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INSECTICIDAL ACTIVITY AND BIOCHEMICAL STUDIES OF EGYPTIAN SESBAN, *SESBANIA aegyptica* ; JYNIT. SEED EXTRACTS AGAINST RICE WEEVIL, *SITOPHILUS oryzae* L.

[35]

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ABSTRACT

The toxic effective of wheat grains treated with Egyptian sesban, *Sesbania aegyptica* seed extracts offered to rice weevil, *Sitophilus oryzae* was determined. Chloroform extract was found to be the most effective. Reproductive potential of treated weevil were strongly affected as number of laid eggs was reduced and no progeny were obtained when adults were fed on wheat grains treated with either Lc_{50} or Lc_{95}. Extracts treatment with Lc_{95} of extracts gave protection up to 10 weeks for petroleum ether, and 9 weeks for both chloroform and acetone extracts. All tested extracts reduced grain germination at the end of 14 weeks storage period. Treated wheat grains with Egyptian Sesban seed extracts reduced the weight loss of grains infested with the rice weevil. Biochemical studies show that some enzymes were affected in treated insects. *S. aegyptica* acetone extract was more effective than the other extracts, in this affect, as it caused a significant reduction in amylase, trehalse and acid phosphatase activity. However, this extract caused an increase in invertase, alkaline phosphatase and cholinestrase activity.

Key words: Egyptian sesbane, Rice weevil

INTRODUCTION

Stored grains are subject to attack by many insect species of which, if not adequately controlled, might cause serious economic damage. The use of insecticides causes many problems, such as harmful residues in the chain of food, pollution of environment, and disruption of biological balance by the destruction of the natural enemies.

The use of plant or their extracts exhibiting an insecticidal or insecticide synergistic activity against several insect species have been widely reported, (e.g. **Makanijuola 1989, Afifi *et al* 1989; Jilani & Su, 1983 and Ahmed 2001)**.

The rice weevil, *Sitophilus oryzae*. (Coleoptera : Curculionidae) is an insect of economic importance as it infests stored products causing a damage in the grains.

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The present work was conducted to evaluate the bioactivity of *Sesbania aegyptica* ; Jynit (Egyptian sesban) seed extracts on the rice weevil, *Sitophilus oryzae* L. Also, several researchers showed that some enzymes in several insect species were inhibited or activated by feeding them on a diet treated with plant extract, **(AbouelGhar, *et al* 1994 and Rizvi *et al* 2001).**

For this reason, some enzymes in adult weevils were estimated to clarify if they were effected in treated insects.

MATERIAL AND METHODS

Insect culture

The rice weevil, *S.oryzae* L. (Coleoptera : Curculionidae) were obtained from a well established laboratory culture maintained at the Stored Grains Insects, Research Division, Plant Protection Research Institute. Rearing of the insects was conducted under laboratory condition of 27 ± 1°C and 65 ± 5% R.H., insects were offered wheat grains.

Preparation of *Sesbania aegyptica* seed extract

Egyptian sesban seeds more obtained from sesban trees planted in Giza Governorate. The seeds were washed thoroughly with water and dried. Dry seeds were ground to fine powder by a high speed micromill. The ground powder was extracted first with pet-ether (40-60) in a flask and left for 48 hours, the extract was then filtered and the solvent was evaporated under pressure by using a rotary evaporator. The detatted powder was thoroughly dried before being extracted next with chloroform, then acetone solvent, as adopted from **Afifi *et al* (1988)**.

Evaluation of *S. aegyptica* seed extract toxicity

Toxicity of the three organic extracts was determined by adding different concentrations ranging from 2.0 to 5.0 ml/kg, 0.50 to 2.00 ml/kg and 2.0 to 7.0 ml/kg for pet-ether, chloroform and acetone, respectively, to gm. of wheat grains.

Twenty five, 1-2 weeks old *S. oryzae* adults, obtained from the maintained stock culture were placed on the treated wheat grains, placed in glass tubes, the tubes were covered with muslin fixed with rubber band. Acetone was prepared containing untreated wheat grains. After 3, 5, 7 and 14 days the tubes were investigated and the number of live and dead weevils counted. Accumulated mortality percentages Lc_{50} or Lc_{95} and regression line were determined and corrected by **Abbott's formula, (1925)**, calculated according to **Finney, (1952)**.

Effect of *S. aegyptica* seed extracts on reproduction of *S, oryzae*

Twenty *S. oryzae* 1-2 weeks old adults placed in glass tubes each containing 10 g. wheat grains treated with each of the determined at Lc_{50} and Lc_{95} *S. aegyptica* seed organic extract.

