Sudan University of Science and Technology

College of Agricultural Studies

Department of Plant Protection

Effectiveness of Propolis powder and Neem leaves powder against the larvae of khapra beetle (*Trogodermagranarium*Everts): (Coleoptera :Dermestidae) on Peanuts(*Arachishypogaea*).

تأثيربدرةالبروبوليسومسحوقاوراقالنيمضديرقةخنفساءالخابرا

A thesis submitted in partial fulfillment of the Requirement for the B. Sc. DegreeHonaors in Plant Protection.

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Dedication

To that pure spirit that wandered around me, and from there my strength was drawn to the spirit of my precious mother .

To the man from whom I have learned the meaning of strength and steadfastness, a man like the Propolis in all his attributes to my beloved Father.

To my sweet sister Salma.

To my dear brothers and sisters.

To my second mother Manal.

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Firstly, thanks to God for giving me health and kept me well to finish this work.

Grateful thanks to the honorable supervisor Abdul Baki Al Sayed for his support and urged us to become active researchers such as bees.

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Abstract

The study was conducted under the laboratory conditions of the laboratory of insects and agricultural animals, Department of Plant Protection Faculty of Agricultural Studies University of Sudan for Science and Technology Shambat in the period from September 2017 to November 2018 to study the effect of Neem powder and propolis on the biological and the mortality rate of larvae Khabrabeetle*T. granarium*.

Three factors were used in this experiment in addition to the witness, and the effect of these parameters on the lesion was evaluated.

The results showed that the coefficients used in comparison with the control showed no significant effect on the growth and development of the larval stage.

In contrast, the neem and propolis mixture gave the best effect on larval stage mortality at an average of 0.135 compared to the neem treatment, which gave an average of 0.09. The treatment with propolis powder gave an average of 0.06 compared to the average 0.03

Summation: Treatment with the mixture of neem and propolis leaves contains some ingredients that have proven the experience of the ability of the mixture to kill the larvae of the beetle at different times, which is considered useful for the continuation of this type of research.

ملخص البحث

أجريت هذه الدراسة تحت الظروف المعملية معمل الحشرات والحيوان الزراعي, قسم وقايه النبات كليه الدراسات الزراعية جامعة السودان للعلوم والتكنلوجيا شمبات في الفترة من سبتمبر2017 الى نوفمبر2018 لدراسة تأثير مسحوق اوراق النيم وبدرةالبروبوليس بيولوجية ونسبة موت يرقات خنفساء الخابرا*T.granarium*على

استخدمت ثلاثة معاملات في هذه التجربة إضافة الى الشاهد, ثم تم تقييم اثر هذه المعاملات على الافة.

اوضحت النتائج ان المعاملات المستخدمة بالمقارنة مع الشاهد لم تظهر اي تاثير معنوي على نمو وتطور الطور اليرقي.

وبالمقابل اعطت معاملة خليط النيم والبروبوليس افضل تاثير على نسبة موت الطور اليرقي بمتوسط0.135 مقارنة بمعاملة النيم التي اعطت متوسط 0.09 اما المعاملة بمسحوق البروبوليس لقد اعطت متوسط 0.06بالمقارنة مع الشاهد الذي اعطى متوسط 0.03

الخلاصه :المعاملةبخليط اوراق النيم والبروبوليس يحتوي على بعض المكونات التي اثبتت التجربة مقدرة خذا الخليط على قتل يرقات خنفساء الخابرا في اوقات متباينة الأمر الذي يعتبر ذو فائدة لإستمرار هذا النوع من البحوث.

