hardy stored products pests (Fields, 1992 and Eliopoulos,*et al.*, 2011). Recent experiments have shown that 24–48 h is necessary for complete mortality even under ‘unrealistic’ freezing temperatures (Eliopoulos,*et al.*, 2011).

2.1.9.4.3. Controlled atmosphere

Carbon dioxide-based atmospheres are less effective against Khapra beetle than most other stored product pests, requiring much prolonged exposure for control of diapause larvae. It was documented that, 16 days exposure at 80% CO₂ (20-30°C) was required to eliminate Khapra beetle (Spratt, *et al.*, 1985, Verma and Wadhi, 1978, and Annis, 1987).

Low-oxygen atmospheres however appear to be quite effective against Khapra beetle, including eggs and diapause larvae (Verma and Wadhi 1978), requiring the same exposures as other tolerant stored product insect pests. Annis (1987) suggests 0.1% oxygen at 20-29°C for more than 20 days. High pressure CO₂ may be effective with only brief exposures (a few hours). No data is available on effectiveness of the new technique on Khapra beetle.

2.2. Wheat

2.2.1. Scientific classification

Subkingdom : Tracheobionta

Division : Magnoliophyta

Class : Liliopsida

Order : Cyperales

Family : Poaceae

Genus : *Triticum*

Species : *aestivum*

2.2.2 Distribution

Wheat crop is grown in Sudan in different locations particularly; Northern State, River Nile State, Jabel Mara and Gizera Scheme. In the Nile River State, wheat used to be high profitable crop for the farmers. The most important varieties have been grown in the River Nile State namely; wadielneil, Giza 168, condor, Emam, and El Nileen (Elsir, 2004).

Storage in its overall connotation covers some complex and critical functions. Firstly it provides for the physical safety of food grains for longer periods. Secondly it prevents losses and prevents the quality of the grain including its nutritional value. Thirdly storage is the king pin for a sound food security system. Thus storage management, forms an integrated part of post harvest operations, has at the hub of management of food economy of any country (Krishnamurthy, 1989).

2.2.3 Pest of wheat

The post harvest losses of wheat grains have been estimated to about 30%. In countries like Sudan, insects cause a lot of damage to stored grains and flour where the prevailing climates create conditions favorable to insect

multiplication. (Lal, 1990). A recent estimate, in Pakistan, puts the total preventable post - harvest losses of food grains at about 20 million tons a year, which is nearly 10 – 25% of the total production (Khushk and Memon, 2006). However, Kabbashi and Suliman (2006) reported, in a survey, that insect infestation reduces sorghum prices by 10 – 50%.

The stored flour, unlike grain, deteriorates rapidly with increase in time and temperature. According to Elnazeer, (2000), Elshazali (in personal communication) stated that the damage in flour is due to its essential pest (*Tribolium castaneum* Hrbst) which is itself considered a minor pest of intact grains since it is incapable of invading them. Yet it is responsible for rather the major losses in grains ,due to grain cracking for a variety of reasons; the khabra beetle [*Trogoderma granarium* (Everts)], the flour beetle [*Tribolium castaneum* (Hrbst)], the long – headed flour beetle [*Latheticus oryzae* (Water House)], the saw– toothed grain beetle [*Oryzaephilus surinamensis* (Linnaeus)], the lesser grain borer [*Rhyzopertha dominica* (Fabricus)], the warehouse moth [*Ephestia (Anagasta) kuehnilla (Cautella)* Walker], the grain weevil [*Sitophilus granaries* (Linnaeus)], the Angoumois grain moth [*Sitotroga cerealella* (Olivier)], the flat – headed flour beetle (*Cryptolestes* sp.) the acarus mite (*Acarus siro* Linnaeus), and unidentified mites. All these insects and acarids were reported in some or all of the following states. Khartoum, Gezira, River Nile, Northern, White Nile, Kassala, Gadarif, Red Sea, Blue Nile, North Kordofan and South Darfur.

2.3. Eucalyptus (Kaphor)

2.3.1. Scientific Classification

Kingdom	Plantae
Phylum	Angiosperms
Class	Rosids
Subclass	Archionydeae
Order	Myrtitorae
Family	Myrtaceae
Genus	Eucalyptus
Species	<i>Eucalyptus spp</i> (Engla).
Common name	Camphor

Eucalyptus is a genus of more than 500 species, and it has become the most planted genus of trees in the world (Demel 2000).

2.3.2. Distribution

Most of *Eucalyptus* spp. (kaphoor) is endogenous to Eastern Australia, Tasmania and is cultivated in Southern Europe and California. The commercial supply is largely from North France, Spain, Portugal, Angola and South Africa (Varro, *et al*; 1981).

In Sudan the major species is *Eucalyptus microthica* (Muel) followed by *E. camaldulensis* (Dehn) and *E. treticornis* (Sm) synonymous *E. umbellata* (Gaertn). The species were characterized by quick establishment, wide adaptability and high biomass production. A large collection was built up in Wad Madani arboretum including 55 species, other promising sides were

established in Southern Kordofan *E. critriodora* (Hook) and Jebel Marra *E. camaldulensis* and *E. umbellata* (Ibrahim, *et al*; 1998).

2.3.3. Description

Median to moderately tall tree, 30m high; leaves are narrow and up to 20cm long with short twisted petiole, the lamina is thick, quite glabrous but punctuate from the presence of numerous oil gland situated in the mesophyll. The surface is frequently marked with a number of minute, warty spots. These are groups of cork cells that fill the ruptured oil glands. The odor of fresh leaves is strong and comphraceous while that of the dry leaves is less perceptible until they are crushed. The test of aromatic, pungent and slightly bitter .The tree requires much water and has been used to dry up marsh lands (Wallis, 1960).

2.3.4. Medicinal uses

Volatile oils which are introduced into medical use contain 55-70% lineol, plus lesser amounts of volatile aldehydes (Varro, *et al*; 1981). Essential oils of *Eucalyptus* spp. were used as an antibacterial, antimicrobial and acaricidal agent (Bagherwl, 1999; Harkenthal, *et al*, 1999; and Lisin, *et al*, 1999). The antioxidant activities of the volatile oil and the ethanol extract as well as that of the tree bark were evaluated by the thiocyanate method. The ethanol extract of *Eucalyptus* fruit exhibited considerable activity compared with butylated hydroxyanisole and tertiary butylated hydroquinone. The high inhibitory effect of the fruit ethanol extract on linoleic acid after 12 days might be related to the higher ellagic acid content. (Al-Ghorab, *et al.*, 2002).

