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

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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 Lc₅₀ or Lc₉₅. Extracts treatment with Lc₉₅ 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, trehalase and acid phosphatase activity. However, this extract caused an increase in invertase, alkaline phosphatase and cholinesterase activity.

Key words: Egyptian sesban, 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 F1 adults offspring were counted.
All of theforementioned 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 LC95 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 LC95’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):

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

**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 LC50 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 digestion 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). Lc50 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, Lc95 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 F1 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 F1 adult emerged progeny (Table, 3). At Lc50 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 F1 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 F1 progeny.

At Lc95 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 Lc95 level (Table, 4) showed that the effect of these extracts was relatively stable up to 8th weeks of storage. Soon after treatment mortality of weevils ranged between 95-96% and was only reduced between 94-95 after 8th week. By the 10th 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 9th 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 Lc95 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 F1 adult progeny |
|------------|---------------------|--------------------------|----------------------------|--------------------------------|--------------------------------|
| **Petroleum ether** |                     |                          |                            |                                |                                |
| LC50 (3.5) | 5.33±1.2            | 94                       | 3.0±0.58                   | 90                             | 100                            |
| LC95 (9.2) | 0.00±0.00           | 100                      | 0.00±0.00                  | 100                            |                                |
| Control    | 97.33±3.67          |                          | 30.0±1.16                  |                                |                                |
| **Chloroform** |                   |                          |                            |                                |                                |
| LC50 (0.8) | 17.00±1.00          | 82                       | 9.33±0.43                  | 75                             |                                |
| LC95 (1.3) | 6.66±2.07           | 93                       | 0.00±0.00                  | 100                            |                                |
| Control    | 97.66±3.66          |                          | 38.33±3.18                 |                                |                                |
| **Acetone** |                     |                          |                            |                                |                                |
| LC50 (3.3) | 2.66±0.66           | 96                       | 0.00±0.00                  | 100                            |                                |
| LC95 (10.0)| 0.00±0.00           | 100                      | 0.00±0.00                  | 100                            |                                |
| Control    | 97.66±3.67          |                          | 26.00±2.08                 |                                |                                |

± Mean standard error

Table 4. Mortality percentages of *Sitophilus oryzae* fed on wheat treated with LC95 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 Lc50 level. Meanwhile, when treated with Lc95 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 Lc50 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 6th 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 6th 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<sub>50</sub> (3.5) | 1.90              | 66                     |
|                | Lc<sub>95</sub> (9.2) | 0.0004            | 99.99                  |
|                | Control             | 2.98              |                         |
| Chloroform     | Lc<sub>50</sub> (0.8) | 1.11              | 62.75                  |
|                | Lc<sub>95</sub> (1.3) | 0.24              | 91.95                  |
|                | Control             | 2.98              |                         |
| Acetone        | Lc<sub>50</sub> (3.3) | 1.03              | 65.44                  |
|                | Lc<sub>95</sub> (10.0) | 0.23              | 92.28                  |
|                | Control             | 2.98              |                         |
Table 7. Activity of *S. oryzae* digestive enzymes treated with *S. aegyptica* extracts

| Solvents      | 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 | |
|               | +13.70        | +54.54 | +11.84 | +16.36 |  

± mean standard deviations  
-Inhibition  
+Activation

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

| Solvent      | Phosphatase (mg phosphate/min/ml) | Acetylicholine esterase (m acetylcholine/min/ml) |
|--------------|----------------------------------|-----------------------------------------------|
|              | Acid Activity   | Change in percentage | Alkaline 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 | |
|              | +13.70        | +54.54 | +11.84 | +16.36 | 

± 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 *Clerodendreem inerme* leaf extract.

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التأثير الإبادى لمستخلصات بذور السيسبان المصرى ضد حشرة سوسة الأرز

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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 لـ95%.

مستخلص الكلوروفورم كان أكثر كفاءة عند استخدامه في كلا المستويين القاتل لـ50% وـ95%.

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

الحبوب المعاملة بالتركيزين القاتلين 50% وـ95% بالمستخلصات أدى إلى انخفاض الفقد في الوزن مقارنة بالغير معامل.

الدراسات البيوكيميائية المدرسية أوضحت أن مستخلص الأسيتون كان أكثر فاعلية مقارنة بالباقي المستخلصات حيث أدى إلى انخفاض نشاط كل من إنزيم الأميليز، تريبالاز والفسفاتاز الحمضي. وكذلك أدى إلى زيادة نشاط إنزيم الإنفرتاز والفسفاتاز القلوي والكولين استيريز.

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