TOOTH-PICK SEED (*AMMI VISNAGA* L.) EXTRACTS AS GRAIN PROTECTANTS AGAINST THE GRANARY WEEVIL (*SITOPHILUS GRANARIUS* L.)

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Abstract

Treating wheat grains treated with chloroform, Acetone and petroleum ether extracts of tooth-pick seed extracts against *Sitophilus granarius* L. was determined and LC_{50} and LC_{95} levels were estimated .

Results showed that *S. granarius* adults was more sensitive to chloroform extract than other extracts. Chloroform, acetone and pet-ether extracts at 10000 ppm concentration which gave 96, 90 and 92% kill respectively after 3 days from treatment. All extracts at LC_{50} and LC_{95} gave complete inhibition in F1 progeny up to 60 days from treatment.

When using of LC_{50} and LC_{95} of chloroform and LC_{95} of pet-ether extracts gave complete inhibition eggs laid, also LC_{95} for pet-ether extracts, while LC_{50} for pet-ether and LC_{50} and LC_{95} for acetone extracts gave significantly reduced in numbers of deposited reaching to eggs 0.83, 2.33 and 0.33 to both extracts, respectively.

LC_{95} for all extracts lead to complete protection of wheat grains up to 60 days from treatment, and the same concentrations had a detrimental significant reduction on germination of wheat grains after 105 days from treatment compared with non-treated seeds.

INTRODUCTION

Insect infestation of stored grains and their products is a serious problem throughout the world. Chemical control of these insects created several problems to the environment, human health and non-target organisms. There is no doubt that the wide spread of indiscriminate pesticide applications have sometimes caused a contamination of the environment and some disastrous ecological damage. Moreover, heavy use of conventional pesticides enhances the potential for the development of pesticides resistance. Also, residues of pesticides in stored products could cause a health hazards to ultimate consumers.

So, using of plant products as oils, dusts and extracts are a new trend preserves the environment from contamination with harmful toxicants; Jaipal *et al.*, 1984; Su, 1985; Mahgoub and Ahmed, 1996; El-Lakwah *et al.*, 1998; Abd El-Latif, 1999; Ahmed, 2001; El-Lakwah and Abd El-Latif, 1999.

The present work is mainly concerned with the bioactivity of tooth-pick seeds extracts to protect wheat grains against the granary weevil, *Sitophilus granarius* L.

MATERIALS AND METHODS

Test insect: *Sitophilus granarius* L. adults were taken from a laboratory culture reared in Giza 172 wheat variety at 26 ± 2°C and 60 ±5% R.H. Adults of 1 – 2 weeks old were used to carry out the present work.

Preparation of extracts: 200 gm of dried tooth-pick seeds were ground by a high speed micromill. The powder was successively extracted with 400 ml petroleum ether (40 – 60) in a glass flask and left for 48 hr. as described by Su (1985). The extract was filtered and the solvent was evaporated at 50°C under reduced pressure by using a rotary evaporator. The defatted powder was thoroughly dried before extraction with chloroform and again dried before extraction with acetone.

Evaluation of extract toxicity: primary experiments were carried out to determined different concentrations used to clarify their toxicity. Toxicity of each extract was determined by adding one ml of each crude extract to 10 ml solvent (chloroform, acetone and pet-ether). Different dilutions were made for each extract using the appropriate solvent of extraction. One ml of different dilutions of each extract was mixed thoroughly with 10 grams wheat grains in glass vials, and left for two hours to evaporate the solvent .

A group of 25 adults of the tested insects was kindly introduced into the vials then covered with muslin fixed with a rubber band.

Each concentration was represented with three vials (replicates) and all vials were kept at 26 ± 2°C and 60 ± 5% R.H. Similar three vials contained untreated grains were used as a check and also kept under the same conditions. Mortality counts were recorded after 1, 3, 7 and 14 days and percentages of corrected mortality were calculated by Abbott's formula (1925). These three days after treatment percentages

were statistically computed according to Finney (1971) to produce LC_{50} and LC_{95} and toxicity slope for each extract.

Assessment of residual efficiency: Firstly, on the adults: a group of fourty eight glass vials, each containing 10 gm wheat grains treated with LC_{95} concentration of the petroleum ether extract was kept under insactry conditions . Each week three vials were taken and 25 *S. granarius* adults were introduced into each. The same technique was done for both chloroform and acetone extracts. Another 42 vials contained wheat grains, only treated with the solvent were death by the same way. Mortality counts were recorded after 72 hr. of introducing the adults. Secondly, on the egg: six vials (10 gm wheat in each) were used for each extract, three for LC_{50} concentration and three for LC_{95} one. Also, another three vials with untreated grains were used as a control. Each vial received 20 adults then covered with fixed muslin. Two weeks later, the insects were removed to count all deposited eggs in each vial according to the methods of Franken feld (1948) and Howe (1952).