CHAPTER ONE

1.1 INTRODUCTION

The increase in human population led automatically to increase in mand for food production.This necessitates more efforts to be or mitigating the expected negative impacts of plant pests crops. However, at the present time pesticides were ion, in devoted f on food considered indispensable for sustainable agriculture prod addition to their role in the protection of human health especially in the tropics. But, the increasing and irrational use of synthetic pesticides has become a source of great concern because of their possible effect on human health and non-target components of the environment. This concern is heightened by the non-specificity and high toxicity of some pesticides and development of resistant strains of microorganisms against other ones. Khapra beetle *.trogoderma granaritum*.is a serious pest of stored grain products in Africa, the Middle East, the Near East, and pockets of Europe and eastern Asia (USDA,1983; CABI/EPPO, 1997; CAB, 2004 ; EPPO, 2005).It has been nominated as one of the 100 worst invasive species worldwide (Lowe et al, 2000).Infestations by Dermestids were 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 At the last half of the century scientific researchers on botanical pesticides was increased Various adverse effects may occur from consuming moringa bar roots or chemicals that appear to be toxic when eaten.The plant have always been vital for mankind irrespective of the era and area all over the globe since the beginning of life.So that, The main objective of this study was to evaluated the effect of the Propolis and neem leave bowder as alternative to synthetic insecticide against one of the major storage insect pests, Khapra beetle *Trogodermagranarium*, (Evarts).k, flowers and their extracts, as these components contain

1.2 The Objective of the research:

To Compare the effect of Neem leaves powder and Propolis powderonbiology , damage and mortality of *Trigodermagranarium(Everts).*

CHAPTER TWO

LITERATURE REVIEW

2.1 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 racks on open spaces in the hive its color varies from green to brown and reddish, depending on its botanical source. Honey bees use propolis to seal any gap inside the hive that smaller than 3/16 or 1/4 (5mm or 6mm) while they leave themselves a bee space or approximately 9.5mm or 38 longer space being filled with wax conib (Burdock , 1998)

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- Bes may also use it to prevent infection with diseases and parasites in the hive.

2.1.1 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 tests virtually impossible (Banskota et al, 2001 and Bankova, 2005).

The source of propolis varies with the latitude In temperate 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, fungi and insects.

In tropical regions, bees gather propolis from flowers, especially clusia, that have adapted propolis and tropical are different. poplar propolis is rich in flavonoids . Clusiapropolis contains polyprenylatedbenzophenones . 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 very brittle (Burdock, 1998).

2.1.2 Physical characteristics:

Th e colour of propolis ranges from yellow to dark brown depending on the origin of the resins but even transparent propolis has deported A+ 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 hand and brittle. It remains brittle after such treatment even at higher temperature Above 45 c , it becomes increasingly sticky and gammy. Typically, propolis become liquid at 60 to 70 c but for some samples the melting point may be as high as 100 c the most common solvent used for commercial extraction are ethanol (ethylalcohol) ether , glycol and water for chemical analysis a large variety of solvents may be used in other to extract the various fractions many of the bactericidal components are soluble in water or alcohol (Arvouet et. al 1993)

2.1.3 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 know 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 quereetin, pinoeembringalangin, 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)

2.1.4 Medical uses:

Given the enormous revenues generated by traditional medicines like propolis.(WHO 21 September 2013) and modern pharmaceutical drugs like acyclovir(WHO 21 September 2013) it is not surprising that the medicinal use of propolis has both its proponents and opponents. Proponents of propolis argue that it has been used for thousand of years(Fearnely, 2001)and is unlikely to have maintained its popularity as a traditional medicine if its use was ineffective Opponents argue that propolis composition varies geographically, seasonally its use without extensive in vitro, in vivo, and clinical investigation to establish or associated with frequent or severe adverse reactions. with bee species.(Toreti, et.al., 2013) and that it is irresponsible to promote both safety and efficacy For impartial information, it is advisable to consult organizations like the National Institutes of Health. Based on the available scientific evidence, the National Institutes of Health rates propolis as "possibly effective" for treating cold sores, genital herpes, and post-surgery mouth pain. Currently, there is insufficient evidence" to rate the effectiveness of propolis in treating canker sores, tuberculosis, common colds and other infections, nose and throat cancer, improving the immune response, ulcers, stomach and intestinal disorders wounds, inflammation, minor burns, or other conditions.(NLM, 2012)

2.1.5 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.

