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Effect of some medicinal plants extracts and cypermthrin against Khapra Beetle (*Trogoderma granarium* Everts)

Karzan Omar[1], Nask Muhammad Faraj[1], Sahar A. A. Malik[2*] and Iman M. Al- Farhani[3]

[1]*Agricultural Technical Institute, Sulaimanya, Iraq*
[2]*Department of Biology, College of Science, University of Basra, Iraq*
[3]*Department of Plant Protection, College of Agriculture, University of Basra, Iraq*

Abstract

Effects of ethanol extracts of *Datura stramonium*, *Solanum nigrum*, *Quercus infectoria* and *Xanthium strumarium* on the mortality, egg hatching, reduction in F1 progeny and repellents on the red flour beetle were investigated. The results showed that cypermethrin had the best effect on the mortality of adults (100%) on all concentrations, whereas the fruits of *Datura stramonium* and *Xanthium strumarium* had a mortality rate of 100% in 2% and 4% concentrations. Egg hatching was also delayed, and began after four days of treatment, in *Quercus infectoria* and *Xanthium strumarium*. Ethanol plant extracts of *Datura stramonium* caused 100% mortality of larvae at a concentration of 1% after eight days of treatment, followed by the alcohol extract of *Solanum nigrum* with 97.43% mortality in 1% concentration. Mix of ethanol plant extract with food reduced F1 progeny. Cypermethrin and *Datura stramonium* were given the best effect that reaches 17.06% and 18.11% respectively. Repellents percentage showed that ethanol extracts of *Datura stramonium* and *Solanum nigrum* showed repellents of 91.87% and 91.45% in 4% concentration after 24 hours of treatment.

Key words: *Trogoderma granarium*, Plant extracts, Repellent, Toxicity

Introduction

The Khapra beetle, *Trogoderma 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 *Trogoderma 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 *Trogoderma granarium* (Saxena and Sinha, 1995). The larval period of development of Khapra beetle had been prolonged after treatment with DDT (Shantaram, 1958).

Local alternatives such as the natural products are cheaper, easily available way for controlling pests, which are safe for humans and environment. Most pesticide plants also have medicinal values, and some are consumed by humans as spices (Okonkwo and Okoye, 1996). There is a growing interest in entomological research to identify and evaluate plant species with insecticide properties for control of various insect pests, including *S. zeamais* (Hassan et al., 1990; Owusu, 2001; Akob and Ewete, 2007; Odeyemi et al., 2008).

Plant insecticides are often alternatives effective as organophosphates or other neurotoxins for pest control due to multiple modes of action. It included toxicity, anti-feedant and anti-oviposition effects (Sutherland et al., 2002). Natural products contain secondary plant compounds such as terpenes (monoterpenes, sesquiterpenes and triterpenes), steroid, alkaloids, phenol and cardiac glycosides (Duke, 1990; Baser et al., 1998).

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*Corresponding Author

Sahar A. A. Malik
Department of Biology, College of Science, University of Basra, Iraq

Email: saharmalik2010@gmail.com

Identifications of plant extracts exhibited the above described deleterious effects on pest insect physiology, behavior and represents a potential alternative strategy for development of directional controls that could replace synthetic neurotoxins (Duke, 1990).

Khalif and Al-Farhani (2008) studied the effects of leave's powders of *Nicotiana tabacum, Nerium oleander, Ziziphus spinachristi, Vitex agnus–castus, Lantana camara, Myrtus communis, Clerodendron inerum* and *Eucalyptus globules* on the mortality reduction in F1 progeny and repellents. Leaves powder on the red flour beetle showed that the leaves powders of N. *tabacum, C. inerum* and *Z. Christi* had the best effect on the mortality of adults after seven days (100%).

Nadi (2001) studied the toxicity of aqueous, methanol and acetone extracts of three plants *Rhazya stricta*, *Azadirachta indica*, and *Heliotropium bacciferum* on Khapra beetle (*Trogoderma granarium* Everts) larvae.