2.3.3. Allelopathy

Allelopathy is the release of chemicals from leaves or litter that inhibits the germination or growth of other plant species (FAO 1985), and consequently reduces the output of crops. An allelopathic effect of eucalyptus is among the issues dominating agroforestry literature (Suresh and Rai, (1988).

Allelopathic exudates from eucalyptus tree components showed an inhibiting effect on undergrowth vegetation regeneration and growth (Poore & Fries, 1985). Allelopathic or phytotoxic compounds are known to be mainly phenolic acid (Rice,1984). These phenolic compounds are degraded with decomposition of plant residues, resulting in alleviation of phytotoxicity of the decomposing plant residues. Eucalyptus leaves have been reported to have phenolic acids, tannins and flavonoids (Babu and Chapius-Lardy *et al*, (2002). Bioassay experiment with eucalyptus litter extracts and leaf leachate showed a high level of phytotoxicity (Bernhard-Reversat, 1988). According Souto, et al (2001) a soil Bioassay showed clear inhibitory effect on germination and growth of under-storey plants, particularly soils from *Eucalyptus globules labill.*, and *acasia melanoxyton*. Stands compared to *Pinus radiata* in Spain. However, much of the work mentioned in the literature lack experimental precision, in particular, they lack proper controls and insufficient replications (Jagger and Pender, 2006). Most of the studies put forward as "evidence" for eucalypts being strongly allelopathic involve laboratory studies of artificial extracts on germination of seeds or early growth of potted plants which may not accurately represent the field conditions. However, inter-cropping eucalyptus with other species, especially leguminous plants has many advantages in establishing eucalypt plantations. For example, improvement of soil fertility, cure and control of insect and pests damages, water and soil conservation was observed due to intercropping of eucalyptus with other species in China (Zhao Tingiang 1988).

2.4. Damas

2.4.1. Scientific Classification

Kingdom Plantae

Phylum Tracheophyta

Class Magnoliopsida

Order Myrtilales

Family Combretaceae

Genus *Conocarpus*

S. N : *Conocarpus lancifolius* Engl.

Common name: Buttonwood

2.4.2. Description

The species is usually a shrub 1.5 to 4 m in height but can become a tree up to 20 m or more in height (Pennington and Sarukhan, 1968) . The root system consists mainly of laterals and fine roots that are dark brown, weak and brittle, and have a corky bark. Plant usually has an erect trunk or multiple trunks, but it may assume prostrate form and have limbs that layer and become new individuals. The bark is gray or brown, furrowed, fibrous, and moderately thin (about 8 mm)(Little and Wadsworth ,1964). The inner bark is dark cream in color. Stem wood (specific gravity of 1.0) is hard, heavy, and strong branches are brittle (Stevens, *et al.*, 2001). The twigs are slender, yellow-green, angled, flattened, or winged Howard, (1989).The spirally arranged, elliptic to lanceolate leaves are cretaceous to somewhat fleshy, 2 to 10cm long, with petioles 3 to 9 mm long. Inflorescences are terminal or maxillary panicles of tiny greenish-white flowers grouped in 3 to 5 mm in

diameter Nelson (1996). The thin, dry, 5- to 15-mm, two-winged seeds are densely packed into globose clusters Liogier (1994).

2.4.3. Geographical distribution

The geographic range of Buttonwood includes the shores of central and southern Florida, including the Florida Keys; Bermuda; most of the West Indies; both coasts of continental tropical America from Mexico south through Central America and northern South America to Ecuador and the Galapagos Islands and Brazil; and tropical West Africa. (Nettel *et al.* 2008; Howard, 1989; Little and Wadsworth, 1964).

Semple (1970) gives the distribution as "along the coasts of west tropical Africa, the Atlantic and Pacific coasts of tropical and subtropical North and South America and throughout the West Indies". Semple notes that pubescent-leaved individuals (as well as the typical glabrous or smooth-leaved ones) are restricted to the northern West Indies, southern Florida, and northern Central America.

Buttonwood has been introduced to Hawaii at least twice and the silver-leaved variety *sericeus* is still commonly planted as an ornamental. The green-leaved variety was introduced to Ohio before 1910, possibly from Florida, and the variety *sericeus* was introduced to Oahu from the Bahamas in 1946. Both forms of Buttonwood have escaped cultivation and established small wild populations on some islands. In contrast to Red Mangrove, which was introduced to Hawaii and is now very common and widespread there, Buttonwood has not shown much tendency to spread beyond the initial introduction sites. In Hawaii it is cultivated and sparingly naturalized in coastal areas of Kauai, Oahu, Lanai, and Molokai. (Allen, 1998).

2.4.4. Uses

The plant is used as folk remedy in anemia, catarrh, conjunctivitis, gonorrhoea, diabetes, diarrhoea, fever, headache, bleeding, tumors, orchids, prickly heat, swellings, and syphilis. The leaves are eaten, or their decoction drunk, for fever (Irvine, 1961). Wood is used for fencepost, crossties, turnery, boat building, firewood and landscaping purposes.

2.4.5. Chemical structural

In the HPLC analysis of ethyl acetate and n-butanol extracts of leaves, stem, flowers and fruits of *C. erectus* revealed the presence of gallic acid, catechin, apigenin, quercetin, quercetin-3-O-glucoside, kaemferol-3-O-glucoside, rutin and quercetin-3-O-glucoside-6-O-gallicacid (Hameed, *et al.*, 2012).

The leaves, stem, flowers and fruits of *C. erectus* has been studied for the total phenolic, flavonoids, and tannin contents. The total phenolic and tannin contents were estimated by Folin-Ciocalteu's reagent (FCR) and expressed as the number of gallic acid equivalents (GAE) and flavonoid contents were determined by aluminium chloride method and is expressed in rutin equivalents (RE) (Hameed, *et al.*, 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Insect culture

The larvae of khapra beetle *Trogoderma granarium* (Everts) for this study were collected from infested materials. They kept in the Laboratory of Entomology - Department of Plant Protection at the College of Agricultural Studies, Shambat.

