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LUPIN SEED (*LUPINUS TERMIS*) EXTRACTS AS GRAIN PROTECTANTS AGAINST THE RICE WEEVIL (*SITOPHILUS ORYZAE* L.) AND THE LESSER GRAIN BORER [*RHIZOPERTHA DOMINICA* (F.)]

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Abstract

The effectiveness of treating wheat grains treated with petroleum–ether or chloroform of lupin seed extracts against *S. oryzae* and *R dominica* was determined and Lc_{25} ,Lc_{50} and Lc_{95}'s levels were estimated .

Results showed that *S. oryzae* adults were more sensitive to both extracts than *R. dominica*. Treatments with both tested extracts caused severe reduction in number of eggs deposited and in the percentage of progeny, especially at Lc_{95}'s level. At this level no offspring was produced by the adults and complete protection of wheat grains was obtained against *S. oryzae* and *R. dominica* up to 90 days .

Lupin extracts had a detrimental effects on germination of wheat grains especially at the high concentrations.

Tested extracts had a different effects on both cholinestrase and peroxidase enzymes level in *S. oryzae* and *R. dominica*.

INTRODUCTION

Wheat grains are subject to attack by several insects. The rice weevil *Sitophilus oryzae* and the lesser grain borer *Rhizopertha dominica* are the most common and cause a great loss.

Synthetic insecticides cause several problems, such as harmful residues in the chain of food, risk of hazards and pollution of environment, thus disruption of biological balance and destruction of the natural enemies of certain insect pest resistance.

The use of natural products from plant origin is a new trend that preserve the environment from contamination with harmful toxicants. Several studies suggested the use of plant dust and extracts (Petterson, 1975; Jaipal *et al.*, 1984; Su, 1985; Mahgoub and Ahmed, 1996) .

The present work is mainly concentrated with the bioactivity of lupin seeds ex-

racts with petroleum-ether and chloroform to protect wheat grains against the rice weevil, *Sitophilus oryzae* L. and the lesser grain borer, *Rhizopertha dominica* (F.).

MATERIALS AND METHODS

Test insects: *Sitophilus oryzae* L. and *Rithopertha dominica* (F.) adults provided from laboratory cultures reared at 26 ± °C and 65 ± 5 R.H were used. All cultures were reared in Egyptian wheat variety Giza 172. Experiments were carried out with adults having 1-2 weeks old.

Test extracts: 500 gm of lupin, dry seeds were cleaned thoroughly to remove any impurities, and were ground by a high speed micromill. The ground powder was successively extracted with petroleum ether (40-60) in flask and left for 48 hr. as described by Su (1985). The petroleum ether extracts was filtered. 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.

Evaluation of extracts toxicity: Toxicity of each extracts was determined by adding different concentrations to wheat grains. The appropriate amount of extracts to give a certain concentration was added to ten grams of wheat in a glass tube, kept at 26 ± 1°C and 65 ± 5 R.H.

Batches of 25 adults of the tested insects were introduced into the tubes. Each treatment consisted of three replicates. A similar treatment with untreated wheat was included. Tubes were covered with muslin fixed with rubber band.

Mortality counts were carried out after 3, 5, 7 and 14 days. Percentages of insects mortality were corrected for natural mortality by means of Abbott's formula (1925). The corrected mortality percentages were statistically computed according to Finny (1952). Computed mortality percentages after 72 hr. exposure were plotted versus the corresponding concentration of log-probability paper and the required concentration to produce 25% , 50% and 95% kill and slopes were determined and shown in table 2.

Assessment of residual efficiency: Tubes each having 10 gm of wheat grains treated with Lc_{95} concentration of each insect, were divided into several groups. Each group consists of three replicates for each storage period. The tubes were kept under laboratory condition.

Twenty five adults of *S.oryzae* or *R.dominica* were introduced into each tube

(3replicates) at 15 day intervals and up to 90 days. Mortality counts were carried out 72 hr. after introducing the insects .

Mortality percentages were corrected according to Abbott's formula (1925). Similar three replicates of untreated wheat were used as control.

