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INSECTICIDAL ACTIVITY OF THREE PLANT EXTRACTS AGAINST *Trogoderma granarium*(Khapra beetle) IN WHEAT GRAINS WITH TOXICITY EVALUATION

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ABSTRACT

Extracts of three plant species were tested under laboratory conditions for their ability to protect wheat (*Triticum* spp.) grains against the insect *Trogoderma granarium* . The insect was reared and tested on whole wheat grains with respect to the larvae,pupae and adult's mortality, and emergence.. Furthermore, the safety of the most effective plant extracts was evaluated with respect to biochemical changes in rats. The results revealed that *Chenopodium ambrosioides* followed by *Eucalyptus gluaca* extracts were the most effective against *T. granarium* with respect to insect mortality and progeny relative to the control. These extracts also showed low toxicity on treated rats relative to control with respect to biochemical changes . The results suggested that these extracts may be a safe alternative to insecticides
Keywords: Control; stored products; safety; insect plant extracts, Khapra beetle

INTRODUCTION

The Khapra beetle, *T. granarium* (Coleoptera, Dermestidae) is considered to be one of the most serious pests of stored grain products, various leguminous crops, rice, oat, barley, and rye throughout the world (Lowe *et al.*, 2000). It is originally occurred in India, and spread to Africa, Europe, South America and East Asia (Harris, 2009). The Khapra beetle occurs in very low numbers and can survive for a long period as an inactive state (Dwivedi and Shekhawat, 2004). According to FAO estimate, 10 to 25% of the world harvested food is destroyed annually due to insects and rodent pests (Anonymous, 1980). Losses caused by *T .granarium* have been reported to range from 0.2 to 2.9% over a period of 1 to10.5 months (Irshad *et al.*, 1988). Chemical insecticides such as malathian, cypermethrin, bifenthrin are used for rapid control, but are expensive, not readily available and may be poisonous to humans and environment (Tsumura *et al.*, 1994). Moreover, malathian and cypermethrin have gone ineffective due to development of resistance in insect pests of stored grain, particularly in *T. granarium* (Saxena and Sinha, 1995).

Local alternatives such as the natural products are cheaper, easily available way for controlling pests, which are safe for humans and environment.

Control of stored-product insect populations is primarily dependent upon continued applications of insecticides (White and Leesch,1995) .In spite of its efficacy, their repeated use for several decades has disrupted biological control system by natural enemies and led to outbreaks of insect pests, widespread development of resistance, undesirable effects on nontarget organisms, and environmental and human health concerns (White and Leesch,1995, . Subramanyam and Hagstrum 1995).

Most of the alternatives insecticides substances were tested against insects attacking stored products in order to establish new control practices with lower mammalian toxicity and lower persistence in the environment relative to insecticides. Therefore, studies should conduct not only on the evaluation of botanical extracts against the target pests but also on their safety on human health that are in demand.

Therefore, this study attempted to find natural and cheaper methods for the control of stored-grain insect pests, aimed to evaluate insecticidal activity of three plant extracts (*C. ambrosioides, E. gluaca , Ocimum basilicum*) compared to the recommended compound malathion against *T. granarium* in wheat grains with respect to progeny and mortality of the insect larvae ,pupae and adults and finally to evaluate the toxicity of the most effective plant extract on rats with respect to biochemical changes relative to control.

MATERIALS AND METHODS

The Insect

T. granarium (Everts) was obtained from the Department of Stored Product Pests Control, Plant Protection Research Institute of Sakha, Kafr El-Shiekh. This strain was reared free of insecticidal contamination for several years at 30 ± 2°C and 70% ± 5 relative humidity. The cultures were maintained under the same conditions in the Pesticide Department, Faculty of Agriculture, Kafr El-Shiekh University, Egypt. The culture was raised by infesting 30 pairs of newly emerged *T. granarium* adults into 500 g of wheat grains in large box. After that, 35 days newly emerged (F1) adults were collected and used to infest the wheat samples.

The Stored Product

Wheat grains were used of culture *T. granarium* and to evaluate the efficacy of tested plant extracts as well as malathion against the same insect. Wheat grains were stored in airtight tins until being required for experiments. The experiments were carried out in a room kept at a constant temperature of 25± 2°C and 70%± 5 R.H .