The collected larvae were kept in glass jars (30cm ×17cm) and were supplied with wheat seeds *Triticum sp* as a rearing food. The glass jars were covered with muslin cloth fixed with rubber band (Plate 1 and 2). The rearing of insects was continued for several weeks to obtain the tested stages of larvae for the experiment.

3.2. Plant materials

3.2.1. Collection of plant leaves

Kaphoor and damas leaves were collected from kaphoor and damas trees from Shambat area during May 2016 (plates 3 and 4). From each plant, 1Kg of leaves were collected, thereafter they washed and dried under shade for two weeks and then kept separately at laboratory.

3.2.2. Preparation of plant extracts

3.2.2.1. Powder extract

From the dried leaves of each plant, about (1kg) were grounded by hand and then sieved by mesh. Then the sample was kept in glass jar and closed tightly in laboratory for the experiment for powder extract and the preparation of other two extracts. Plates (5 and 6).

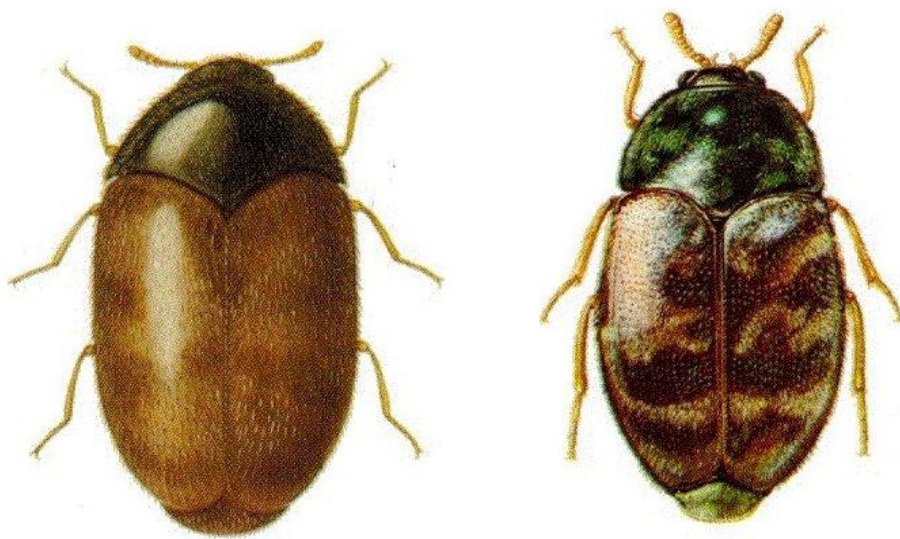


Plate 1: Adults of *Trogoderma granarium*



Plate 2: The larvae of *Trogoderma granarium*

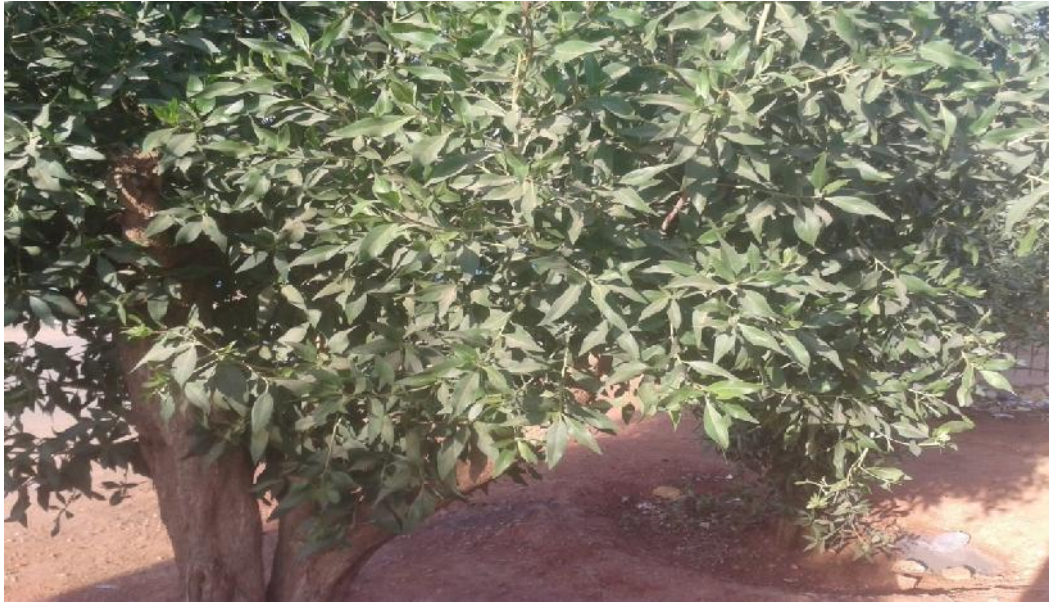


Plate 3: Leaves of *Conocarpus lancifolius* plant.



Plate 4: Leaves of *Eucalyptus sp* plant



Plate 5: Powder of *Eucalyptus sp* plant



Plate 6: Powder of *Conocarpus lancifolius*

3.2.2.2. Ethanolic extracts:

The ethanolic extracts was prepared followed the method of cooled extraction .From the grounded powder of leaves for each plant 50gms were weighted into conical flask (500ml) and 200ml of ethanole were added. The crude mixture then was put in Shaker (for extraction) for 16 hours - 169 rpm (plate 7). After that, the mixture was filtered under reducing pressure using Vacuum Pump to obtain the ethanolic extracts (plate 8).

3.2.2.3. Aqueous extracts

The same method at (3.2.2.2) was followed to prepare the aqueous extracts, however, 200ml of water was added instead of the ethanolic solution (plate 9).



Plate 7: The Shaker apparatus



Plate 8: Ethanolic extract



Plate 9: Aqueous extract

3.3. Methods of treatment

3.3.1. Treatments with ethanolic and aqueous extracts:

To test the mortality rate of *T. granarium* (third instar larvae), the crude plant extracts, was used, in which 1 mL solution of each plant extract was dropped on filter paper placed inside a petri dish (9 cm diameter) with the help of a pipette. The filter paper was then air dried for a few minutes. Then third instar larvae were released into each petri dish, and the same number was also confined to filter papers treated with water and ethanol as an untreated check. Three replications were made for each treatment and control (Sahaf, *et al.*, 2008). Plate (10) showed the experimental design . The insect mortality was recorded after 24 hrs and 48 hrs.

