Use Of *Sesbania Sesban* (L.) Merr Seed Extracts for the Protection of Wheat Grain Against the Granary Weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae)

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Abstract :

Studies on the efficacy of petroleum ether, chloroform and acetone extracts of *Sesbania sesban* (L.) Merr seeds against *Sitophilus granarius* in wheat grain revealed that there was significant toxic effects of all extracts on the adults. Based on LC_{95} concentrations, chloroform extract was the most toxic (3.7 ml/kg), followed by petroleum ether extract (7.4 ml/kg) and acetone extract (15.0 ml/kg).

All extracts prevented oviposition and adult emergence at LC_{95} concentrations, and reduced oviposition by (89-94%) at LC_{50} concentrations. Adult emergence was reduced (88-97%) in the petroleum ether and acetone extracts by 97% and 88%, respectively, however, chloroform extract completely prevented adult emergence.

Infested grain loss was significantly lower in all extracts when compared with the control. All treatments gave high protection for up to 6 weeks after treatment.

Water absorption was significantly increased in all treatments, and germination was significantly decreased in all treatments.

Key Words: seed extracts, grain protectants, Sesbania sesban

Introduction :

Wheat is an important food in the Mediterranean region and worldwide. It is attacked by several insect pests during storage (Nakakita, 1998). Use of synthitic chemical insecticides for grain protection is a common practice, but it may have drawbacks including toxicity, attendant resistance problems, and environmental pollution (Georghious and Lagunes-Tejeda, 1991; Yusof and Ho, 1992). In fact, management of stored product pests, using materials of natural origin, is nowadays the subject which received much research to overcome there problems, because of their little environmental hazards and low mammalian toxicity (Isman, 1994).

Botanical materials are a rich source of bioactive organic chemicals. Over 2000 plant species around the world are known to possess pest control properties (Ahmed *et al.,* 1984). Previous research indicated that some plant

powders and extracts have strong effects on stored grain insects such as toxicity and the inhibition of reproduction (Regnault - Roger and Hamraoui, 1993; Talukder and Howse, 1995). Various plant by-products from Asia, Africa and America have been tested recently with a good degree of success as protectants against a number of stored grain insect pests (Rajapakse, 1990; Regnault – Roger and Hamraoui, 1991, 1993).

Some indigenous plants in the kingdom of Saudi Arabia are known to possess some biological activity against insects (Elhag *et al.*, 1996; Al-Moajel and Al-Dosary, 2002, 2003; Al-Moajel and Al-Fuhaid, 2003).

Sesbania sesban is a native plant in Saudi Arabia (Abdel Magid *et al*., 1988). It can provide a wide range of products: forage, green manure, firewood, gum, pulp and paper, edible leaves and flowers (NAS, 1979).

This paper describes experiments to assess the efficacy of *S. sesban* seed extracts for protection stored wheat against attack by *S. granaries,* a serious pest of a great variety of stored products in tropical and sub-tropical parts of the world.

Materials and Methods

Cultures:

Test insects were drawn from laboratory cultures reared in jars. New cultures were obtained by removing about 100 adults (unsexed) and placing them in a glass jars (size 14x10.5x30cm) containing 300g of wheat grains, and left to bread until new adults emerged. New adults (2-14 day old) were used for the experiments.

The cultures were kept in the incubator under controlled conditions of 27+1^{o}c and 60 + 10% r.h. The vails of all cultures were kept closed by perforated lids to prevent the insects from climbing outside the vails.

Plant extracts:

Seeds of S. sesban were obtained from the local markets, washed, air-dried, ground and then extracted in a Soxhlet apparatus separately with petroleum ether (at 55oc), chloroform (at 60oc) and acetone (at 65oc) for 6 hrs in each case (Talukder & Howse, 1995). The extracts were dehydrated with anhydrous sodium sulphate, then evaporated.

These extracts were stored in screw-capped glass vails at 4oc until needed (Islam, 1983).

Treatments:

A stock solution of every extract was prepared by redissolving 1ml of the extract in 10 ml of its solvent to obtain different concentrations of each extract. Wheat grains treated with petroleum ether, chloroform and acetone, separately, were considered as negative controls.

For each experiment, the extracts were evenly spread over the grains in each vail (replicate) by shaking manually for 2 min. 25 insects were introduced into each vail after evaporating the solvents in air. The vails of all experiments were kept under controlled conditions of 27 ± 1^{o}c and $60\pm10\%$ r.h.

Mortality assessment:

To investigate the effect of *S. sesban* on *S. granarius,* adult mortality, different concentrations (3,4,5,6 and 7 ml/kg of petroleum ether extract, 1.5, 2, 3 and 4 ml/kg of chloroform extract and 2,4,5,7 and 8 ml/kg of acetone extract) were prepared and each mixed with 10 g of wheat grain in tubes of 3x10 cm size.