Plants and Preparation of Crude Extracts

The leaves of plant species (*C. ambrosioides , E. gluaca, O. basilicum*) were collected from a local nursery at Kafr El-Sheikh Governorate, Egypt., 250 g each, from the tested plant species were submitted to hydrodistillation for 6 h using a Clevenger-type apparatus to produce the essential oils. The obtained oils, which were dried over anhydrous sodium sulfate after filtration, were weighed and stored at 4_C until analyzed and tested.(Marie.*etal.*,2009).

Effect of Tested Plant Extracts and Malathion on Progeny of *T. granarium*

The tested plant extracts at concentration levels of 150, 250, and 400 ppm were used to evaluate its efficacy against *T. granarium*. Malathion was used as recommended compound against *T. granarium* at concentration levels of 5, 10, and 20 mg/L. Each concentration was applied in three replicates, and each replicate contained 20 g of wheat grains. The treatment

of wheat grains was carried out by dipping wheat grains in water solution of malathion and botanical extracts at tested concentration levels twice consecutively for 5 seconds and subsequently spread on top of plastic sheets to dry for 90 mins. The control treatment was carried using water only and replicated three times. Then, 10 adults of *T. granarium* were transferred to treated wheat grains which were put in a 85 × 75 mm plastic jar and kept at 30 ± 2°C and 70% ± 5 R.H , according to the method described by Kestenholz *et al.*(2007). The emerged adults from the hatched eggs were recorded after 6 weeks of treatment. These adults were used to calculate the reduction percentages in *T. granarium* progeny from the use of the tested plant extracts as well as malathion compared to the control as shown in the following equation as described by (Tapondjou *et al.* 2002).

% IR = (Cn - Tn) 100/Cn, where,

Cn is the number of newly emerged insects in the untreated (control) jar,

Tn is the number of newly emerged insects in treatments

Efficiency of the Tested Plant Extracts and Malathion on Mortality of Adults, Larvae , and Pupae of *T. granarium* .

Wheat grains were treated with the tested plant extracts and malathion for protection against larvae, pupae, and adults of *T. granarium* at concentration levels mentioned before. Each concentration was applied in three replicates and in each replicate contained 20 gm of wheat grains. The treatment of wheat grains was carried out by dipping wheat grains in aqueous solution of malathion and botanical extracts at the tested concentration levels twice consecutively for 5 seconds and subsequently spread on top of plastic sheets to dry for 90 mins. The control treatment was carried using water only and replicated three times. Then, 10 adults, pupae, and larvae of *T. granarium* were transferred to treated wheat grains which were put in a 85 × 75 mm glass jar and kept at 30 ± 2°C and 70% ± 5 R.H, according to the method described by Kestenholz *et al.* (2007). The glass jars were covered with cotton cloths held on with rubber bands. The number of dead adults, pupae, and larvae in each jar was counted after one and two weeks and the percentage of insect mortality was recorded.

Toxicity Assessments

Animal Treatment

Adult Wistar male rats (*Rattus norvegicus*), eight weeks old and 80–100 gm in weight were obtained from Faculty of Medicine, Tanta University. Rats were housed in wire cages under standard conditions with free access to drinking water and food in a temperature controlled room with 14 hs light and 10 hs dark cycle. The rats were given a standard diet as describe by Romestaing *et al.* (2007). Before treatment, rats were left two weeks for adaptation. The animals were randomly divided into three groups of three. Two groups were treated with the most effective plant extracts and the third group was the control. The most effective plant extracts *C. ambrosioides and E. gluaca* were administered once orally at a concentration of 500 mg kg^{-1} body weight. The control group rats were administrated once orally with an equal amount of almond oil. After 21 days the rats were sacrificed under

anesthesia. Then, blood samples were taken, under anesthesia, by cardiac puncture and stored in vials containing heparin.

Enzymes assays

Blood samples were centrifuged at 4500 rpm for 15 mins at 4°C and the blood serum was used to determine the Glutamate Pyruvate Transaminase (GPT), creatinine and alkaline phosphatase (ALP) according to the methods described by Barham and Trinder (1972), Reitman and Frankel (1957) and Wilkinson *et al.* (1969), respectively.