3.3.2. Treatments with powder extract

Twenty grams of wheat grain seeds were weighted and placed in plastic pots. The leaves powders of each plant were tested at 2, 4, 6 grams to obtain the (10%, 20%, 30%) (W/W) concentrations and were added to the plastic pots containing wheat grain. All the treated seeds were put inside Petri-dishes (9cm).

Then thereafter, 10 larvae of khapra beetle *T. granarium* were added to the treated wheat seeds for each Petri-dish.

Same number of larvae was added to some Petri-dishes contains wheat seeds without powder extract which considered as control.

Each treatment was replicated three times. Plate (11) showed the experimental design. The mortality percentages was recorded daily for one week and two weeks.

7. Data analysis

The experiment was conducted in Completely Randomize Design (CRD). Data were analyzed by Analysis of Variance (ANOVA) using Statistical Analysis Software (SAS). Comparison between means was calculated using Multiple Duncan Range Test (MDRT).



Plate 10: The experimental design of ethanolic and aqueous extract



Plate 11: The experimental design of powder

CHAPTER FOUR

RESULTS

The study was focused on the effect of some plant extracts on mortality of khapra beetle *T. granarium* (Everts). The results were shown in the following tables and figures.

From table (1) and figure (1) it was clear that, the concentration 30% of eucalyptus (Kaphoor) ethanolic extract after 24 hours of treatment, achieved the highest percentage of insect mortality which was significantly not different from the concentration 20% of the same plant, however, it was significantly different from the control and the concentration 10% kaphor and concentration 10% 20% 30% of damas ethanolic extract. The concentration 10% of kaphor and 20% of damas attained the same result. For more details see Appendix (1 and 2).

Table 1. The effect of three different concentrations of two plants ethanolic extracts on insect mortality after 24hr of treatment

Concentration	Mortality %
Damas 10%	16.7 ^c
Damas 20%	30.0 ^b
Damas 30%	33.3 ^b
Kaphoor 10%	33.3 ^b
Kaphoor 20%	40.0 ^{ab}
Kaphoor 30%	46.7 ^a
Control	0.0 ^d
C.V	21.8
SE±	6.2

Means followed by the same letters within the same column are not significantly different (P= 0.05)

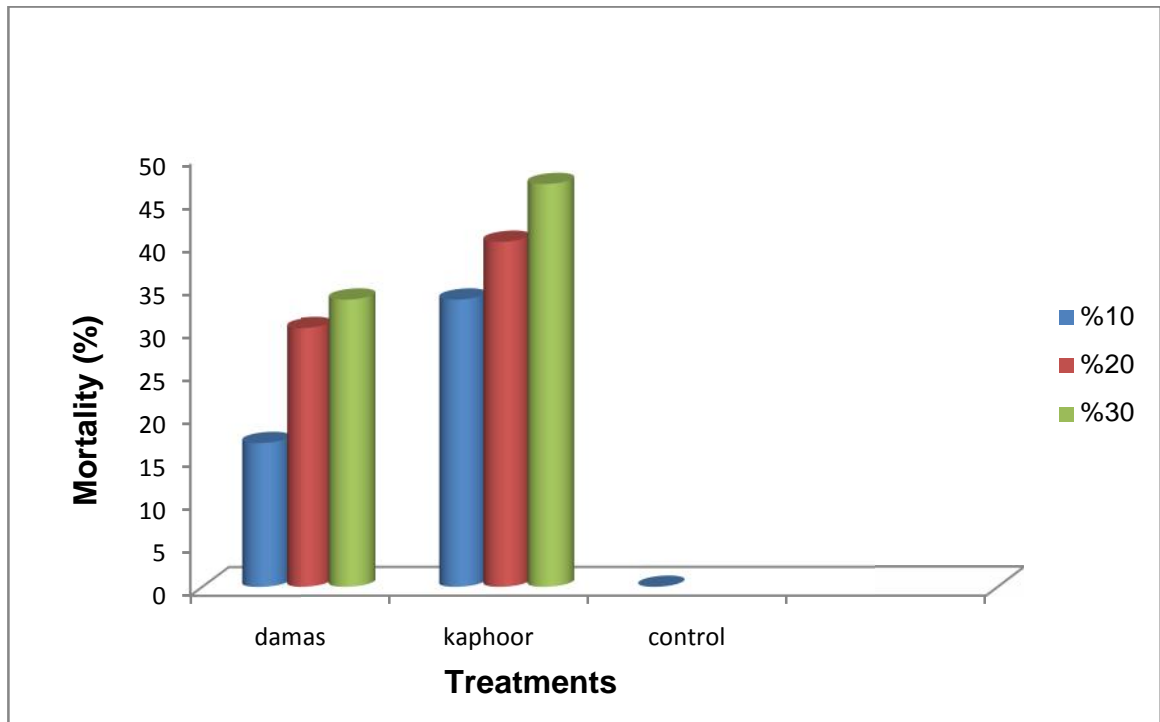


Figure 1. The effect of two different plants ethanolic extracts on mortality of *T. granarium* after 24hr of treatment

The effect of ethanolic extracts of kaphoor and damas on mortality of the insect larvae was represented in table (2) and figure (2). It was clear, that the concentration 30% of eucalyptus (Kaphoor) ethanolic extract after 48hr of treatment, achieved the higher percentage of insect mortality which was significantly different with all other concentrations . Concentration 20% eucalyptus (Kaphoor) and concentration 30% damas are not significantly different, concentration 10% eucalyptus are not significantly different with concentration 20% and 30% of damas. Concentration 10% of damas obtained lower mortality of the insect.