A number of plant species like *Azadirachta indica* A. Juss, *Melia azedarach* L., *Lantana camara* L., *Cannabis sativa* L., *Nerium indicum* Mill., *Eucalyptus* sp., *Ricinus communis* L. as well as *Solanum nigrum* L. are known to possess insecticidal properties, although only a few of these have been exploited commercially. The compounds from these plants have a number of useful activities like toxicity, repellence, feeding and oviposition deterrence and insect growth regulate activity (Mordue, 2004).

Recently some indigenous plants have been reported to possess repellent property against Khapra beetle. Dwivedi and Bajaj (2000) assessed of the *Cassia* leaf extract on the repellent activity on Khapra beetle, while Dwivedi and Sharma (2002) investigated repellents of five plant extracts against Khapra beetle.

Present trends in the world directed to the use plants that have insecticides properties. Some plant extracts are highly effective and safe for human beings and environment, convenience and inexpensive for protection of stored grains. The toxicity of plant extracts had been checked against a number of stored product insects (Hasan et al., 2006). We need in this time, to research programmers to determine the response of *Trogoderma granarium* to different doses of plant and synthetic insecticide.

Materials and Methods

Rearing of test insect

Mixed population of *Trogoderma granarium* (Everts) was collected from infected stored wheat for rearing in the laboratory. The insects were collected and kept in wide jars covered with muslin cloth. The insects were kept in the laboratory for two months for rearing. The adults of *T. granarium* were sieved and placed in breeding containers in the medium of uninfected wheat grains. The samples were placed in an incubator at 30± 2°C and 65± 5% relative humidity for three days (72 hours). Then, the insects were transferred from the jars to new containers.

The media (wheat grains) in which the adults of *T. granarium* were initially retained for 72 hours contained enough eggs laid by females. In these jars, the first larvae appeared after four days and the highest number of larvae appeared after seven days. Same age and size progeny were removed from these stocks. It was further kept for another period of five days before testing (Hasan et al., 2006).

Collection of plant materials

Samples were collected from plants included fruit of *Datura stramonium* L. and *Quercus infectoria* DL. As well as leaves of *Solanum nigrum* L. and *Xanthium strumarium* L. from different places Sulaimanya, Arbile and Basrah in 2008-2009 (Table 1). All the identified plant materials were dried by using air for 6-7 days, while others were dried by using the oven at a temperature of 30°C for a period of 24 hours. The dried materials were macerated and powdered in blender machine type (Moulinex, 241, France).

Table 1. Plants and insecticide used against *Trogoderma granarium.*

Family name	Plants	Tissue used
Solanaceae	*Datura stramonium* L.	Fruit
Fagaceae	*Quercus infectoria* DL.	Leaves
Solanaceae	*Solanum nigrum* L.	Leaves
Compositae	*Xanthium strumarium* L.	Fruit
Cypermethrin	-	-

Preparation of ethanol plant extracts and cypermethrin solutions

Extracts of leaves and fruits were prepared by mixing 50g from sample in 500 mL of ethanol in a flask. The flask was closed with a piece of cotton and aluminum foils and placed in rotary shaker at 30°C for 24 hours or soxhlet for extraction. The extract was filtered to obtain the stock solution. From the stock solution, 1, 0.5, 2 and 4% concentrations were prepared.

10% master stock solution of cypermethrin 25% EC was prepared by dissolving technical grade of cypermethrin in primary non-volatile solvents (olive oil). Further concentrations such as 0.5, 1, 2 and 4% were prepared from 10% stock solution for non-volatile solvents.

Seventy two Petri dishes were taken, and filter papers were cut according to the size of Petri dishes for both test solutions. Plant extract and cypermethrin were spread uniformly on the filter papers (Whatman No. 1.7 cm diameter). Then, 0.5 millimetres of the extract and cypermethrin were placed on the filter paper by the syringe. Insect mortality was recorded after seven days of treatment. Three replicates were calculated for each concentration.