Effect of tested extracts on number of eggs of *S.oryzae*: Ten grams of wheat grains treated with test extracts at two levels of Lc_{50} and Lc_{95} (i.e . 2.9 and 12.0 ml/kg in case of pet-ether, 2.0 and 7 ml/kg in case of chloroform), were placed in glass tubes (1 x3 inches).

Twenty adults 1-2 weeks old were placed in each tube and covered with muslin fixed with rubber band. Two weeks later, the insects were removed and the number of deposited eggs on the grains were determined according to the method described by Franken feld (1948) and Howe (1952). Three replicates were made for each concentration. In addation, three replicates of untreated grains were used as control.

Effects of tested extracts on number of progeny of tested insects: Twenty adults (1-2) weeks old of *S.oryzae* or *R.dominica* were placed in glass tubes with 10 grams of wheat grains treated with Lc_{50}'s and Lc_{95}'s for each tested extracts against *S. oryzae* and *R.dominica*.

Two weeks later, the insects were removed. The tubes were kept in the incubator at 26 ± 1ºC and 65 ± 5 R.H. After seven weeks, the total number of emerged F_1 offspring was counted. Three replicates were made for each concentrations and three control.

The effect of test extracts on grains germination: Germination tests were carried out according to the International rules for seed testing (Anonymous ,1966) to find out the effect of the two tested extracts on the germination of treated wheat grains at the period of 45 and 90 days. Concentrations used were 2.9, 12.0 and 19.0 ml /kg for pet.ether extract and 2.0, 7.0, 12.0 and 17.0 ml/kg for chloroform.

Determination of enzymatic activity

a. Determination of cholinesterase activity: Acetylcholinesterase activity in *S. oryzae* and *R. dominica* exposed to wheat grains treated with tested extracts, was determined colourimetrically according to the method adopted by Ellman *et al.*, (1961) as following.

b. Determination of peroxidase Activity: The peroxidase activity in *S.oryzae* and *R . dominica* under examination was determined according to the method of Ogawa and Vritani (1970).

RESULTS AND DISCUSSION

Effect of lupin seed extracts against adults of *S.oryzae* and *R. dominica.*

Data shown in table 1 indicate that pet-ether at all tested concentrations gave complete mortality to *S.oryzae* adults within 14 days. The extracts at the rate of 8 ml/ kg gave 100% kill to the insects after 5 days exposure, while with *R. dominica* the same mortality percentage was achieved only at the concentration of 16 ml/kg after 14 days exposure.

In the case of chloroform extract, only the concentration of 5ml/kg gave complete mortality to *S.oryzae* adults after 5 days exposure. All other concentrations gave 100% mortality after 14 days. Meanwhile, *R. dominica* complete mortality was achieved only at the concentration of 16 ml/kg after 7 days exposure .

Lc_{50} values shown in table 2 indicate that *R.dominica* adults were much more tolerant than *S.oryzae* adults to the effect of both extracts, since these values were 12 and 2.9 ml/kg for pet. ether extracts compared with 12 and 2 for chloroform extract against the two insects respectively.

On the other hand, in this study chloroform extract was more effective on the two tested insects than pet. ether. These results agreed with Afifi *et al.*, (1989), they proved that chloroform extract of lupin was more effective than pet.ether extract on *S.oryzae* and *R.dominica.*

Values of slopes showed that the rate of effectiveness for chloroform was lower than the pet-ether.

Effect of tested extracts on some biological aspects

1. Effect of tested extracts on the number of eggs laid by *S.oryzae*

Data shown in table 3 indicate that pet.ether and chloroform extracts affected strongly the number of eggs laid by *S.oryzae* females. Pet.ether extract at Lc_{25}'s and Lc_{50}'s and Lc_{95}'s levels was more effective than chloroform. The mean number of eggs

were 90, 44.7 and 3.3, respectively. Meanwhile, the corresponding numbers with chloroform extract were 101, 61 and 13 eggs /10 female, respectively.