Table 2. The effect of two different plants ethanolic extracts on mortality of *T. granarium* after 48hr of treatment

Concentration	Mortality %
Damas 10%	26.7 ^d
Damas 20%	40.0 ^c
Damas 30%	46.7 ^{bc}
Kaphoor 10%	43.3 ^c
Kaphoor 20%	53.3 ^b
Kaphoor30%	63.3 ^a
Control	0.0 ^e
C.V	12.1
SE±	4.7

Means followed by the same letters within the same column are not significantly different (P= 0.05)

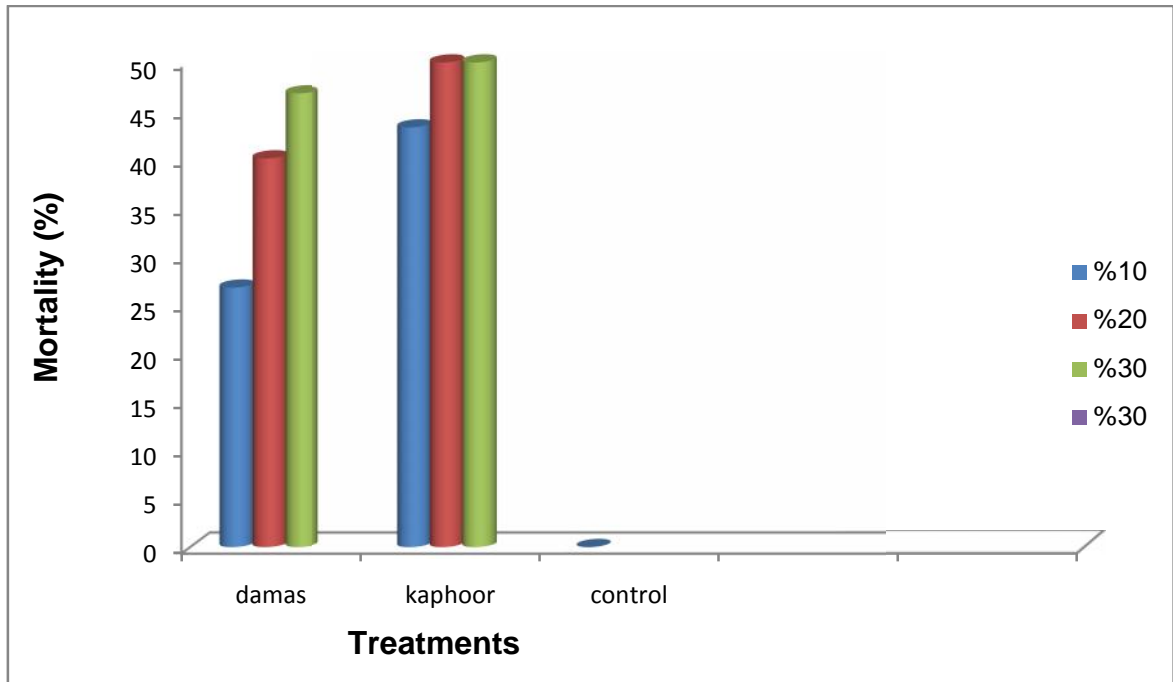


Figure 2. The effect of two different plants ethanolic extracts on mortality of *T. granarium* after 48hr of treatment

From table (3) and figure (3) it was obvious, that the concentration 30% of eucalyptus (Kaphor) aqueous extract after 24hr of treatment, achieved the higher percentage of insect mortality which was significantly not different from the concentration 20% of same plant, however, it was significantly different from the control and concentration 10% eucalyptus (Kaphoor) and concentration 10%, 20% and 30% of damas aqueous extract.

Table 3. The effect of two different plants aqueous extracts on mortality of *T.granarium* after 24hr of treatment

Concentration	Mortality %
Damas 10%	13.3 ^c
Damas 20%	20 ^{bc}
Damas 30%	23.3 ^b
Kaphoor 10%	20 ^{bc}
Kaphoor 20%	26.6 ^{ab}
Kaphoor30%	33.3 ^a
Control	0.0 ^d
C.V	23.7
SE±	4.6

Means followed by the same letters within the same column are not significantly different (P= 0.05)

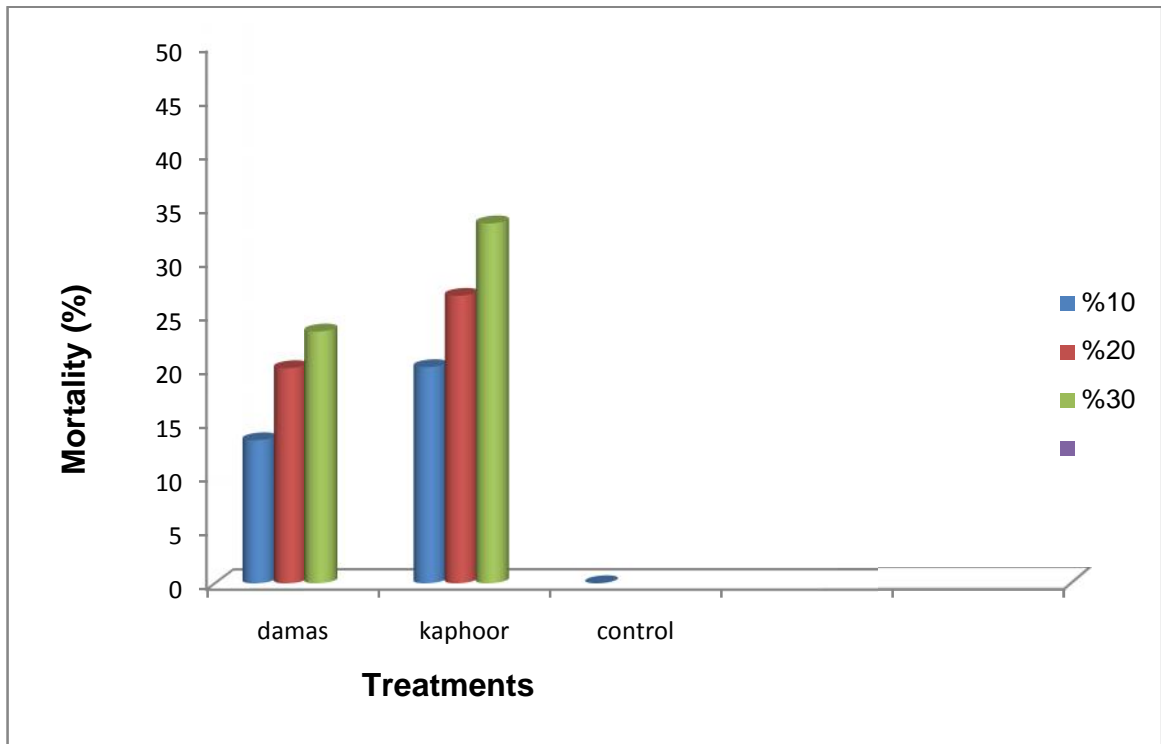


Figure 3. The effect of two different plants aqueous extracts on mortality of *T. granarium* after 24hr of treatment

The influence of the aqueous extracts of the two plants on khapra beetle larvae was showed in table (4) and figure (4). From the result, the concentration 30% of eucalyptus (Kaphoor) aqueous extract achieved the higher percentage of insect mortality which was significantly not different from the concentration 20% of same plant and concentration 30% of damas. However it was significantly different from the control and concentration 10% eucalyptus and concentration 10% and 20% of damas. Concentration 20% eucalyptus and 30% damas has attained the same result. Anova tables were represented at appendix 3 and 4.