2.1.6 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.

2.1.7 Acaricidal effect of bee propolis extracts:

Anumber 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. A, 2007).

In Nigeria, Osipitan et. al, (2010) tested propolisethanolic 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 need 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.

2.2 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).

2.2.1 Classification:

Kingdom:Planta

Subkingdom:Tracheobionta

Super division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Sub class: Rosidae

Order: Rutales

Family: Meliaceae

Genus: Azadirachta

Species*: Azadirachtaindica*

S.N*: Azadirachtaindica*A.juss

E.N: Neem

2.2.2 Origin:

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

2.2.3 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)

2.2.4 Distributions

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

2.2.5 Ecology :

Theneem trees is famous forits drought resistance, normally it thrives in areaswithsub_areas to sub humid conditions with an annual rainfall between 400 and200mm . it can also grow in regions with an annual rainfall 400MM. but in suchcases it depends largely on the ground water levels. Neem can grow in manydifferent types of soil, but it seems to develop best on well drained, deepsandy soils. It is a tropical and subtropical tree, and exists at annual meanswith sub-arid 200 mm. temperatures and does not tolerate (Ganguli, 2002).

2.2.6 Chemical Compounds of Neem tree :

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

2.2.7 Uses of Neem in pest and diseasecontrol:

Neem is deemed very effective inthe treatment of scabies although only preliminary scientific proof existswhich still has to be corroborated and is recommended for those who aresensitive to Permethrin. A known insecticidewhich might be irritantsand also the scabies mite has yet to become resistant to Neem ,so in persistent cases Neem has been shown to be very effective, there isalso 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 alone. Herbal medicine is the oldest from 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' (Griga , 1981).

Siddig (1993) report from sudan that neemseed water extracts at 1kg/1liter of water repelled folige pest of potato including ***B tabaci***, ***Aphis gossypii*** and ***J. lybica***and yield increased to 5 ton/ha.

Mohammed (2002) report that neem seed showed good performance against ***A. gossypii, B .tabaci***, and ***J. lybica***on Okra.

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

2.3 Khapra Beetle (*Trogodermagranarium*Everts)

2.3.1 Classification:

Kingdom Animalia

Phylum: Arthropoda

Class: Insecta

Order Coleoptera

Family Dermestidae

Scientific name:*Trogodermagranarium*Everts.

Common names: khapra beetle (English)

(Anon ,1993) .

2.3.2 Distribution:

Khapra beetle is a native of the Oriental region, but has become established tin a number of Asian, Middle East and African countries, as well as some European countries. In the USA a Khapra beetle infestation in 1954 resulted in a successful eradication program The USDA now regulates the importation of certain items from 25 countries (Dillon, 1968)

Khapra beetle is established within an area broadly limited north by the 35° parallel, south by the Equator, west by West Africa and east by Myanmar; i e. the warm dry regions along the Suez route from the Indian subcontinent to Europe. it has been introduced into areas of similar climatic conditions elsewhere, especially the alternative route between India and Europe around Africa Initially, these introductions caused severe damage but outbreaks have been local and have, in

most cases, been eradicated. In general. Khapra beetle is only successful in competition with other major stored product pests in conditions of low humidity. Ii has also established in some areas of unfavorable climate, in protected environments only. for example in Western Europe and Japan (OEPP EPPO, 1981)

2.3.3 Description :

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). Laid loosely and singly in the host material (APHIS, 1984)

Total length of the first-instar larva is 1 6-18 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). Morphologically, the mature larva of Khapra beetle can be

separated from that of ***T. versicolor*** by the absence of a dark pretergal line on the

7th and 8th abdominal segments, such a line being faint or absent on the 7th segment and never present on the 8th segment in Khapra beetle OEPP/EPPO, 1981). 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 exarate type; male smaller than female, average lengths being 3.5 mm and 5 mm, respectively OEPP/EPPO, 1981). Whitish colour (APHIS, 1984).