2. Effect to tested extracts on F progeny of *S.oryzae* and *R. dominica*

Concerning *S.oryzae*, Table 3, the mean number of progeny emerged at Lc_{25}'s and Lc_{50}'s were 18.0 and 4.7 for pet. ether treatments compared with 24.0 and 12.0 for chloroform treatments. At Lc_{95}'s level within both extracts, no emergence of adults was observed.

Data in Table 4 showed that the numbers of F_1 progeny for *R.dominica* within petroleum ether extract treatments were much more than that of chloroform extract treatments, since the mean numbers were 5.5 and 2.3 for pet.ether compared with 19 and 11.7 in case of chloroform at the level of Lc_{25} and Lc_{50}, respectively. At Lc_{95} level within all treatments no emergence was observed. For the control, the mean number was 65.0.

Residual efficiency of lupin seeds extracts

Table 5 indicated that pet.ether and chloroform extracts at the concentration of Lc_{95} gave 95% kill against *S.oryzae* and *R.domincia* up to 60 days and decreased slightly till 90 days (69 and 90% kill) for pet.ether and (71 and 85% kill) for chloroform, respectively.

Results in Table 6 indicated that *S.oryzae* and *R.dominica* adults exposed to wheat grains treated with pet.ether and chloroform extracts at Lc_{95} level and stored up to 90 days gave no emergence.

Effect of lupin seed extracts on germination of wheat grains

Table 7 indicated that the extracts tested had detrimental effect on the germination of wheat grains. This effect was much more obvious in case of the higher concentration (Lc_{95}'s) and the longer period of storage (90 days).

Enzyme activity in *S. oryzae* and *R. dominica* exposed to sublethal concentration of tested extracts

A. Acetyl cholinesterase activity: The acetyl cholinesterase (AchE) activity in *S.oryzae* and *R. dominica* exposed to (Lc_{50}) of *lupin termis* extracts for different exposure times shown in tables 8 and 9 showed that chloroform extract affected the ace-

tyl cholinesterase activity of both *S. oryzae* and *R. dominica*. The inhibitory effect after 12 hrs. of exposure was 50% and 42.5 % for *S.oryzae* and *R.dominica,* respectively. The effect declined gradually and reached 21.62% and 6.52%, respectively at the end of the experimental period (48 hrs).

On the other hand, pet. ether extract showed a different pattern, where an activation appeared first after 12 hrs. of exposure especially for *S.oryzae* (17.65%), then a gradual recovery appeared thereafter with the increase in the period of exposure.

It could be concluded that pet. ether extract had no effect on the cholinesterase activity of both *S.oryzae* and *R.dominca* after 48 hours of exposure.

B. Peroxidase activity: Table 10 showed that peroxidase activity in *S.oryzae* was not affected by tested extracts (Lc_{50}) especially after 48 hrs. of exposure. Chloroform extract showed an activation till 24 hrs. of exposure (42.86%), but a decline happened later on.

Pet.ether extract showed an activation in the peroxidase activity of *R.dominca* who reached 33.33% after 12 hrs. of exposure, then an inhibition appeared and become more pronounced with the increase in the exposure period and reached 40% and 41.67% after 36 and 48 hrs, respectively.

Chloroform extract affected highly the peroxidase activcity of *R. dominica* being 233.33 after 12 hrs. of exposure, then declined later on to reach the normal activity of untreated insects after 48 hrs. of exposure.

It could be concluded that pet.ether extract did not affect the peroxidase activity of *S.oryzae* and caused a slight inhibition in case of *R.domincia*.

Chloroform extract did not affect the peroxidase of *S.oryzae*, but affected highly the enzyme of *R.dominica* which revealed to normal later on.

Table 1. Effect of lupin seed extracts against adults of *Sitophilus oryzae* L. and *Rhizopertha dominica* F.