After two weeks, the tubes were opened and the insects removed. The number of deposited eggs, on the grains were counted according to the methods described by **Frankenfeld, (1948) and Howe, (1952)**.

The same previous experiment was replicated, but laid eggs were left undisturbed to hatch. After two weeks the weevils were removed and the tubes left for seven weeks, up to F_1 adult progeny

emergence. The number of emerged F_1 adults offspring were counted.

All of the forementioned experiments were replicated three times and a control containing untreated wheat included each time.

Residual activity of *S. aegyptica* seed extracts on wheat grains

Tubes containing 10 g. of wheat grains treated with the determined Lc_{95} concentration of each extract, were divided into several groups.

Twenty five adults of *S. oryzae* were introduced into every three tubes at a weekly interval and up to 12 weeks. Similar three replicates of untreated wheat were used as control. In all cases, mortality percentages were corrected with **Abbott's formula (1925)**.

Effect of Egyptian sesban seed extracts on germination

Germination of seeds treated with sesban seed extracts at the determined $Lc_{95's}$ of each of the three organic extracts was calculated at the initial time and at the end of 12 weeks storage period according to the International rules of seed testing **(Anonymous, 1966)**.

Weight loss of wheat treated with *S. aegyptica* seed extract

Weight loss of wheat grains treated with Egyptian sesban seed organic extracts and infested with *S. oryzae* was determined according to the equation reported by **Khare and Johari (1984)**:

$$\text{Weight loss \%} = \frac{\text{Initial dry weight - Final dry weight}}{\text{Initial dry weight}}$$

Effect of sesban seed organic extracts on the activity of some enzymes of the rice weevil, *S. oryzae*

One-two weeks old *S. oryzae* weevils were offered wheat grains treated with Lc_{50} of sesban seed pet-ether, chloroform and acetone extracts. After 48 hours of feeding the insects were removed and a weight of 0.2 g. of these weevils were homogenized in buffer solution. This solution was there filtered and the enzymatic activity were determined in the supernatant.

The following enzymes were considered:

1- Carbohydrate enzymes : amylase, trehalase and invertase

The method of **Ishaaya and Swiriski (1976)** was adopted. This method was based on the digection of starch and sugar by amylase, trehalase and invertase, respectively, using spectrometer (550 nm).

2-Acid and Alkaline Poshatases

These two enzymes were determined by measuring the optical density of the produced colour as described by **Powell and Smith (1954)**, using spectrophotometer, (510 nm).

3-Acetylcholine esterase (AchE)

This enzyme was determined according to method described by **Simpson *et al* (1964)**, where the optical density was

measured spectrophotometrically at 515 nm.

RESULTS AND DISCUSSION

Toxicity of Egyptian sesban seed extracts on *S. oryzae*

Sesban seed organic extracts at 4.0, 4.5 and 5 ml/kg for pet-ether, 0.75, 1.0 and 2.0 ml/kg for chloroform and 4.0, 5.0 and 7.0 ml/kg for acetone gave 100% mortality after 7 days for *S. oryzae* fed on treated wheat grains (Table, 1). Lc_{50} of seed organic extracts on the rice weevil was 3.5, 0.8, and 3.3 ml/kg, when pet-ether, chloroform and acetone, respectively were used in seed extraction (Table, 2). Meanwhile, Lc_{95} was 9.2, 1.3 and 10.0 ml/kg for the respective mentioned solvents. Values of slopes showed that the rate of acetone extract effectiveness was the lowest, meanwhile chloroform extract the highest.

Effect of sesban seed extract on egg fecundity and F_1 progeny of *S. oryzae*

Wheat grains treated with *S. aegyptica* organic extracts and offered to *S. oryzae* weevils reduced their fecundity as well as number of F_1 adult emerged progeny (Table, 3). At Lc_{50} level, *S. aegyptica* acetone extract proved to be the most effective in this respect, as the number of laid eggs by 5 couples were 2.66 eggs as compared to 97.66 eggs in the control, equal to 96% reduction. Also, no F_1 progeny were obtained. This was followed by pet-ether extract as 5.33 eggs per 5 females were recorded, i.e. 94% reduction. Meanwhile, chloroform seed extract was found to be the least effective, as it caused 82% reduction in egg fecundity and 75% in F_1 progeny.

At Lc_{95} level, no eggs were laid when either acetone or pet-ether, either were used as solvent for sesban seed extraction and only 6.6 eggs by 5 females were recorded when chloroform was used (Table, 3).