Abbott's formula (Abbott, 1925) was used to correct the mortality. Corrected mortality (PT) was calculated using the following formula:

$$PT = [(Po - Pc) / (100 - Pc)] \times 100$$

Where Po = observed mortality and Pc = controlled mortality.

Toxicity on F1 progeny

The effects of treated wheat grains on the F1 progeny of *Trogoderma granarium* were studied using cypermethrin and ethanol plant extracts of the four plant species diluted with ethanol (95%) to five different concentrations, 0 (control), 0.5, 1, 2, and 4%. Twenty-five grams of wheat grains were placed in five jars and treated with 0.5 mL to each of the five different concentrations of plant extracts and cypermethrin. The jars were well shaken manually for optimum coverage of grain surface. Five pairs (five male and five female) of Khapra beetle were put together into each jar, and then covered with mesh. Replicates of the treatments (four plant species extract as well as cypermethrin and four concentrations) and then the samples were analyzed in a randomized complete block (RCB) design.

After seven days all adult of Khapra beetles were removed, and the jars with grains were left undisturbed for 30 days. This was observed daily and F1 progeny emergence were recorded.

Repellent effect

The repellent effect of the cypermethrin and four ethanol plant extracts against *Trogoderma granarium* were evaluated using the method as described by McDonald et al. (1970). The treated and untreated grains were placed adjacent to each other in the Petri dish (11 cm diameter). Ten adult *T. granarium* of three – five days old were introduced into the middle of the Petri dishes. Each treatment was replicated four times and the number of insects present in control (Nc) and treated (Nt) grains were recorded after one hour and up to six hours. A percent repellent (PR) was computed as:

$$PR = [(Nc - Nt)/ (Nc + Nt)] \times 100$$

And the data were analyzed using the ANOVA test after converted into arcsine values. All negative PR values were treated as zero (Obeng-Ofori et al., 1997).

The five concentrations of the extracts and the five replications were arranged in a completely randomized design (CRD). The numbers of insects present on the control (untreated) and treated were recorded after 30 minute exposure.

Statistical analysis

Statistical analysis of variance was carried out with the Statistical Package for Social Science (SPSS), using a factorial completely randomized design (CRD). Treatment means showing significant difference ($P<0.01$) were separated by using Least Significant Difference.

Results

Toxicity of cypermethrin and ethanol plant extracts on adult insects

Ethanol extracts of fruits of *Datura stramonium* and *Xanthium strumarium*; as well as leaves of *Quercus infectoria, Solanum nigrum* and cypermethrin, using various concentrations had significant ($P<0.01$) contact toxicity on *Trogoderma granarium*.

The data in a table (2) showed mean percent mortality of *T. granarium* after eight days under the various treatments of the ethanol plant extract and cypermethrin. Cypermethrin gave maximum mortality 100% in all concentrations. In the concentration of 2% and 4% of *Datura stramonium, Solanum nigrum* extracts gave maximum mortality 100%, while 0.5% concentration yielded the minimum mortality.

Table 2. Toxicity of cypermethrin and ethanol plant extracts on *Trogoderma granarium* after eight day of treatment.

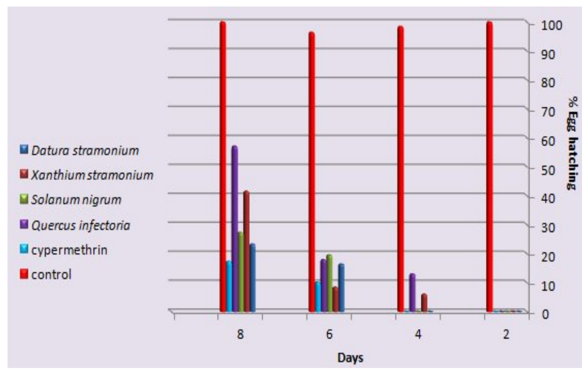
Plant extracts	Concentration %				Mean extract plant
	0.5%	1%	2%	4%	
Datura stramonium	11.34	65.34	100	100	69.17
Quercus injector	10.43	33.64	46.43	46.43	34.23
Solanum nigrum	13.23	52.08	100	100	66.32
Xanthium strumarium	15.67	55.78	80.91	85.87	59.55
cypermethrin	100	100	100	100	100
Control	0	0	0	0	
Mean concentration	12.13	61.36	85.46	86.46	
L.S.D. (0.01) of plant extract	9.3				
L.S.D. (0.01) Interaction between plant extract and concentration	15.3				