Adult Oblong-oval beetle, about 1.6-3.0 mm long by 0.9-1.7 mm wide; males brown to black, with indistinct reddish-brown markings on the wing covers; females are slightly larger than males, and lighter in colour, antennae are 11-segmented; head is small and usually deflexed.(Hinton ,1945. Beal 1956and Faber, 1971).

2.3.4 Life cycle:

The adults are short-lived, mated females living 4-7 days , unmated females 20_days and males 7-12 days. They do not fly and feed very little. Mating occurs about 5 days after emergence. The beetle can lay a full complement of eggs following a single mating, but a second mating greatly increases the total number eggs of eggs produced: once-mated females lays about 60 eggs, whereas twice- mated individuals laid about 60 and then 500 eggs after the respective mating. Delay i n mating of 15-20 days results in up to 25 % reduction in fecundity . The preoviposition period, which is not affected by humidity, is negligible at 40°C, I day at 35°C, 2 days at 30°C, 2-3 days at 25°C, and, at 20° C, no eggs are produced. Under optimum conditions, the female lays an average of about 50-90 eggs loosely in the host material. The eggs hatch in 3-14 days (OEPP/EPPO, 1981). Optimum conditions for development are 33-37 ° C , 45-75 % R.H (Howe , 1958) . Kapra 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 % RH , 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) Both short- and long-

lifecycle larvae can develop. Larvae may enter diapause under certain conditions, which then make it difficult to control them chemically, if the temperature falls below 25 C for any period of time and, sometimes, if the larvae are very crowded, they may enter diapause and development ceases (OEPP/EPPO ,1981). The larvae are cold- hardy, surviving temperatures below -8°C. Diapause often occurs at constant temperature, below 30°C. In diapause, the larva can moult but is relatively inactive and rarely feeds. It tends to seek out crevices in the fabric of buildings. A larva can remain in this state for several years, but the provision of a new consignment of food, especially in warm conditions, may stimulate renewed development and pupation. Young larvae are unable to feed on whole grains and depend on damaged grains or grain products for food (they readily attack softer foods such as nuts). Such damaged grains are always present in practice in lots of store grain. Older larvae can feed on whole grains. The amount and condition of the food present affects the speed of development, but larvae can survive long periods (at least 13 months) without food. These starving larvae pupate within a week on the return of favourable conditions such as high temperature and availability of food. Starvation of dormant larvae for 3 months, followed by a brief period of feeding , results in the production of 40 % of the normal number of eggs . However, this percentage is ample for the survival of the pest. One to 3 months of starvation does not affect the pupation rate of dormant larvae. 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 . 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 *Rhyzoperthadominica* and 25 times at 27 . 31 ° C (minimum RH 50 %) for *Sitophilusoryzae* , 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 larva develop rapidly into the pupal stage, e-g. in 15 days at 35°C (OEPP/EPPO, 1981) .

2.3.5 Economic importance:

The khapra beetle is principally a serious pest of stored products under hot dry conditions; complete destruction of grain and pulses may take place in a short time. In humid climates, the rates of increase of its competitors are so much greater that it has difficulty in establishing itself. However, in such areas, it lives at the inner edge of the expanding hot zone of stacks or bulks, in which heating has been induced by the activity of other species. In the EPPO region in the 1970s, Khapra beetle was rated as of considerable economic importance in Cyprus, Tunisia and Turkey. khapra beetle, depending upon existing conditions, may cause losses to stored grain of S to 30 percent and losses have been known to reach as high as 74 percent,the most favourable condition for multiplication and damage are in bulk grain under extended storage. Despite this, it is likely that direct losses to stored grains in Australia by susceptible Khapra beetle strains would be limited. This is because current phosphine treatments used to control endemic grain pests would also control non-resistant Khapra beetle populations.

Unlike most Khapra beetle feeds by preference on grain and cereal products (particularly wheat, barley, oats, rye, maize, rice, flour, malt, and noodles it can be a serious pest of such commodities in store. Khapra beetle will attack almost kind of material (Cotton, 1956) The damage is done in the larval stage as the adults do not normally feed such as dried blood, dried milk, fish meal, wool, goat skins and many more (Dillon , 1968) .