Statistical Analysis

Data from the experiments were statistically analyzed using one-way repeated measurement analysis of variance. Duncan's multiple range test (Duncan 1955) were used to separate means using SAS program (Version 6.12, SAS Institute Inc., Cary, USA).

RESULTS

Effect of Plant Extracts and Malathion on Progeny of *T. granarium*

The numbers of emerged adults of *T. granarium* were significantly decreased in all treatments (the tested plant extracts and malathion) relative to the control, as shown in Table 1. Moreover, the tested plant extracts delayed the progeny of the tested insect three weeks relative to control treatment. Increasing the concentration levels of all tested treatments reduced the emergence of *T. granarium* even more (concentration dependent). *C. ambrosioides*, extract followed by *E. gluaca* , *O. basilicum* extracts and malathion were the most effective treatment .

Table 1: Effect of the tested plant extracts and malathion on progeny of *T. granarium*.

Treatment	Concentration mg/L	% Reduction
C. ambrosioides	150	96bc
	250	100a
	500	100a
E. gluaca	150	92.5de
	250	96bc
	500	100a
O. basilicum	150	85.7g
	250	93d
	500	97.5ab
Malathion	8	82.5h
	16	90f
	32	100a
control	0.0	0.00i

A Letters indicate differences between means at P = 0.05 using Duncan's multiple range test (Duncan 1955)

Table 2:Effect of the tested plant extracts and malathion on adult's mortality of *T. granarium*.

Treatment	Concentration mg/L	%mortality after one week	%mortality after two weeks
C.ambrosioides	150	87.5de	98b
	250	92bc	100a
	500	95a	100a
E. gluaca	150	83g	95bc
	250	90d	98b
	500	93b	100a
O. basilicum	150	65k	90e
	250	82gh	95bc
	500	87.5de	100a
Malathion	8	65k	88.5fe
	16	80i	93d
	32	85f	100a
control	0.0	0.00l	0.00g

A Letters indicate differences between means at P = 0.05 using Duncan's multiple range test (Duncan 1955).

Efficiency of Tested Plant Extracts and Malathion on *T. granarium* Adults Determined by Mortality Values

Effect of Plant Extracts and Malathion on Mortality of *T. granarium* Adults.

The efficacy of the tested plant extracts and malathion against *T. granarium* adults by means of mortality was presented in Table 2. The results showed that *C. ambrosioides* extract was the most effective treatment against *T. granarium* adults followed by *E. gluaca* and *O. basilicum* extracts respectively. The morality percentages of *T. granarium* were significantly increased in the second week relative to the first week at all tested treatments. Increasing the concentration levels of all tested treatments increased the mortality of *T. granarium* adults even more concentration dependent.

Efficiency of Tested Plant Extracts and Malathion on *T. granarium* Pupae by Mortality

The efficacy of the tested plant extracts and malathion against *T. granarium* pupae by means of mortality was presented in Table 3. The results showed that *C. ambrosioides*, extract was the most effective treatment against *T. granarium* pupae followed by *E. gluaca and O. basilicum* extracts respectively. The morality percentages of *T. granarium* pupae were significantly increased in the second week relative to the first week at all tested treatments. Increasing the concentration levels of all tested treatments increased the mortality of *T. granarium* pupae even more concentration dependent.

Table 3 :Effect of the tested plant extracts and malathion on mortality of *T. granarium* pupae.

Treatment	Concentration mg/L	%mortality after one week	%mortality after two weeks
C. ambrosioides	150	73hi	95c
	250	88.5cd	98ab
	500	93a	100a
E. gluaca	150	66.5j	92de
	250	88.7bc	95c
	500	90b	100a
O. basilicum	150	55k	87f
	250	83g	92de
	500	90b	95c
Malathion	8	45.8	85fg
	16	75h	93d
	32	86.7e	100a
control	0.0	0.0l	0.0h

A Letters indicate differences between means at P = 0.05 using Duncan's multiple range test (Duncan 1955).