Toxicity on egg hatching

Ethanol extracts of all the selected plants, delayed the hatching of eggs of *Trogoderma granarium* (Figure 1). The effect of different concentration levels and time exposure on hatching of eggs varied with material and exposure time, we also noticed that the insects did not lay the eggs in 2% and 4% concentration, and that the female insect did not lay the eggs until the fourth day after the treatment in 1% concentration. In addition, hatching started just after four days in *Quercus infectoria* and *Xanthium strumarium*, as for the other treatments, no hatching was observed until six days. Inhibition in egg hatching was significantly higher in treatments than control. The percentage of hatching eggs were 17.34% and 23.31% in cypermethrin and *Datura stramonium* respectively, while the percentages of hatching in the control was 100%.

Toxicity on larvae

The results showed that cypermethrin larval mortality reached 100% after eight days of exposure in all concentrations (Table 3). Ethanol plant extracts of *Datura stramonium* showed 100% larval mortality in 1%, 2% and 4% concentration, while *Quercus infectoria* and *Xanthium strumarium* extracts showed 76.54% and 92.43% mortality of larvae at a concentration in 4% after eight days of exposure.



L.S.D. of plant extracts = 0.9.

L.S.D. of time = 4.5

Figure 1. Toxicity of cypermethrin and plant extracts on *Trogoderma granarium* in 1% concentration.

Table 3. Toxicity four plants extract and cypermethrin on larvae *Trogoderma granarium* after eight-day treatment.

Plant extracted	Concentration %				Mean extract plant
	0.5	1	2	4	
Datura stramonium	80.43	100	100	100	95.10
Quercus infectoria	30.76	43.86	66.31	76.54	54.36
Solanum nigrum	81.45	97.43	100	100	94.72
Xanthium strumarium	15.32	60.23	85.24	92.43	63.30
cypermethrin	100	100	100	100	100
Mean concentration	61.59	80.30	90.31	93.79	
L.S.D. of plant extract	1.2				
L.S.D. of concentration	15.08				
L.S.D. interaction between plant extract and concentration	23.11				

Toxicity on F1 progeny production

Pesticide and plant extracts applied to wheat grains using various concentrations were significant (*P*<0. 01) and the toxic influence against F1 progeny of *Trogoderma granarium* infesting wheat was shown in (Table 4). Adult emergence from the control was significantly higher than the rest of the other treatments at all concentrations irrespective of plant extracts. However, the lower emergence was obtained from grains treated with *Datura stramonium*, *Solanum nigrum* and cypermethrin by using 4% concentration, after eight days of treatment was 8.34, 9.34 and 5.43 respectively. The data analysis showed higher significant differences in 4% concentration and gave 93.79% mortality.

Table 4. Effect plant extracts and cypermethrin on F1 progeny of *Trogoderma granarium* after eight day treatments.

Species / Concentration	*Datura stramonium*	*Quercus infectoria*	*Solanum nigrum*	*Xanthium strumarium*	cypermethrin	Mean of concentration
zero	92.54	90.23	93.11	91.45	90.55	
0.5	34.75	28.60	33.23	25.21	21.89	28.73
1	17.36	25.90	19.50	24.45	20.65	21.57
2	11.56	20.56	16.87	20.45	15.29	16.94
4	8.34	12.42	9.43	14.67	5.43	11.05
Mean concentration	18.11	21.12	20.50	21.19	17.06	
L.S.D. (0.01) plant extract	0.82					
L.S.D. (0.01) concentration	18.22					

Repellents of cypermethrin and ethanol plant extracts against *Trogoderma granarium*

Pesticide and ethanol plant extracts repellent results have been tabulated in Table 5. It had been shown that the ethanol extract of *Datura stramonium* exhibited maximum percent repellents (91.87%) in 4% concentration, while the lowest percent repellence (77.65%) was recorded in *Quercus infectoria*, and the cypermethrin gave 93.76% in the same concentration. Similar results were found by Mohiuddin et al. (1987) who observed that the repellents rate was 75% at the treatment of *Xanthium strumarium* against *Trogoderma castaneum*.