Table 4. The effect of two different plants aqueous extracts on mortality of *T. granarium* after 48hr of treatment

Concentration	Mortality %
Damas 10%	20 ^c
Damas 20%	30 ^{bc}
Damas 30%	36.7 ^{ab}
Kaphor 10%	30 ^{bc}
Kaphor 20%	36.7 ^{ab}
Kaphor 30%	46.7 ^a
Control	0.0 ^d
C.V	22.3
SE±	6.3

Means followed by the same letters within the same column are not significantly different (P= 0.05)

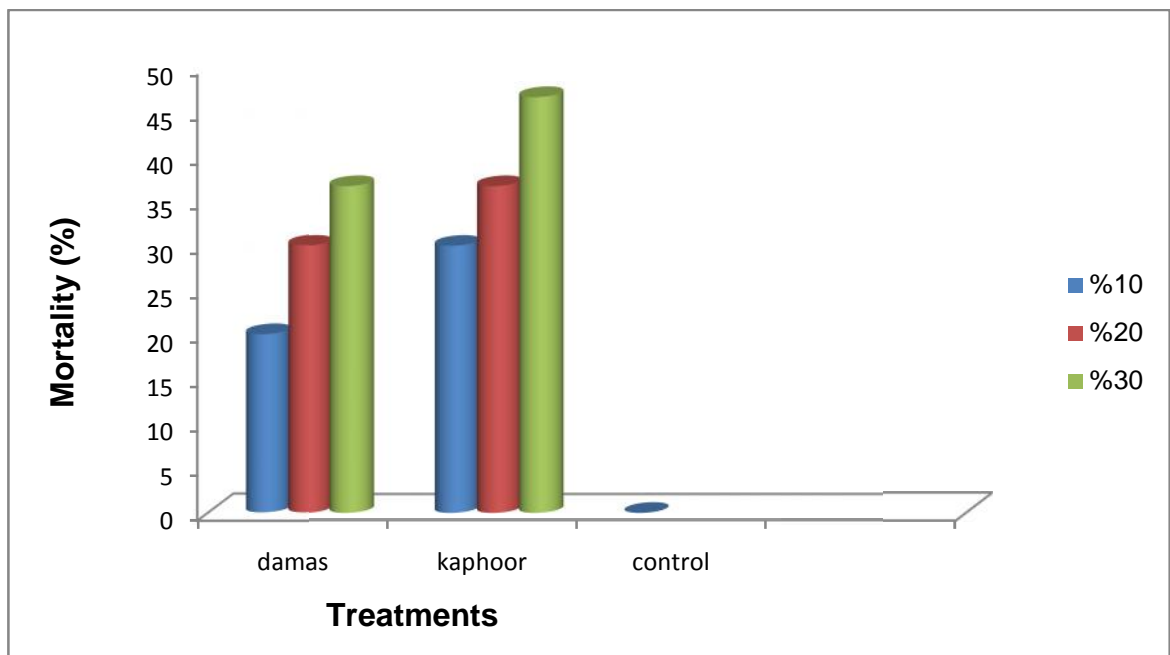


Figure 4. The effect of two different plants aqueous extracts on mortality of *T. granarium* after 48hr of treatment

Table (5) and figure (5) showed the effect of powder extract of the two tested plants on mortality of insect larvae after one week of treatment. The results conducted that, the concentration 30% of eucalyptus (Kaphoor) powder extract achieved the higher percentage of insect mortality which was significantly not different from the concentration 20% of same plant and concentration 30% of damas. However, it was significantly different from the control and concentration 10% eucalyptus and concentration 10% 20% damas. Concentration 20% eucalyptus and 30% damas has attained the same result.

More details in appendix (5).

Table 5. The effect of two different plants powder on mortality of *T. granarium* after one week of treatment

Concentration	Mortality %
Damas 10%	16.7 ^b
Damas 20%	20.0 ^b
Damas 30%	26.7 ^{ab}
Kaphor 10%	20.0 ^b
Kaphor 20%	26.7 ^{ab}
Kaphor30%	33.3 ^a
Control	0.0 ^c
C.V	26.1
SE±	6.3

Means followed by the same letters within the same column are not significantly different (P= 0.05)