Effect of extracts on number of progeny:

Two insect groups each twenty adults of *S. granarius* were used for each extracts. The first group was placed into a vial contained 5 gm. wheat treated with LC_{50} concentration while the second was for LC_{95}.

Each treatment was represented with three vials (replicates) in addition to another three vials had untreated grains as a control. All vials were kept in the incubator under the same previous conditions. Two weeks later, the insects were removed and the vials were returned to the incubator. After 60 days, the total number of emerged F1 offspring was counted.

Seed germination :

Wheat grains were treated with extracts at LC_{95} level. Germination's % were tested after 4 hrs. and 105 days from treatment with extracts, 25 grains placed in petri dishes, four dishes (9cm) for each extracts. Germination were recorded 7 days later, four replicates of untreated wheat grains were used as control for comparison .

Statistical analysis:

Differences between means of the different treatments were statistically analyzed using ANOVA and Duncan tests. .

Table 1. Corrected mortality percentages of *S. granarius* adults resulted from treatment with tooth-pick seed extracts at 26 ± 2°C and 60 ± 5% R.H.

Extracts	Concentration (ppm)	Cumulative mortality after				Mean
		1	3	7	14 days	
Chloroform	6000	0.0 d	40.0d	80.0b	100.0a	55.0
	7000	22.0 c	51.0cd	98.0A	100.0a	67.8
	8000	34.0 b	61.0c	100.0a	100.0a	73.8
	9000	41.0 a	77.5b	100.0a	100.0a	79.6
	10000	46.0 a	96.0a	100.0a	100.0a	85.5
LSD 5%	--	5.71	13.81	9.20	2.91	--
Acetone	6000	0.0c	8.0e	83.0b	96.0b	46.8
	7000	0.0c	22.0d	88.0b	100.0a	52.5
	8000	24.0b	36.0c	92.0b	100.0a	63.0
	9000	40.0a	76.0b	92.0b	100.0a	77.0
	10000	40.0a	90.0a	100.0a	100.0a	82.5
LSD 5%	--	7.49	6.13	6.07	0.57	--
Petroleum ether	6000	0.0d	21.0d	74.0c	86.0c	45.3
	7000	8.0cd	36.0c	75.33c	100.0a	54.8
	8000	18.0c	42.0c	84.0b	100.0a	61.0
	9000	40.0b	64.0b	100.0a	100.0a	76.0
	10000	78.0a	92.0a	100.0a	100.0a	92.5
LSD 5%	--	10.79	11.55	6.0	4.91	--

-Means within a column followed by the same letter are not significantly different at 5% level .

Table 2. LC_{50}, LC_{95} (ppm) and slope of tooth-pick seed extracts against *S. granarius* adults at 26 ± 2°C and 60 ± 5% R.H. after three days of treatment.

Extracts	LC_{50}	LC_{95}	Slope
Chloroform	6970	10880	8.02
Acetone	8210	10770	13.92
Petroleum ether	8780	11780	9.10

Table 3. Effect of LC_{50} and LC_{95} of the extracts on some biological aspects of *S. granarius* adults at 26 ± 2°C and 60 ± 5% R.H.

Extracts	Concentration	Eggs per 5 females	Reduction (%)	No of emerged adults	Reduction (%)
Chloroform	LC_{50}	0.0b	100.0	0.0b	100.0
	LC_{95}	0.0b	100.0	0.0b	100.0
	Control	28.0a	--	10.0a	--
LSD 5%	--	3.46	--	2.62	--
Acetone	LC_{50}	2.33b	88.72	0.0b	100.0
	LC_{95}	0.33b	98.40	0.0b	100.0
	Control	20.67a	--	10.67a	--
LSD 5%	--	2.20	--	3.29	--
Petroleum ether	LC_{50}	0.83b	97.40	0.0b	100.0
	LC_{95}	0.0b	100.0	0.0b	100.0
	Control	32.0a	--	9.0a	--
LSD 5%	--	9.03	--	6.26	--

-Means within a column followed by the same letter are not significantly different at 5% level .

Table 4. Corrected mortality percentages of *S. granaries* adults after 3 days of exposure to wheat grains treated with LC_{95} of extracts and kept for different periods under insectary conditions.

Period (days)	% Mortality of exposed insects		
	Chloroform	Acetone	Petroleum ether
4(hrs)	96.0a	96.0a	95.0a
15	95.0a	96.0a	95.0a
30	94.0a	95.0a	96.0a
45	95.0a	95.0a	95.0a
60	95.0a	95.0a	95.0a
75	94.0a	81.0b	95.0a
90	80.0b	60.0c	90.0b
105	52.0c	30.0d	82.0c
LSD 5%	4.23	3.89	3.61

-Means within a column followed by the same letter are not significantly different at 5% level .

Table 5. Effect of the LC_{95} extracts on the percent of wheat grains germination after different periods of storage under insectary conditions.