Table 5. Percentage repellents of cypermethrin and ethanol plant extracts against *Trogoderma granarium.*

Species / Concentration	*Datura stramonium*	*Quercus infectoria*	*Solanum nigrum*	*Xanthium strumarium*	cypermethrin	control	Mean concentration
0.5	50.45	44.11	69.34	51.98	70.65	0	57.30
1	77.87	54.34	78.56	60.43	83.76	0	70.99
2	85.39	65.21	88.45	80.54	90.54	0	82.02
4	91.87	77.65	91.45	86.21	93.76	0	88.18
Mean concentration	76.39	60.32	81.95	69.79	84.67		
L.S.D. (0.01) plant extract	11.24						
L.S.D. (0.01) concentration	3.5						

Discussion

Ethanol plant extracts showed that the mortality rate increased with increasing concentration as well as the length of exposure times. The stored grains with high insect mortality could be attributed to the presence of toxic secondary metabolites. It has been reported that some secondary metabolites may act as insecticides or anti-feedants against *Trogoderma castaneum* (Nawrot et al., 1989). *Datura stramonium* contains alkaloid compounds such as atropine, scopolamine in the leaves, stems and fruits in addition to essential oils, while *Solanum nigrum* contains solanine and solasodine (Chakravarty, 1976) which may be the direct reason of killing the insects. Furthermore, similar studies indicated that the effectiveness of insecticides activity of *Acacia nilotica* extracts in controlling pests of *Trogoderma granarium, T. castaneum, Callosobruchus maculatus* and *Sitophilus zeamaiz* (Chairat et al., 2002). The present results showed that pesticide cypermethrin gave maximum mortality than plant extracts and the result supports the findings of Hasan et al. (2006) who reported that pesticide deltamethrin was more toxic to kill insects (25%) than the extract of the plant *Haloxylon recurvum* (17%) mortality. The activity of crude plant extracts on insects is composed of both toxic and anti-feedant effects (Koul, 1993; Akhtar and Isman, 2004). In some studies it has been found that the compound Azadirachtin, isolated from the neem tree (*Azadirachta indica*), is very important both as toxicant and anti-feedant and has been one of the most widely, tested and successfully implemented plant insecticides over the past two decades (Schmutterer, 1995). Moyin-Jesu (2010) report that the neem leaf extracts decrease the insect population, number of damaged leaves and number of holes per plant in maize plant.

Extract of *Datura stramonium* produced larvae mortality rates after one day compared with other plants extract *Quercus infectoria*, *Xanthium strumarium* and *Solanum nigrum*. Some of the larvae did not die after two days of exposure, but instead they died at the pupal or at the adult stage, this is due to the chronic effects of chemical compounds isolated from plant extract (Sukumar et al., 1991).

Conclusion

In general, it could be concluded that, plant extracts used in the present study act as larvicidal, adulticidal, and possess growth and emergence inhibiting, repellent activities against the *Trogoderma granarium*. Furthermore, this study suggested that ethanol extracts of plants belonging to families taxonomically unrelated possess toxic effects with significant insecticidal effect and could be a potential tool to protect stored grains against *T. granarium*, and this may contribute to a reduction in the application of synthetic insecticides, which in turn increases the opportunity for natural control of various medically important pests by plant pesticides. It is often active in specific target insects, less expensive, easily biodegradable to nontoxic products and potentially suitable for use in a *Trogoderma granarium* control program (Bream et al., 2010).

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