Residual effect of of *S. aegyptica* seed extracts on wheat grains offered to *S. oryzae* weevil

The residual toxic effect of *S. aegyptica* seed extracts at Lc_{95} level (Table, 4) showed that the effect of these extracts was relatively stable up to 8^{th} weeks of storage. Soon after treatment mortality of weevils ranged between 95-96% and was only reduced between 94-95 after 8^{th} week. By the 10^{th} week the toxic effect of *S. aegyptica* seed extracts to *S. oryzae* deteriorated slightly to reach 88 and 70%

After 12 weeks of grain storage, mortality percentage of *S. oryzae* was only 30, 37 and 45% for *S. aegyptica* acetone, pet-ether or chloroform extracts, denoting the ineffectiveness of these extracts past the 9^{th} week of storage.

Effect of *S. aegyptica* extracts on germinate of treated wheat grains

The germination of wheat grains soon after treatment was slightly reduced following treatment with Lc_{95} of *S. aegyptica* seed extracts. This effect was more apparent when pet-ether was used as solvent for extraction, followed by acetone then chloroform i.e 87, 88 and 90% respectively. Meanwhile after 12 weeks of storage of treated wheat grains, germination was 82, 84 and 86% for acetone and pet-ether and chloroform, respectively (Table, 5). In this respect, Shemais and **Al-Moajel, (2000)** found that wheat

Table 1. Mortality percentages of *S. oryzae* fed on wheat grains treated with *S. aegyptica* seed extracts

Solvent used for treatment	Concentrations ml/kg	Mortality percentage after period (days)				
		1	3	5	7	14
Petroleum ether	2.0	00±0.00	20±1.53	39±4.05	57±2.57	86±3.06
	3.0	00±0.00	40±0.37	68±4.17	80±4.59	98±1.16
	4.0	8±1.53	45±5.52	100±0.00	100±0.00	100±0.00
	4.5	12±0.8	60±4.51	100±0.00	100±0.00	100±0.00
	5.0	20±1.16	85±2.31	100±0.00	100±0.00	100±0.00
Chloroform	0.50	00±0.00	20±3.06	41±1.16	62±0.58	92±2.31
	0.75	3±1.16	50±1.00	63±2.23	100±0.00	100±0.00
	1.00	6±1.53	80±3.06	100±0.00	100±0.00	100±0.00
	2.00	10±1.16	90±4.17	100±0.00	100±0.00	100±0.00
Acetone	2.0	00±0.00	28±1.53	36±4.05	54±2.31	82±0.00
	3.0	00±0.00	35±2.52	50±3.61	79±3.06	100±0.00
	4.0	10±1.53	65±3.52	88±3.41	100±0.00	100±0.00
	5.0	10±0.85	70±3.51	100±0.00	100±0.00	100±0.00
	6.0	14±2.00	80±3.06	100±0.00	100±0.00	100±0.00
	7.0	20±2.52	90±2.52	100±0.00	100±0.00	100±0.00

± Mean standard error

Table 2. Lc_{50} and Lc_{95} values and slopes of regression line for tested extracts against *Sitophilus oryzae*

Solvents	Lc_{50} (ml/kg)	Lc_{95} (ml/kg)	Slopes
Petroleum ether	3.5	9.2	3.91
Chloroform	0.8	1.3	9.07
Acetone	3.3	10.0	3.38

Table 3. Fecundity and F1 progeny of *Sitophilus oryzae* fed on wheat grains treated with *S. aegyptica* extract

Solvent	Concentration ml/kg	Mean no. of eggs/5 pairs	(%) Reduction of fecundity	Mean of adult progeny emergence	(%) Reduction F_1 adult progeny
Petroleum ether	Lc_{50} (3.5) Lc_{95} (9.2) Control	5.33±1.2 0.00±0.00 97.33±3.67	94 100	3.0±0.58 0.00±0.00 30.0±1.16	90 100
Chloroform	Lc_{50} (0.8) Lc_{95} (1.3) Control	17.00±1.00 6.66±2.07 97.66±3.6	82 93	9.33±0.43 0.00±0.00 38.33±3.18	75 100
Acetone	Lc_{50} (3.3) Lc_{95} (10.0) Control --	2.66±0.66 0.00±0.00 97.66±3.67	96 100	0.00±0.00 0.00±0.00 26.00±2.08	100 100

± Mean standard error

Table 4. Mortality percentages of *Sitophilus oryzae* fed on wheat treated with Lc_{95} of *S. aegyptica* seed extracts