They 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 (Freeman, 1980). Apart from the destruction of grain products by Khapra beetle, ingesting products contaminated with body parts, setae and cast larval skins can result in gastro-intestinal irritation, Asthmatics and sensitised individuals are also at risk, as contaminants are highly allergenic.

Since infestations would most likely be confined to grain storage facilities and other buildings, this pest is not expected to have significant impacts on natural

environments or endangered /threatened species (Pasek, 1998) .

2.3.6 Control:

2.3.6.1 Chemical control:

Worldwide, the fumigant of choice for quarantine treatment against Khapra beetle is methyl bromide. This is despite its known high level of tolerance to the fumigant, particularly when in diapause. Recommended dosage rates against the pest tend to be twice as high or more than against typical stored product pests (Bond, 1984). Practical problems with fumigating some oily, high risk commodities eg. (Expeller cake) with methyl bromide further compounds the difficulties Despite the importance of Khapra beetle to a number of quarantine authorities there is remarkably little modern (post, 1960) literature on the response of the pest to insecticidal measures. This is particularly so for the response of diapause larvae - assumed here to be the main form of the pest requiring quarantine control. Many of the available scientific studies do not take adequate precautions to ensure the at least part of their test samples is well established in diapause. There is a risk that these studies will give rise to dosage schedules that will not eliminate diapause larvae, though they may well control the more susceptible active larvae and other developmental stages. The Australian Department of Health, a predecessor of AQIS, commissioned studies in the UK to remedy the lack of reliable data on diapause larvae (Rees & Banks, 1999).

2.3.6.1.a- Methyl bromide:

Historically, dosage recommendations for control of Khapra beetle from Methyl bromide have been based on ,,double the normal dosage" for typical stored product pest control. The latter are often aimed at ct =200 g h m-3 at 20°C, implying ct =400 g h m-3 at 20°C for Khapra beetle. This is close to the value of 480 gh m -3 for 100 % kill at 20 ° C given by Bell *et al* (1985) , but less than the Russian quarantine dosage implied by Mordkovitch and Sokolov (1992) of 600 g h m-3. give a dosage of 600 g h m-3 for >15°C, which may be the origin of this r

recommendation (Bogs ,1976). During the apparently successful Khapra beetle

eradication campaign in the 1950s in USA, the dosage recommendations finally adopted correspond to a minimum ct of about 1200 g h m -3 at unspecified temperatures (initial dosage 80 g m-3, 32 g m-3 remaining after 24 hours) .

Exposures exceeding ct =400 g h m-3 are easily obtained at 20°C with dosages of 48 g m-3 for 24 hours with most commodities in well sealed enclosures(Amritage, 1958). Oily and finely divided commodities may need additional dosage or topping up to achieve target ct-products and as a result residual bromide levels may exceed established tolerances (eg. 50 ppm in cereals, Australian MRL). There is also a risk of taint or quality change in some materials. Alternative treatments may be considered more appropriate despite long history of methyl bromide use in such situations.

The use of methyl-bromide or other fumigants to eradicate or control Khapra beetle will likely produce adverse effects to the environment and human health.

Methyl bromide is an ozone-depleting substance, and human exposure to high concentrations can result in the failure of the central nervous and respiratory systems (Pasek, 1998).

2.3.6.1.b- Phosphine:

Phosphine fumigation is not currently approved by AQIS as a quarantine treatment against Khapra beetle. Reasons for this appear to be historical - with suitable precautions to prevent leakage and exposure times of 12 or more days, depending on conditions. Khapra beetle, even as a larva in diapause, is quite sensitive to phosphine.

While susceptible Khapra beetle, even as diapause larvae, appear to be controllable with standard dosage rate and exposures to phosphine, there is evidence that resistance to phosphine has already developed in this species. Prolonged exposures at substantial phosphine concentrations are required to control the tolerant strains so far identified. There has been no substantial recent

survey of resistance levels to phosphine in Khapra beetle (Bogs ,1976).