Extracts	Concentrations ml/kg	*Sitophilus oryzae*				Concentrations ml/kg	*Rhizopertha dominica*			
		% mortality after indicated days					% mortality after indicated days			
		3	5	7	14		3	5	7	14
Petroleum ether	1	20±1.0	61±3.0	77±3.5	100	10	20±2.0	44±3.5	56±5.3	80±2.0
	2	27±2.0	59±3.0	84±2.0	100	12	33±4.2	62±2.6	72±4.0	88±3.0
	4	53±2.6	80±2.6	93±2.7	100	14	65±5.0	72±5.0	85±5.0	92±2.0
	5	77±2.0	97±1.7	97±4.4	100	16	89±2.8	93±3.5	93±3.4	100±0.0
	8	91±3.0	100±0.0	100±0.0	100					
Chloroform	1	24±2.0	49±4.6	87±1.7	100	9	21±1.7	39±2.0	61±1.0	92±2.0
	2	27±3.0	73±3.0	88±3.0	100	12	24±1.7	60±1.0	77±3.5	96±3.4
	4	59±3.6	89±1.7	97±3.5	100	14	63±3.5	72±2.6	85±4.3	96±2.6
	5	96±3.0	100±0.0	100±0.0	100	16	94±1.4	96±3.5	100±0.0	100±0.0
	Control	-	0.3±0.1	0.7±2.0	1.0±3.0		-	-	-	1.0±0.0

Table 2. Lc_{25}, Lc_{50} and Lc_{95} values and slopes of regression lines for petroleum ether and chloroform extracts of lupin seed against *Sitophilus oryzae* (L.) and *Rhizopertha dominica* (F.) adults, 72 hrs. after treatment.

Extracts	Pet. Ether				Chloroform			
	Lc_{25} ml/kg	Lc_{50} ml/kg	Lc_{95} ml/kg	Slope	Lc_{25} ml/kg	Lc_{50} ml/kg	Lc_{95} ml/kg	Slope
Sitophilus oryzae	1.5	2.9	1.0	2.43	1.2	2.0	7.0	2.97
Rhizopertha dominica	10.4	12.0	19.0	9.00	10.1	12.0	17.0	10.60

Table 3. Some biological aspects of *S. oryzae* L. as afected by the tested extracts.

Concentration ml/kg	Pet.ether				Chloroform			
	mean no. of eggs/10 pairs	reduction %	mean no. of progeny emergency	reduction %	mean no. of eggs/10 pairs	reduction %	mean no. of progeny emergency	reduction %
Lc_{25}	90.0	45.5	18.0	51.35	101.0	43.58	24.0	52.29
Lc_{50}	44.7	72.9	4.7	87.3	61.0	65.92	12.0	76.48
Lc_{95}	3.3	98	-	100	13.0	92.27	-	100
Control	165		37		179.0		51	

Table 4. Effect of tested extracts on F_1 progeny of *R. dominica* (F.).

Concentration ml/kg	Pet.ether		Chloroform	
	Mean no. of F_1 progeny/10 pairs	Reduction %	Mean no. of F_1 progeny/10 pairs	Reduction %
Lc_{25}	5.5	91.78	19.0	70.77
Lc_{50}	2.3	96.53	11.7	82.00
Lc_{95}	-	100	-	100
Control	66.3		65.0	

Table 5. Mortality percentages of *S. oryzae* (L) adults and *R. dominica* (F) exposed to wheat grains treated with Lc_{95} of test extracts after different post treatment periods.

Period after Treatment Days	*S. oryzae* % mortality of exposed insects		*R. dominica* % mortality of exposed insects	
	Pet.-ether	Chloroform	Pet.-ether	Chloroform
Initial	96 ± 3.1	95 ±3.5	96 ± 0.0	95 ±4.6
15	95 ± 2.6	96 ± 1.0	95 ± 0.0	95 ±0.0
30	95 ± 1.0	95 ± 0.0	96 ± 4.6	95 ±5.3
45	96 ± 4.0	95 ± 1.0	95 ± 1.0	96 ±0.0
60	95 ± 2.0	95 ±2.0	95 ± 2.6	95 ±1.2
75	88 ± 5.3	83 ±5.3	95 ± 4.4	90 ±7.2
90	69 ± 2.6	71 ±3.0	90 ± 2.0	85 ±5.2

Table 6. Influence of test extracts treatment of wheat grains with Lc_{95} on adult emergence of *S. oryzae* and *R. dominica*.