Weeks	Mortality percentage of *S. oryzae* weevils		
	Petroleum ether	Chloroform	Acetone
Initial	96±1.56	95±0.00	96±0.58
1	96±0.00	96±1.16	95±0.00
2	95±0.58	96±1.53	95±0.58
3	94±2.00	96±2.00	95±1.00
4	95±1.53	95±0.58	94±0.58
5	95±0.00	95±5.20	95±0.00
6	96±0.58	94±1.53	94±1.16
7	94±1.57	95±0.00	95±1.16
8	95±0.58	94±2.00	94±0.00
9	93±1.23	95±1.53	90±0.58
10	90±0.00	88±0.58	70±4.00
11	72±4.05	62±2.08	69±3.52
12	37±2.52	45±0.00	30±0.00

± Mean standard error

grains treated with capparis seed extract lost viability especially at the end of storage.

Weight loss of wheat grains treated with *S. aegyptica* extracts

Wheat grains treated with *S. aegyptica* seed extracts caused a weight loss ranging between 62.75-66% in wheat grain weight than the control when treated at Lc_{50} level. Meanwhile, when treated with Lc_{95} this loss was between 91.95-99.99% than the control. Treated with pet-ether extract gave the most efficiency, meanwhile, chloroform extract the lowest effect (Table, 6). These results agree with **Abdel-Latif, (2003)**, which found that treatment the cowpea and chickpea seeds with some natural oils reduced the weight loss in the seed.

Effect of tested extract on activity of some enzymes

1- Amylase, Trehalase and Invertase

The results exhibited in (Table, 7) show that there was a significant decrease in amylase activity in *S. oryzae* fed on *S. aegyptica* extracts at Lc_{50} level. The highest reduction in this enzyme activity was induced after treatment with chloroform extract, followed by pet-ether then acetone extracts (1255.39, 1309.66 and 1328.25 mg glucose/min/ml) respectively, compared to 1813.12 mg glucose/min/ml in the control. These results are in agree with **Ayyangar and Rao, (1990)** who reported that digestive enzymes activity was reduced in 6^{th} instar larvae of *S. littoralis* injected with azadirachtin.

Also, there was a significant decrease in trehalase activity, the highest reduction was recorded after treatment with acetone extract followed by pet-ether (56.54 and 103.3 mg glucose/min/ml respectively). On the other hand chloroform extract caused a significant increase in trehalase activity (198.31 mg glucose/min/ml) compared to untreated insects (128.32 mg glucose/min/ml). Similarly, **Abou El-Ghar *et al* (1994)** found that acetone extract of *Melia azedrach* caused an increase in trehalase activity of *A. ipsilon* larvae.

On the other hand, all the tested extracts caused an insignificant increase in invertase activity. Acetone extract caused the highest increase (486.39 mg glucose/min/ml) followed by pet-ether and chloroform extracts (476.24 and 468.44 mg glucose/min/ml respectively), compared the control insects (418.84 mg glucose/min/ml). **El-Skeikh (2002)** found an increase in trehalase activity after treating 6^{th} instar larvae of *A. ipsilon* with acetone extract of *Melia azedrach* seeds.

2- Phosphatase activity

Data in (Table, 8) revealed that Egyptian sesban seed extracts decreased acid phosphatase activity in treated weevils. Acetone extract, caused the lowest decrease followed by pet-ether and then chloroform (170.66, 195.83 and 198.31 mg phosphate/min/ml, respectively) compared to 248.76 mg phosphate/min/ml in untreated insects.

Acetone extract caused a significant increase in alkaline phosphatase activity. Meanwhile, pet-ether and chloroform caused an insignificant decrease. **Imtiaz, (2001)** reported a decrease in alkaline

Table 5. Germination of wheat grains stored for 12 weeks after treatment with *S. aegyptica* seed extracts

Solvent	Concentration ml/kg	Initial time		After 12 weeks storage	
		Germination (%)	Reduction %	Germination (%)	Reduction %
Petroleum ether	9.2	87 ± 1.16	9.38	84 ± 1.00	11.58
Chloroform	1.3	90 ± 1.53	6.25	86 ± 1.73	19.47
Acetone	10.0	88 ± 0.33	8.33	82 ± 1.16	13.68
Control		96 ± 0.58		95 ± 1.53	

Table 6. Effect of tested extracts on grains weight loss

Solvent	Concentration ml/kg	Dry weight loss %	Dry weight reduction %
Petroleum ether	Lc_{50} (3.5)	1.90	66
	Lc_{95} (9.2)	0.0004	99.99
	Control	2.98	
Chloroform	Lc_{50} (0.8)	1.11	62.75
	Lc_{95} (1.3)	0.24	91.95
	Control	2.98	
Acetone	Lc_{50} (3.3)	1.03	65.44
	Lc_{95} (10.0)	0.23	92.28
	Control	2.98	