When the solvents had dried up, 20 newly emerged adults of mixed sex were introduced into each replicate (three replicates for each treatment). Mortalities were assessed at 1,3,5,7 and 14 days after treatment.

Fecundity:

The effect of each extract on biology of *S. granarius* adults was tested.

Two groups of 27 glass tubes, each containing 5g of wheat grains, were treated separately with three plant extracts at LC_{50} and LC_{95} concentrations with three replicates for each concentration and control (three solvents control). Ten sexed *S. granarius* adults (5 males + 5 females), were introduced into each treated wheat grain tube, and left for two weeks, then removed. Egg count in the first group of treated tubes was carried out. Acid fuschin stain was used for the detection of the eggs (Frankenfeld, 1950). The second group was kept undisturbed until adults emerged, where the number of newly emerging adults were determined daily when the earliest emergence was observed, to prevent overlap of first and second generations.

Percent grain weight loss:

Grain weight loss was also estimated (Khare and Johari, 1984). Batches of 10g wheat grains were treated with LC_{50} and LC_{95} concentrations of *S. sesban* and controls (three replicates each), then the tubes were shaked vigorously for optimum coverage of the grain surface. After solvents evaporation, 20 *S.*

granarius adults were introduced into each tube (replicate). After 45 days, the grains were shifted and reweighed.

Ten gram of untreated wheat grain (3 replicates) where heated at 105^{o}c for 18 hrs and reweighed to determine moisture content.

Weight loss was calculated as the difference between the final and initial weights of treated or untreated grain, corrected for changes in moisture content, and expressed as a percentage of the initial weight of grains.

Residual effect:

Persistence of *S. sesban* seed extracts on treated grains was determined by treating 6 kg of wheat grain with each extract at LC_{95} concentrations plus a controls, then storing in the incubator (3 extracts + 3 controls). From each treatment, 30 g were taken weekly and divided between 3 vails (3 replicates). Twenty adults were introduced into each vail, and mortality counts were made after 3 days for 8 weeks.

Grain germination and water absorption:

grains ability to germinate was investigated according to the International Seed Testing Methods (Anonymous, 1966) to find out the effect of the extracts on wheat grains treated by LC_{95},^{S} concentrations.

Germination was tested at initial time and after the end of storage period (8 weeks) in 90 dia Petri-dishes containing wet cotton wool. Ten grains randomly selected from each flask, treated with LC_{95} concentrations of each extract and controls, were placed in 36 trays (three replicates each and moistened daily with distilled water. Percentage of germination were determined after 10 days.

Effect of these plant extracts on percentage grain water absorption was also evaluated by adding 4ml of distilled water to 2 g of previously treated grains (36 Petri dishes, 90 mm dia). After various time exposures, 1,5,24 hr, grains were weighed for each treatment. (Sighamony *et al*., 1986).

Statistical analysis:

The percentage mortalities of adults were subjected to one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1968) after correction of the percentage mortality by Abbott's (1987) formula. Means were compared with Duncan's multiple range test (DMRT) (Duncan, 1951) ($P < 0.05$). Data obtained from the various concentrations were subjected to probit analysis in order to estimate LC_{50} and LC_{95} values (SPSS, 1999). Percentages of reduction

in egg laying and F_1 progeny were calculated according to the following equation:

$$\frac{C-T}{C}\times 100$$

where C: Number of eggs layed or adults emerged in the control.

T: Number of adults emerged in treatments.

To calculate the grain weight loss index (WL), the following equation was used:

%WL = (IW – FW) ×100 / IW

where IW is the initial weight and FW is the final weight.

Means of all determinations (+SEM) were recorded.

For comparisons between treatments and control of oviposition, adult emergence and percentage weight loss, T-test Student's was used. Germination and water absorption were analyzed by one-way analysis of variance, and means were compared using DMRT.

Table (1)

Mortality of the granary weevil, *Sitophilus granarius* adults fed on wheat grains treated with *Sesbania sesban* seed extracts. .

Treatment	Extract applied on wheat (ml/kg)	Corrected mortality (%) after days*				
		1	3	5	7	14
Petroleum ether extract	3.0	0^{abd}	20^{a}	49^{ab}	60^{a}	72^{ab}
	4.0	0^{ae}	40^{bc}	75	100^{b}	---
	5.0	0^{af}	70^{def}	98^{d}	100^{b}	---
	6.0	21^{g}	80^{dg}	100^{d}	---	---
	7.0	23^{g}	96^{g}	100^{d}	---	---
Chloroform extract	1.5	0^{ah}	30^{abh}	52^{ae}	64^{ac}	80^{a}
	2.0	0^{aI}	65^{Id}	100^{d}	---	---
	3.0	18^{g}	85^{gJe}	100^{d}	---	---
	4.0	22^{bg}	95^{g}	100^{d}	---	---
Acetone extract	2.0	0^{efhI}	30^{ab}	57	69^{c}	77^{a}
	4.0	0^{efhI}	45^{ch}	75	94^{b}	100^{b}
	5.0	4^{efhI}	55^{Ifc}	98^{df}	100^{b}	---
	7.0	16^{bgJ}	70^{fIJ}	100^{df}	---	---
	8.0	20^{dgJ}	90^{fIJ}	100^{d}	---	---
F-value		11.245	15.385	21.268	32.814	12.163
F-tabulated		2.089				

* Seventy five adults in three replicates were used for each concentration.