Period After Treatment Days	*S. oryzae* Average no. of progeny				*R. dominica* Average no. of progeny			
	Pet.-ether		Chloroform		Pet.-ether		Chloroform	
	treated	con.	treated	con.	treated	con.	treated	con.
Initial	0.0	8.6	0.0	7.6	0.0	16.0	0.0	10.0
15	0.0	5.3	0.0	6.6	0.0	10.0	0.0	15.0
30	0.0	6.3	0.0	6.0	0.0	13.3	0.0	12.3
45	0.0	8.0	0.0	6.1	0.0	10.0	0.0	16.6
60	0.0	7.6	0.0	10.0	0.0	15.0	0.0	11.0
75	0.0	10.0	0.0	9.0	0.0	11.0	0.0	13.3
90	0.0	9.0	0.0	8.6	0.0	9.7	0.0	16.6

Table 7. Effect of lupin extracts on the germination of wheat grains stored for 45 and 90 days.

Tested Extracts	Conc. ml/kg	% germination of treated wheat stored for		
		Initial	45 (days)	90 (days)
Pet. Ether	2.9	88 ± 0.82	80 ± 1.41	77 ± 1.5
	12	59 ± 0.96	50 ±1.29	46 ± 1.0
	19.0	38 ± 1.91	33 ± 1.26	32 ± 1.41
Chloroform	2.0	90 ± 0.58	81 ± 0.96	78 ± 0.58
	7.0	53 ± 0.50	38 ± 1.29	38 ± 0.82
	12.0	42 ± 1.29	36 ± 1.41	32 ± 0.82
	17.0	39 ± 0.96	31 ± 0.96	27 ± 0.50
Control		94 ± 0.58	93 ± 0.96	94 ± 1.00

Table 8. Effect of *Lupinus termis* seeds extracts (Lc_{50}) on the acetylcholinesterase activity of *S. oryzae* and *R. dominica.*

Tested Insects	Extracts	Acetylcholinesterase			
		Time of exposure per hours			
		12	24	36	48
S. oryzae	Untreated	0.34×10^{-4}	0.40×10^{-4}	0.60×10^{-4}	0.37×10^{-4}
	Pet.-ether	0.40×10^{-4}	0.32×10^{-4}	0.49×10^{-4}	0.37×10^{-4}
	Chloroform	0.17×10^{-4}	0.26×10^{-4}	0.37×10^{-4}	0.29×10^{-4}
R. dominica	Untreated	0.80×10^{-4}	0.66×10^{-4}	0.43×10^{-4}	0.63×10^{-4}
	Pet.-ether	0.54×10^{-4}	0.69×10^{-4}	0.32×10^{-4}	0.63×10^{-4}
	Chloroform	0.46×10^{-4}	0.69×10^{-4}	0.43×10^{-4}	0.57×10^{-4}

Table 9. Effect of tested extracts on AchE activity of *S. oryzae* and *R. dominica* adults treated by median lethal concentration (Lc_{50}'s).

Tested Insects	Extracts	AchE inhibition % at the indicated post treatment period in hours			
		12	24	36	48
S. oryzae	Pet.-ether	-17.65	21.43	12.22	0.00
	Chloroform	50.00	35.00	38.33	21.62
R. dominica	Pet.-ether	32.50	-4.35	25.58	0.00
	Chloroform	42.50	-4.35	0.00	9.52

Table 10. Effect of *Lupinus termis* seeds extracts (Lc_{50}) on the peroxidase percentage of *S. oryzae* and *R. dominica* treated for different periods.

Tested Insects	Extracts	Acetylcholinesterase			
		Time of exposure per hours			
		12	24	36	48
S. oryzae	Untreated	100	100	100	100
	Pet.-ether	100	114.28	100	100
	Chloroform	106.25	142.86	80	100
R. dominica	Untreated	100	100	100	100
	Pet.-ether	133.33	100	60	58.33
	Chloroform	233.33	140	83.33	100