2.3.6.2 Physical control:

Carbon dioxide - based atmospheres (< 70 % CO2) are less effective against Khapa beetle than most other stored product pests, requiring much prolonged exposure for control of diapause larvae .

Annis (1987) concluded that 16 days exposure at 80 % co2 (20-30°C) was required to eliminate Khapra beetle (data of Spratt .*et al*,(1985). (Vermna and Wadhi, (1978) and Le Tore'h, (1983)).

Low-oxygen atmospheres however appear to be quite effective against Khapra beetle, including eggs and diapause larvae (Verna 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 to us on effectiveness of the new technique on Khapra beetle. In summary, some CA treatments are effective against Khapra beetle at exposure periods only slightly longer than required for phosphine. While the difficulties and costs associated with CA application in large structures and containers may limit its use there, small-scale packaging in nitrogen or CO2 in barrier film packs may be assumed to be fully insecticidal against Khapra beetle inadvertently Included, provided temperatures exceed 20°C (no data available below this), exposures exceed 20 days and the atmospheres in the packs do not exceed 1 % 02.Heat treatment appears to be a potentially useful technique for quarantine treatment of heat tolerant commodities against Khapra beetle. There is a surprising quantity of data available to substantiate this. Much of it is antique, but of good quality.For instance, Husain (1923) studied heat disinfestation of wheat from Khapra larvae. As expected the resulting temperature/time relationship is of the form typical of heat/time curves shown by many stored product pests (Banks & Rees, 1999).

Overall the data shows that, unexpectedly, Khapra beetle is not the most heat

tolerant common stored product pest. Some stages of *R. dominica* are more so (Husain 1923), and heat dosages aimed at complete kill of *R. dominica* can be expected also to eliminate Khapra beetle. This is despite the known unusual tolerance of Khapra beetle to moderately high temperatures, around 41°C, lethal to many species, and its unusually high optimum developmental temperature.

Note; however there are some inconsistencies in the data between authors that give rise to some concern and addition al studies are required for conclusive development of recommendations.

In summary, most of the available laboratory data show temperatures above 55 C are lethal to all stages of Khapra beetle in less than 15 minutes. However response data for diapause l arvae is very limited. The USDA recommendation of 7 minutes at 66°C seems unnecessarily stringent, but may actually include some allowances for time that heat takes to penetrate to the actual target pest through structures, commodities or residues.

It is suggested (Rees & Banks, 1999) that a conservative heat dosage of at least 120 minutes at 55°C at the site of the infestation would be adequate to eliminate Khapra beetle. Due allowance for time to heat the site to the required temperature would need to be added to any specification.

2.3.6.3 Biological control:

Al-Kirshi*et al*. (1997) considers the potential or the larval parasitoid *Laeliuspedatus* (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.

2.3.6.4 Other methods of control :

Data on the effectiveness of other fumigants and for fumigant mixtures is

insufficient to base recommendations for quarantine treatment on. Almost all studies do not adequately show that larvae in diapause have been tested. An exception is Bell *et al.* (1985) who tested a methyl bromide / methyl chloroform mixture. However methyl chloroform is no longer available, as it is an ozone - depletory. Rees & Banks (1999) refer to many laboratory-based studies on use of irradiation to sterilize Khapra beetle for its control. Most of these studies are directed at adult insects, often not the stage of concern in quarantine treatments.

Studies on effectiveness of irradiation on apparently diapause larvae are not adequate to base a sound assessment on, but they suggest diapause larvae are very not tolerant to irradiation at low temperatures(20°C). (Rahalkar and Nair, 1968).

CHAPTER THREE

Materials and Methods

3.1 Site location

This study was conducted in the Laboratory of Entomology and Zoology, Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology, during September/2017 to November/2018.

The study was conducted to know effect and control of Neemleaves powder and propolis powder on larvae of Khapra beetle (*T. granarium*).