Effect of tested plant extracts and malathion on mortality of *T. granarium* larvae

The efficacy of the tested plant extracts and malathion against *T. granarium* larvae by means of mortality was presented in Table 4. The results showed that *C. ambrosioides*, extract was the most effective treatment against *T. granarium* larvae followed by *E. gluaca and O. basilicum* extracts and malathion, respectively. Among the tested plant extracts *C. ambrosioides* extract was the most effective one and *O. basilicum* extract recorded the lowest efficacy against the larvae of *T. granarium*. The mortality percentages of *T. granarium* larvae were significantly increased in the second week relative to the first week at all tested treatments. Increasing the concentration levels of all tested treatments increased the mortality of *T. granarium* larvae even more concentration dependent.

Table 4 :Effect of tested plant extracts and malathion on mortality of *T. granarium* larvae.

Treatment	Concentration mg/L	%mortality after one week	%mortality after two weeks
C.ambrosioides	150	82fg	100a
	250	95b	100a
	500	98a	100a
E. gluaca	150	80gh	97bc
	250	93bc	98ab
	500	95b	100a
O. basilicum	150	65j	87.5fg
	250	75i	90f
	500	92cd	94d
Malathion	8	60k	88fg
	16	82.5f	92de
	32	90de	100a
control	0.0	0.0l	0.0h

A Letters indicate differences between means at P = 0.05 using Duncan's multiple range test (Duncan 1955).

Toxicity Evaluation

Effect of the Most Effective Plant Extract on Rats Liver Enzymes .

The Alkaline phosphatase and GPT activities are known as cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage. In the present study, therefore, both enzyme activities were used as indicators of hepatic damage. The obtained data in Table 5 showed that there were no significant differences in the activity of ALp and GPT after 21 days of rat's administration with the most effective plant extract at dose level of 500 mg/kg body weight relative to control treatment.

Table 5 :Effect of the most effective plant extract *C. ambrosioides,* on serum GPT, ALp, and creatinine of treated rats at dose level of 500 mg/kg body weight.

Treatments	GPT (IU/LB ± SEA)	ALP (IU/L ± SE)	Creatinine (mg dl−1C ± SE)
Control	2.35± 53.1	1.45±97	0.12±0.225
C. mbrosioides)	2.76± 53.7	100 ± 4.43	0.13 ±0.22 9

A Standard Error. B International Units per Litre. C mg dl−1 milligram per decilitre

Effect of the Most Effective Plant Extract on Rats Kidney Function.

Regarding the kidney function, there were no significant differences in creatinine level in rats administration with the most effective plant extract at dose level of 500 mg/kg relative to control (Table 5). The normal creatinine in rats treated with the most effective plant extract relative to control treatment was assumed to be the normal kidney function.

DISCUSSION

The results of the present study implied that the tested plant extracts were effective against *T. granarium* in stored wheat with respect to progeny of adults and mortality of all its stages (larvae, pupae, and adult). The efficacy of the plant extracts against *T. granarium* insect in stored wheat with respect to progeny and adult mortality has been reported by many researchers (Tapondjou,*etal* 2002, Ketoh ,*etal*2005, Kestenholz,*etal* 2007, Derbalah, 2012,Karzan,*etal*2012) . However, the efficacy of the tested plant extracts, especially the most effective ones, has not been reported against *T. granarium* and considered first report.

The insecticidal activity of *C. ambrosioides* extract against *T. granarium* may be due to the presence of the previous compounds(Kapoor, *etal* 1972 and Ahmed. 2000, Park *etal*,2003 Negahban ,*etal*,2006 Rozman *etal*, 2007 Ogendo *etal*, 2008 , López *etal*,2008)

Moreover, the efficacy of the most effective plant extract at higher concentrations might actually have efficacy comparable to the chemical pesticides. In fact, the actual dosage of any one compound identified in this extract could be relatively low, safe, and economically feasible.

Although the insecticidal activity of the most effective plant extract is attributed mainly to its major compounds mentioned before, the synergistic or antagonistic effect of some compounds in the mixture has to be considered (Ragas *etal*,2002) . Each of the plant extract components has its own contribution on biological activity of the extract against the tested insect.