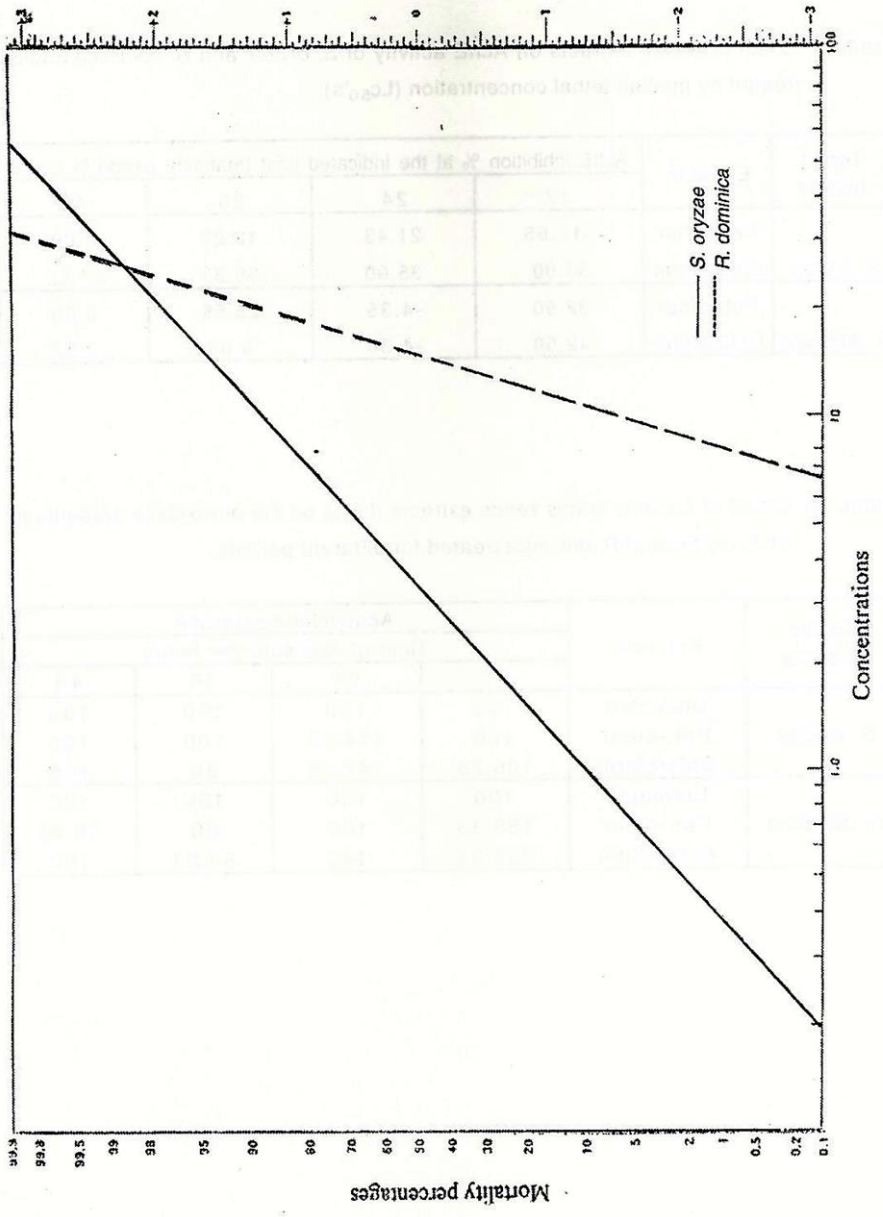

Fig.1. Toxicity regression lines of pet.-ether extract on wheat grains against *S. oryzae* and *R. dominica* after 72 hrs. from exposure.