3.2. Materials of the study

-leaves powder of Neem-propolis -garble

- gloves

- forceps

- hand lens

-sensitive balance

-needle

-electric bladder

3.3 Methodology:

3.3.1 Collection of insects sample:

Adult of *Trogodermagranarium* (Everts.), collected from laboratory . They were breededin a glass container covered with a pace of muslin cloth to allow for aeration and avoid suffocation of the insects and equally prevent escape of the insects, and placed in the laboratory . The breed was raised under ampeint temperature (28-3c) and relative humidity (70 + 5 %) condition .respectively on this enhance availability of *T. granarium* for this experiment.

3.3.2 Collection of plant materials:

Neem leaves were collected during August2018 from the area nearDepartment of Agricultural Engineering(Shambat).

3.3.3Propolis:

The Propolis powder was obtained from the Plant Protection Department

3.3.4 Preparation of powder:

Theneem leaves of test plant were washedand dried under shade foe 7 days , and then carefully crushed by an electric blender powder which was stored in a tightly closed glass jar and kept in the room temperature.

a- drying of leaves **b-electric bladder** **c-forceps**

d-needle **e-hand lens**

f- sensitive balance **g-gloves** **h-muzzle**

Plate(1): tools which used for Preparation:

3.3.5 Experiment layout:

The experiment was conducted in the Laboratory of Entomology and Zoology during September 2017 November 2018, in the temperature conditions 30 C° and 68% R.H. the objective of the experiment control the insect pest (*T. granarium*) . Nine plastic cups prepared for running this experiment. The experiment contains three treatments. Powder concentrations are (1g propolis, 1g datura and 1g mixture) in

addition to the control. ten larvae and Ten grains per plastic cup.

The larvae mortality, damage and other effect were recorded after each 24hours after treated by use (forceps-brush-needle-hand lens).

3.4 Statistical analysis :

These experiments were designed in a complete randomized design. The data were subjected to ANOVA using MASTAT.C program and mean separation was made by using LSD test. Results with $P>0.05$ were considered to be statistically significant.

CHAPTER FOUR

RESULTS

4.1 EffectofPropolispowder ,Neem leaves powderand there mixture against larvae of khaprabettle(*Trogodermagranarium*Everts.)

The table below shows the total percentage of the death of khapra beetle larvae, where the mixture give the highest rate 30%, followed by the neem powder which give 26.6%, after it the propolis powder which gave the rate of death 16.6% and less rate gave from the Control was 10%.

Table No(1) Effect of PropolispowderNeem leaves powder and mixture of them against larvae of khapra beetle(*Trogodermagranarium*Everts.)

Treatment	Mean mortality	Mortality%
A	.06	16.6%
B	.09	26.6%
C	.135	30%
D	.03	10%

FigureNo(1) Mean percent mortality of khapra beetle`s larvae*T.granarium*(Everts) by effect of propolis powder ,Neem leaves powder and there mixture

Keys:

A= Propolis

B=Neem

C= Mixture

D= Control

4.2Effect of Propolis powder ,Neem leaves powder and there mixture on ability of larvae to cause damage on grains.

The following table shows the infection of the grains resulting from a khpra beetle larvae infection. The lowest rate was recorded from the mixture treatment 0.14 followed by the treatment of propolis by the ratio 0.205 and the treatment of neem gives the ratio 0.22, where the control gave the highest ratio of infection 0.26.

Table No(2) Effect of Propolispowder,Neem leaves powder and mixture on larvae in reducing damage to grains

Treatment	Mean number of infected grains
A	0.205
B	0.22
C	0.14
D	0.26
STD	0.387

Figer No(2) Mean number of infected grains by khapra beetle larvae

Keys:

A= Propolis powder

B= Neem powder

C= Mixture

D= Control

4.3 Effect of Propolispowder , Neem leaves powder and there mixture against khapra beetle stages except Eggs.

The table below shows the effectiveness of application of neem powder, propolis powder and there mixture against the larval stage, pupa stage, moulting , grains damage, and effect on adult stage mortality.
The treatment of the mixture gave the highest mortality rate of the larvae stage and the least grains damage Compared with the neem treatment the gives highest rate of Adult stage mortality, the treatment of propolis powder gave the highest rate of moulting of larvae stage and the lowest rate of Pupa development where the control gave the highest rate of grains damage and the lowest rate of larvae mortality.