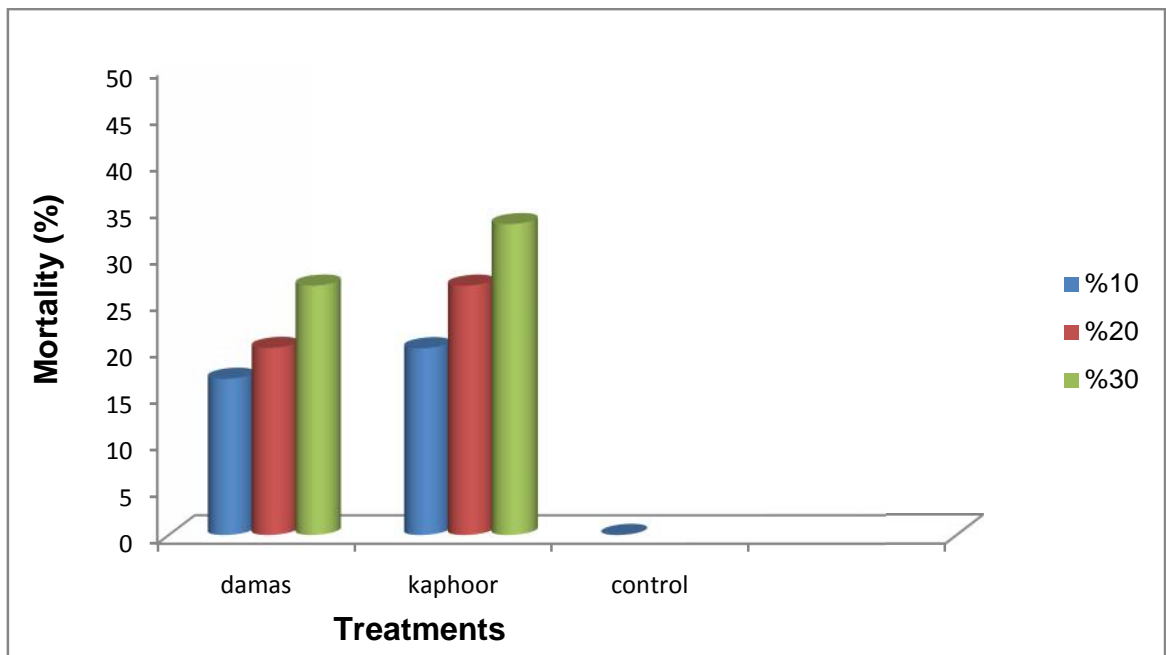


Figure 5. The effect of two different plants powder on mortality of *T. granarium* after one week of treatment

The higher percentage of insect mortality was achieved by the concentration 30% of eucalyptus (kaphoor) powder extract after two weeks of treatment, which was significantly not different from the concentration 20% of the same plant and the concentration 30% of damas (Table 6 and figure 6). However, this result was significantly different from the control and the concentration 10% of eucalyptus and the concentration 10% and 20% of damas. Concentration 20% of eucalyptus and 30% of damas, has attained the same result. For more statistical information see appendix (6).

Table 6. The effect of two different plants powder on mortality of *T.granarium* after two weeks of treatment

Concentration	Mortality %
Damas 10%	23.3 ^c
Damas 20%	30.0 ^{bc}
Damas 30%	33.3 ^b
Kaphor 10%	30 ^{bc}
Kaphor 20%	33.3 ^{ab}
Kaphor 30%	40. ^a
Control	0.0 ^d
C.V	15.3
SE±	4.1

Means followed by the same letters within the same column are not significantly different (P= 0.05)

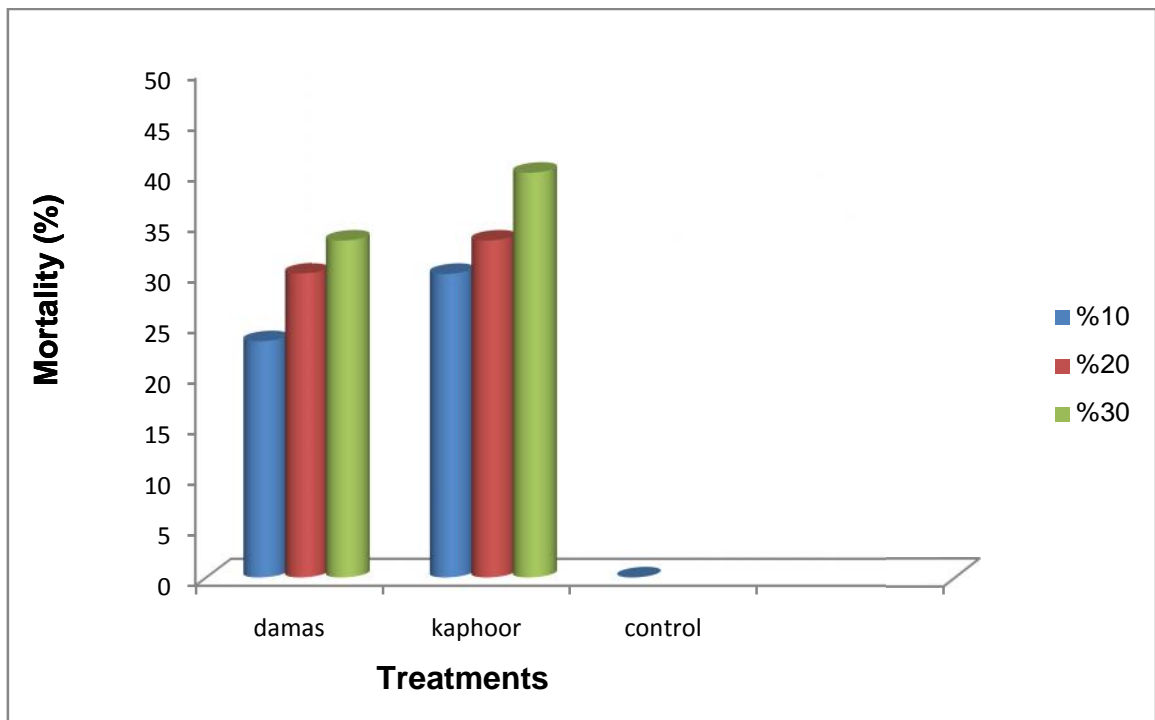


Figure 6. The effect of two different plants powder on mortality of *T.granarium* after two weeks of treatment

CHAPTER FIVE

DISCUSSION

The phytochemical composition of many plants has changed over time, with domestication of agricultural crops resulting in the enhanced content of some bioactive compounds and diminished content of others plants continue to serve as a valuable source of therapeutic compound, because of their vast biosynthetic capacity. A primary advantage of botanicals is their complex composition consisting of collections of related compounds, having multiple activities that interact for a greater total activity (Schimidt, *et al* 2008).

The study was conducted to determine the effect of powder, ethanolic and aqueous extracts of two plants (Damas and Kaphoor) on the mortality of khapra beetle adult (*T.granarium*).

The present result on the effect of ethanolic extract of two plants on Khapra beetle after 24 hours , stated that, kaphoor ethanolic extract at concentration 30% was achieved the best result against *T. granarium*, same as the effect of the concentration 20% of the same plant which was significantly not different.

The result showed a significant difference between the effect of kaphoor plant at concentration (30%) and the effect of damas plant at all concentrations.

No significant difference was observed between the effect of the concentration 20% kaphor and the effected of damas at all concentrations.