Extracts	LC_{50} (ppm)	% Germination after:	
		4 hrs.	105 days
Chloroform	10880	89.33a	56.0c
Acetone	10770	78.33b	57.0bc
Petroleum ether	11780	92.0a	64.0b
Control	--	96.0a	92.0a
LSD 5%	--	9.83	7.80

-Means within a column followed by the same letter are not significantly different at 5% level .

RESULTS AND DISCUSSION

The present work studied effect of three different extracts of tooth-pick seeds on *S. granaries* adults as follows:

Effect on adults:

Data shown in Table 1 proved that mortality values increased with increase of the concentration and time of exposure. After three days from treatment, mortality ranged from 40 – 96, 8 – 90 and 21 – 92% for chloroform, acetone and petroleum ether extracts, respectively. Increased to 80 – 100, 83 – 100 and 74 – 100% after 7 days post-treatment at the various concentrations for all extracts respectively. After 14 days the mortality percentages reached 100, 96 – 100 and 86 – 100 for the same extracts, respectively.

As shown in Table 2 , the LC_{50} values tooth-pick seed extracts, after 3 days post-treatment, was 6970 , 8210 and 8780 (ppm), while the LC_{95} values was 10880, 10770 and 11780 (ppm) for the chloroform, acetone and petroleum ether extracts, respectively. This result showed that *S. granaries* adults are more sensitive to chloroform extracts than acetone and petroleum ether extracts.

Effect on number of deposited eggs and F1-progeny: Table 3 indicated that, the treatment of chloroform extract at LC_{50} and LC_{95} inhibited the oviposition, on the other hand the LC_{50} and LC_{95} for acetone and chloroform extract significantly reduced the rate of oviposition compared with untreated grains (control). Mean number. of deposited eggs per 5 females were in range from (0.33-2.33) and (0.83 – 0.0) for LC_{50} and LC_{95} for acetone and chloroform extracts , respectively. Also, the numbers of emerged adults were observed and the reduction in F1-progeny reached 100% for all treatments compared with untreated grains.

The obtained results are in complete harmony with that obtained by (Helen, 1989) and (El-Lakwah and Mohamed, 1998)

Residual effect: Table 4 indicated that chloroform, acetone and pet. ether extracts at the concentration of LC_{95} gave 95% kill against *S. granaries* adults up to 60 days and decreased highly till 105 days (52, 30 and 82% kill) for chloroform, acetone and pet. ether extracts, respectively.

Effect of LC_{95} on wheat grains germination: Table 5 showed that the effect of

extracts tested on the germination of wheat grains. This effect was much more obvious in case of the higher concentration (LC_{95}) and the longer period of storage 105 days). The germination percentages were 56, 57 and 64% for chloroform, acetone and pet. ether extracts, respectively after 105 days from treatment compared with untreated grains it was 92% germination at the same storage period. This results harmony with that obtained by (S Ahmed, 2001).

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تقييم فاعلية مستخلصات بذور الخلة البلدى كمواد واقية لحبوب القمح ضد حشرة سوسة القمح

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اجريت دراسات معملية لتقييم فاعلية مستخلصات الكلوروفورم ، الإسيتون والإثـير البترولى لبذور الخلة البلدى فى وقاية حبوب القمح ضد حشرة سوسة القمح وذلك عن طريق المعاملة السطحية للحبوب.

أوضـحت النتائج ان سوسة القمح اكثر حساسية لمستخلص الكلوروفورم بالمقارنة بمستخلص الإسـيتون والأثـير البترولـي. حيث انه عند استعمال تركيز ١٠٠٠٠ جزء في المليون من مسـتخلص الكلوروفـورم والإسـيتون والأثير اعطت نسب موت مقدارها ٩٦ ،٩٠ ،٩٢% لمستخلصـات علـي التوالي بعد ٣ يوم من المعاملة ولقد ادى استعمال المستخلصات الثلاثة بتركـيزات LC_{50}،LC_{95} الـى تثبيط كامل للخلفة الناتجة بعد ٦٠ يوما من معاملة الحبوب. بيـنما ادى اسـتعمال تركز الـ LC_{50}، LC_{95} لمستخلص الكلوروفورم وكذلك تركيز LC_{95} لمسـتخلص الأثـير البـترولى الى تثبيط كامل لوضع البيض. بينما اعطت تركيزات LC_{50} لمستخلص الأثير البترولي وتركيزات LC_{50}،LC_{95} لمستخلص الإسيتون انخفاض معنوى في متوسط عدد البيض الناتج فكان ٠,٨٣،٢,٣٣،٠,٣٣لكلا المستخلصين علي التوالي.

وعـند استعمال تركيز LC_{95} للمستخلصات الثلاثة كانت هناك وقاية كاملة للحبوب من سوسة القمـح حـتى ٦٠ يوما ، بينما ادى هذا التركيز الى انخفاض معنوى في نسبة إنبات الحبوب بعد ١٠٥ يوم من المعاملة مقارنا بالحبوب الغير معاملة.