High mortality of *T.granarium* may be due to the chemical composition of the *C. ambrosioides* extract plant is known to contain insecticidal compounds such as scridole ,cymol and alpha- terpine (Quarles,1992).

Tapondjou *etal.*(2002)reported that leaf powders of *C. ambrosioides* were potent against six stored product beetles. It is therefore likely that *C. ambrosioides* has several insecticidal constituents that are active against all Coleopterans.

The mode of action of the bioactive natural monoter-penoids (hydrocarbons, alcohols, and ketones) isolated from plant extracts oils may be due to inhibition of acetylcholinesterase (Gordon *etal.*,1982; Miyazawa *etal.* 1997; Lee *etal*,2000)

Since (Lee *etal*,2000) reported that 1, 8-Cineole was the most potent inhibitor of AChE among the monoterpenes tested. This inhibition may be a mode of action for essential oils and monoterpenes against stored grain insects as well. Also, the mode of action of the tested botanical extracts may be largely attributable to its fumigant action (Shaaya ,*etal*,1997 ,Park,*et al*,2003)

The botanical extracts as pest control agents present two main characters: the first is their safety to the people and the environment, and the second is the less resistance development against it by the tested insect. Regarding the safety, the toxicity evaluation of the most effective plant extract revealed that there were some slight variations that occurred sporadically in treated rats relative to control with respect to enzyme.. With referring to resistance development, it is believed that it is difficult for the insect to develop resistance to such a mixture of bioactive components with, apparently, different mechanisms of insecticidal activity (Wei *etal*,2008).

This study is considered the first step towards more investigation and concern about using these effective botanical extracts as alternative for controlling of stored product pests. This will help to reduce the environmental pollution and the adverse effect on human health resulted from using insecticides since these botanical extracts revealed non significant toxicity relative to the high dosage that were given orally and will not reach human by this dose as a residue under any conditions.

CONCLUSIONS

The insecticidal activity of the tested plant extracts against *T. granarium* indicated that the potential of some plant species to be a natural sources *C., ambrosioides*, *E. gluaca* and *O. basilicum*) of insecticidal material.

Insecticidal activity was confirmed in all the tested plant species, although the results showed that extracts varied in their effectiveness against

T. granarium . It is possible to use botanical products as an alternative to chemical control of *C. maculates* and this may contribute to reducing the amount of insecticides applied and subsequently minimizing their hazards. The ability of using botanical products as alternative of chemical control of *T. granarium* is possible if the problem of cost-effective commercial production can be solved. Moreover, some of these botanical extracts could find a place in IPM strategies, especially where the emphasis is on environmental, food safety and on replacing the more dangerous toxic insecticides. Work in this regard should continue to obtain information regarding its practical effectiveness under natural conditions to protect the stored products without any side effects.

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تقييم النشاط الابادي والسمية لثلاثة مستخلصات نباتية ضد خنفساء الصعيد (خنفساء الخابرا)في حبوب القمح

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اختبرت ثلاثة مستخلصات نباتية تحت الظروف المعملية من ناحية قدرتها علي حماية حبوبالقمح ضد حشرة خنفساء الصعيد بالنسبة لموت اليرقات والعذاري والحشرة الكاملة وكذلك بالنسبة للخلفةأو نسبة الخروج . وأيضا تم تقييم سمية أفضل المستخلصات كفاءة علي الفئران وذلك بدراسة التغييرات البيوكيميائية فيها أشارت النتائج الي أن المستخلص النباتي للزربيح يليه الكافور كانا أكثر فعالية ضد خنفساء الصعيد تبعا لنسبة الموت والخلفة مقارنة بالكنترول.

أ يضا أظهرت هذه المستخلصات النباتية سمية منخفضة علي الفئران المعاملة مقارنة بالكنترول وذلك بالنسبة للكرياتينين في الكلي وانزيمي الفوسفاتيز القلوي والجلوتاميت بيروفات ترانس أمينيز مما يؤكد أن هذه المستخلصات النباتية يمكن استخدامها كبدائل آمنة للمبيدات.

قام بتحكيم البحث

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