Mortality was significantly ($p = 0.0001$) affect by ethanolic extract of kaphor and damas after 48 hours (table 2 and figure 2) . The highest mortality was achieved by the concentration 30% of kaphor which was significantly different from all other treatments. This was proved that kaphoor after 48 hour was the best.

The finding about ethanolic extract confirm that kaphoor and damas plants had biopesticide effect on the insect larvae of *T. granarium*, the result was in agreement with Khalifa, (2014), who found that ethanolic extract of damas had a toxic effect on mortality of red flour beetle *Tribolium casteneum* at concentration 15%.

The results at in agreement with Ali, (2016), who stated that ethanolic extract of kaphor and damas at concentration 30% was effective in controlling adult of sorghum aphid *Rhopalosiphum maidis*.

The result of evaluation of the influence of aqueous extract of damas and kaphoor on khapra beetle after 24 hours of treatment was clearly demonstrated that, aqueous extract of kaphoor at concentration 30% was attained the highest mortality of the insect which was significantly not different from the effect of concentration 20% of the same plant, however, it was significantly different from the concentration 10% of the same plant and also from all other treatments of damas aqueous extract. The positive effect of kaphoor extract may be due to the some bioactive compounds of the plant.

The results of the effect of the aqueous extract of damas and kaphoor after 48 hours of treatment, showed a good impact on the insect larvae. Concentration 30% of kaphoor was achieved the highest mortality of the insect adult which was significantly not different from the effect of concentration 20% of the same plant and the concentration 30% of damas. This result was significantly different from the concentration 10% of kaphoor and the concentration 10% and 20% of damas aqueous extract. The result indicated that, the kaphoor aqueous extract at concentration 30% was the best in controlling *T. granarium* adult. Similar finding was obtained by Farsani,*et al.*, (2011) who stated that, aqueous extracts of eucalyptus caused mortality relative on second instars larvae of *Lycoriella auripila*, the obtained results show that eucalyptus could be considered as potential organic insecticide in control of sciarid flies in mushroom cultivation farm. Elbanna, (2006) found

that the leaf extract contain toxic compounds to mosquitos' larvae, which can be developed and used in the control of mosquitoes. Further studies of these plants as possible agents for mosquito control are recommended.

The effect of powder extracts of damas and kaphoor on the insect mortality, was slow compared with ethanolic and aqueous extracts of the same plants. After 24 and 48 hours of treatments ,the result was not clear regarding the effect on the mortality , so that the data of the mortality was reported after one week and two weeks of treatment.

The result on the effect of powder extracts of damas and kaphoor against khapra beetle after one week of treatment, achieved considerable impact on the insect larvae mortality . The concentration 30% of kaphoor achieved the highest mortality of the insect which was significant not different from the concentration 20% of the same plant and concentration 30% of damas, and it was significant different from the concentration 10% of same plant and 10% and concentration 20% of damas.

The result on the effect of powder extract of two plants on Khapra beetle after two weeks was evaluated. From the result it was obvious that, the concentration 30% of kaphor achieve the highest mortality of the insect which was significant not different from the concentration 20% of the same plant, and it was significant different from all other treatment. Musundire, *et al.*, (2014) who used of eucalyptus powder extract against *Sitophilus zeamais*, found that, the powder reduced grain damage and insect infestation which can be used as sustainable alternative to synthetic insecticide in maize storage especially by smallholder farmers.

Feeny, (1970) reported that tannin content in oak leaves inhibits the growth of winter moth (*Operophtera brumata*) caterpillars and causes death. The results obtained in this study may also be attributed to the tannin content in Eucalyptus and *C. lancifolius* leaves.

CONCLUSION AND RECOMMENDATIONS

This study clearly demonstrates that the tested plants in various formulations have a bio-insecticidal effect on the larvae of *T. granarium*. However, all formulations of Kaphoor were found to be more toxic than the different formulations of Damas.

Based on the above mentioned results, powder, aqueous and ethanolic extract of Kaphor and Damas can be recommended to be used as a control agent for *T. granarium*. The study recommended that, these formulations should be used as stores treatments before the grain storage. However, further experiments should be conducted to evaluate the effects of high concentrations of these plants with other organic solvents and also on other insect pests. Finally, a comprehensive study should be conducted to specify the active ingredients in each plant extract.

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Appendices

Appendix (1): Analysis of variance output for the mortality of khapra beetle larvae affected by two plants ethanolic extract after 24h of treatment.

Source of variation	D.F	S.S	M.S	F
Treatment	6	4390.48	731.75	18.82**
Error	12	466.67	38.89	
Total	20	5057.14		

** Significant at $\rho \leq 0.01$.

Appendix (2): Analysis of variance output for the mortality of khapra beetle larvae affected by two plants ethanolic extract after 48h of treatment.

Source of variation	D.F	S.S	M.S	F
Treatment	6	7647.62	1274.60	57.36**
Error	12	266.67	22.22	
Total	20	7980.95		

** Significant at $\rho \leq 0.01$

Appendix (3): Analysis of variance output for the mortality of khapra beetle affected by two plants aqueous extracts after 24h of treatment

Source of variation	D.F	S.S	M.S	F
Treatment	6	2028.57	338.10	15.78**
Error	12	257.14	21.43	
Total	20	2295.23		

** Significant at $\rho \leq 0.01$.

Appendix (4): Analysis of variance output for the mortality of khapra beetle larvae affected by two plants aqueous extract after 48h of treatment.

Source of variation	D.F	S.S	M.S	F
Treatment	6	2057.14	676.19	16.71**
Error	12	485.71	40.48	
Total	20	4657.14		

** Significant at $\rho \leq 0.01$.

Appendix (5): Analysis of variance output for the mortality of khapra beetle larvae affected by powder of two plants after one week of treatment.

Source of variation	D.F	S.S	M.S	F
Treatment	6	2028.57	338.10	11.83**
Error	12	342.86	28.57	
Total	20	2495.24		

** Significant at $\rho \leq 0.01$.

Appendix (6): Analysis of variance output for the mortality of khapra beetle larvae affected by powder of two plants after two weeks of treatment.

Source of variation	D.F	S.S	M.S	F
Treatment	6	3028.57	504.76	30.29**
Error	12	200.00	16.67	
Total	20	3228.57		

** Significant at $\rho \leq 0.01$.