Table 7. Activity of *S. oryzae* digestive enzymes treated with *S. aegyptica* extracts

Solvents	Digestive enzymes (mg glucose/min/ml)					
	Amylase		Trehalase		Invertase	
	Activity	Change in percentage %	Activity	Change in percentage %	Activity	Change in percentage %
Petroleum ether	1309.66 ± 22.00	-27.76	103.39 ± 5.80	-19.43	476.24 ± 11.78	+13.70
Chloroform	1255.39 ± 55.28	-30.76	198.31 ± 12.23	+54.54	468.44 ± 15.54	+11.84
Acetone	1328.25 ± 38.46	-26.74	56.54 ± 4.67	-55.94	486.39 ± 9.79	+16.36
Control	1813.12 ± 87.12		128.32 ± 7.61		418.86 ± 10.33	

± mean standard deviations
-Inhibition
+Activation

Table 8. Activity of phosphatase enzymes and acetylecholine estrase *in S. oryzae* treated with *S. aegyptica* seed extracts

Solvent	Phosphatase (mg phosphate/min/ml)				Acetylecholine esterase (m acetylcholine/min/ml)	
	Acid		Alkaline			
	Activity	Change in percentage %	Activity	Change in percentage %	Activity	Change in percentage %
Petroleum ether	195.83 ± 6.27	-21.28	4.11 ± 0.24	-32.40	1465.66 ± 46.70	+19.19
Chloroform	198.31 ± 12.23	-20.28	5.35 ± 0.45	-12.01	1248.90 ± 33.44	+ 1.57
Acetone	170.66 ± 5.56	-31.40	11.64 ± 1.050	+91.45	1445.13 ± 47.95	+17.61
Control	248.76 ± 9.42		6.08 ± 0.85		1229.65 ± 31.11	

± mean standard deviations
-Inhibition
+Activation

phosphatase activity in *S. oryzae* treated with neem leaf extract.

3-Choline esterase activity

Data in (Table, 8) revealed that both acetone and pet-ether extracts significantly increased the activity of choline esterase enzyme however, meanwhile, extract caused an insignificant increase. **Rizivi *et al* (2001)**, found an inhibition in the choline esterase activity in *Tribolium castaneum* after treatment with *Clerodenreem inerme* leaf extract.

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مجلة اتحاد الجامعات العربية للدراسات والبحوث الزراعية ، جامعة عين شمس ، القاهرة ، 13(2) ، 537 - 548 ، 2005

التأثير الإبادى لمستخلصات بذور السيسبان المصرى ضد حشرة سوسة الأرز وبعض الدراسات البيوكيميائية للحشرة

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تم تقدير تأثير معاملة حبوب القمح بمستخلصات بذور السيسبان المصرى على حشرة سوسة الأرز وكذلك تم تقدير التركيز القاتل لـ50% وكذا القاتل لـ 95%.

مستخلص الكلوروفورم كان اكثر كفاءة عند استخدامه فى كلا المستويين القاتل لـ50% ، 95% .

تأثرت الكفاءة التناسلية لسوسة الأرز تأثرا شديداً وخصوصا عند استخدام التركيز القاتل لـ95% حيث لم تخرج خلفة من الحبوب المعاملة .

معاملة الحبوب بالتركيز القاتل لـ 95% أعطى حماية عشرة أسابيع فى حالة مستخلص الاثير البترولى و9 أسابيع لمستخلص الكلوروفورم والأسيتون .

تأثر الإنبات فى بداية التخزين حيث انخفضت نسبة الإنبات بنسبة قليلة عن الغير معاملة ولكن زاد هذا الانخفاض فى نهاية فترة التخزين .

الحبوب المعاملة بالتركيزين القاتلين 50% ، 95% بالمستخلصات ادى الى انخفاض الفقد فى الوزن مقارنة بالغير معامل .

الدراسات البيوكيميائية المدروسة أوضحت ان مستخلص الأسيتون كان اكثر فاعلية مقارنة بباقى المستخلصات حيث أدى الى انخفاض نشاط كل من إنزيم الاميليز، تريهاليز والفوسفاتيز الحامضى . وكذلك أدى الى زيادة نشاط إنزيم الإنفرتاز والفوسفاتيز القلوى والكولين استيريز .

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