Table 0No (3) Effect of Propolis powder , Neemleaves powder and there mixture

against Khaprabeetle Stages except Eggs

Treatment	Mortality of larvae	Pupa	Moulting	Damage	Mortality of Adults
Propolis	0.06^{a}	0.36^{a}	0.845^{a}	0.205^{a}	0.23^{a}
Neem	0.09^{a}	0.41^{ab}	0.82^{a}	0.22^{a}	0.35^{ab}
Mixed	0.135^{a}	037^{ad}	0.74^{a}	0.14^{ab}	0.27^{b}
Control	0.03^{ab}	0.37^{d}	0.66^{a}	0.26^{bd}	0.14^{bd}
LSD	0.169	0.405	0.625	0.387	0.401

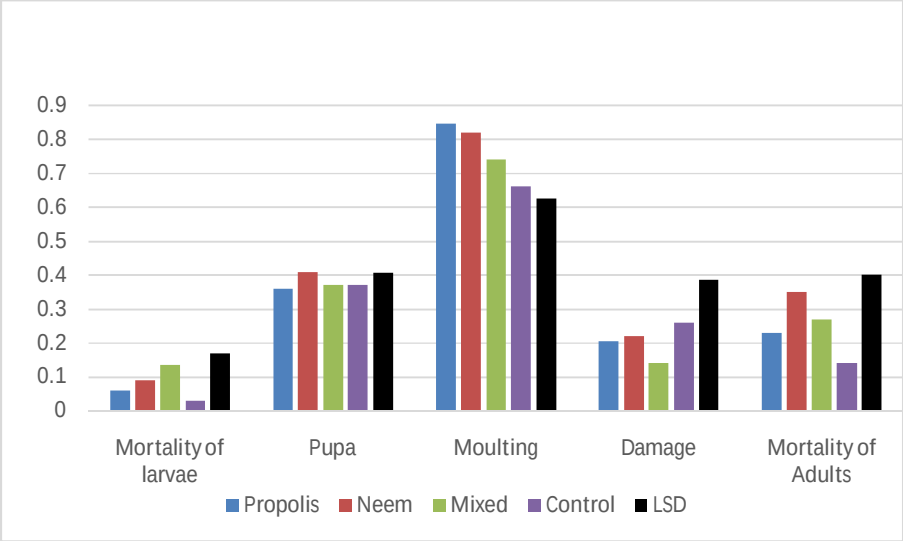

Figure No(3) Mean effect of Propolispowder ,Neem leaves powder on stages of khapra beetle stages except eggs.

CHAPTERFIVE

5.1 Discussion:

The results showed that there are no effect for both the datura leaves powder and propolisin the control of khaprabettle larvae because they gave a small percentage compared to mixture . The rate of Neemleaves powder was 26.6%, the ratio of Propolis was 16.6% , the mixture was 30% and the control was 10% mortality.

The mixture is the best thanNeem and Propolis of attribution for the grains damage is less and higthmortality of adult (0.135 mixture, 0.09Neem and 0.06Propolis) mean.

From these study it is clear to us that the mixture of Neemand Propolis contain on some components that have the ability to prevent the larvae of khapra beetle to cause damage, which is useful for the continuation of this type of research.

5.2 Conclusion:

In this study neem leaf powder , propolis and theremixturewas so effective against Khaprabettle larvae , because they are causes 30% mortality in the mixture Compare with26.6%Neem, 16.6% Propolis and 10% control .

5.3 Recommendations:

The use of both propolis and neem powder each alone did not reduce the rate of damage in the grains and they had low effect on larvae mortality , so need more studies for these results.

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