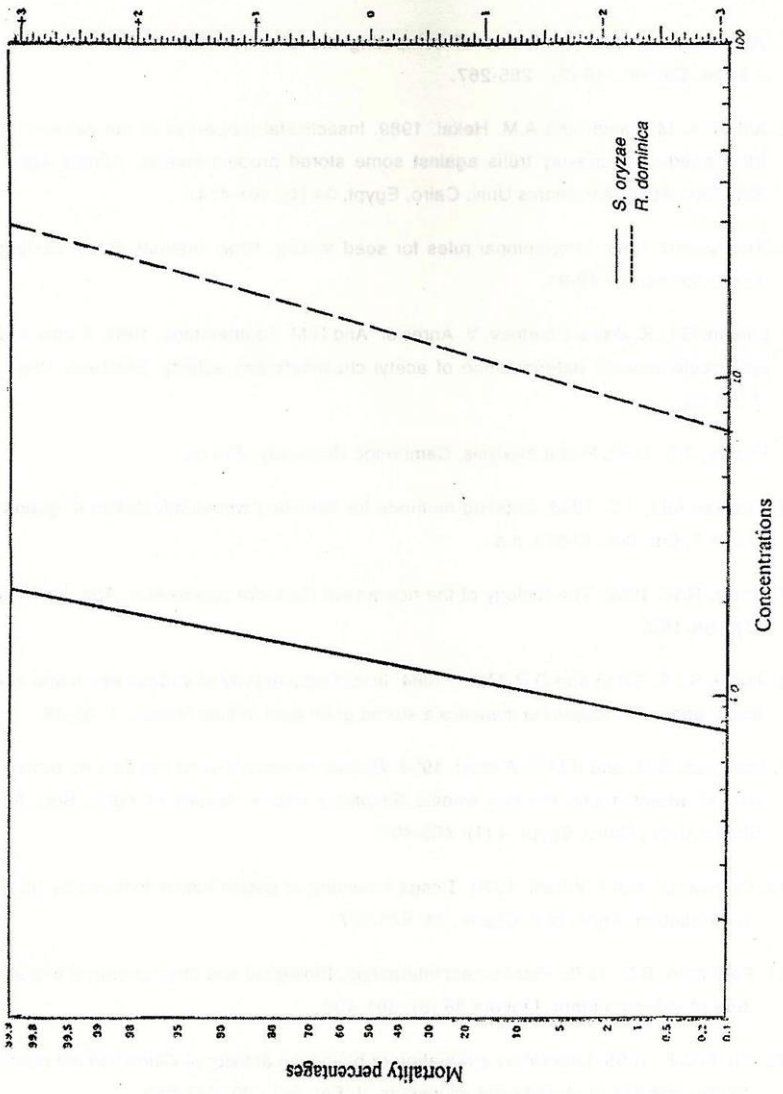

Fig.2. Toxicity regression lines of chloroform extract on wheat grains against *S. oryzae* and *R. dominica* after 72 hrs. from exposure.

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تقييم فاعلية مستخلصات بذور الترمس كمواد واقية لحبوب القمح ضد حشرتي سوسة الأرز وثاقبة الحبوب الصغرى

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أجريت دراسات معملية لتقييم فاعلية مستخلصات الاثير البترولي والكلوروفورم لبذور الترمس كمواد واقية لحبوب القمح ضد حشرتي سوسة الأرز وثاقبة الحبوب الصغرى وذلك عن طريق معاملة الحبوب سطحياً بالجرعات التي تسبب موت LC_{25} وكذلك الجرعات الوسطية المميتة LC_{50} وأيضاً الجراعات التي تسبب موت LC_{95} من كلتا الحشرتين.

أوضحت النتائج أن سوسة الأرز أكثر حساسية من حشرة ثاقبة الحبوب الصغرى لكلا المستخلصين. كما تسبب المستخلصات بكل تركيزاتها المختبرة لكل من الحشرتين في خفض الكفاءة الإنتاجية انخفاضاً شديداً عن حشرات المقارنة. وكذلك لم تنتج أي خلفة نهائية من الحشرات المختبرة لتركيز LC_{95} مما نتج عنه وقاية كاملة للحبوب ضد الحشرتين.

عند استعمال تركيز LC_{95} كانت هناك وقاية كاملة للحبوب من سوسة الأرز وثاقبة الحبوب الصغرى حتى ٩٠ يوماً.

معاملة الحبوب بالمستخلصات المختبرة أدى إلى خفض حيوية الحبوب المعاملة وخصوصاً عند التركيزات العالية.

عند تعريض الحشرات لحبوب معاملة بتركيز LC_{50} تأثر إنزيمي الكولين إستريز والبيروأكسيدز تأثيرات مختلفة.