- Nombers followed by the same letter in the same column are not significantly different (P<0.05) by Duncan's MRT.

Table (2)
Toxicity of *Sesbania sesban* seed extracts to *Sitophilus granarius* adults.

Extract	LC_{50} ml/kg	LC_{95} ml/kg	Slope
Petroleum ether	4.2	7.4	6.55
Chloroform	1.9	3.7	5.5
Acetone	3.8	15.0	2.71

Table (3)
Inhibition of oviposition and adult emergence (F_1) in *Sitophilus granarius* by application of *Sesbania sesban* seed extracts on wheat grains.

Treatment	Conc. (ml/kg)	Mean egg production per 5 pairs ± SEM	Reduction	Mean offspring per 5 pairs ± SEM	Reduction
Petroleum ether extract Control	4.2 (LC_{50}) 7.4 (LC_{95})	2.0±0.58 0.0±0.00 27.0±3.60	92 100	0.3±0.058 0.0±0.0 11.0±2.00	97 100
t-value		**-6.85***		**-5.35***	
Chloroform extract Control	1.9 (LC_{50}) 3.7 (LC_{95})	1.3±0.36 0.0±0.00 24.0±2.40	94 100	0.0±0.00 0.0±0.00 9.0±1.00	100 100
t-value		**-9.2***		**-**	
Acetone extract Control	3.8 (LC_{50}) 15.0 (LC_{95})	2.7±0.33 0.0±0.00 26.0±4.20	89 100	1.00±0.58 0.0±0.00 9.0±1.00	88 100
t-value		**-5.59***		**-6.93***	

* Significant ($\alpha = 0.05$).

Table (4)
Mean percentage of loss in treated wheat grains due to feeding by *Sitophilus granarius* adults after 8 weeks storage period.

Treatment	Conc. (ml/kg)	Mean per cent weight loss of grains ± SEM	t-value	%Weight reduction
Petroleum ether extract	4.2 (LC_{50})	7.27±0.50	3.09*	40.21
	7.4 (LC_{95})	1.45±0.05	7.13*	8.07
Control		12.16±1.50		
Chloroform extract	1.9 (LC_{50})	5.63±0.43	4.19*	53.70
	3.7 (LC_{95})	0.93±0.04	7.48*	92.35
Control		12.16±1.50		
Acetone extract	3.8 (LC_{50})	3.77±0.35	5.42*	69.00
	15.0 (LC_{95})	0.11±0.006	8.03*	99.10
Control		12.16±1.50		

* Significant ($\alpha = 0.05$).

Table (5)
Adult mortality of *Sitophilus granarius* in treated grains with *Sesbania sesban* seed extracts during 8 weeks after treatment

Exposure times (weeks)	% Mortality		
	Petroleum ether	Chloroform	Acetone
Initial	97	98	95
1	95	96	95
2	96	95	96
3	96	95	94
4	94	93	95
5	95	94	95
6	88	90	83
7	87	85	60
8	59	70	42
Slope	-1.1262	-1.6404	-1.7371
LT_{95}	2	2	2
LT_{50}	61	72	17

Table (6)
Percent water absorption and germination of wheat grains treated with *Sesbania sesban* seed extracts and stored for 8 weeks.

Extract and concentration (ml/kg)	Average percent weight increase of grains submerged for (hours)*						Average percent grain germination **			
	Initial			After storage			Initial		After storage	
	1	5	24	1	5	24	G.±SEM	% Reduction	G.±SEM	% Reduction
Petroleum ether (7.4)	23^{a}	38^{a}	61^{a}	21^{a}	36^{a}	59^{a}	92 ± 1.15^{a}	4	84 ± 2.0^{ab}	9
Chloroform (3.9)	24^{a}	38^{a}	59^{b}	22^{a}	35^{a}	55^{a}	90 ± 0.00^{b}	6	80 ± 2.52^{c}	13
Acetone (15.0)	22^{ab}	36^{a}	56^{c}	20^{a}	36^{a}	57^{ab}	90 ± 1.15^{c}	6	78 ± 1.15^{ad}	16
Control	18^{b}	32^{b}	51^{d}	16^{b}	30^{b}	50^{c}	96 ± 0.58^{abc}		93 ± 0.00^{bcd}	
F-value	4.37	16.00	113.50	16.60	4.71	10.53	10.67		14.75	
F-tabulated	4.066									

* Nombers followed by the same letter in the same column are not significantly different at the 5% level ($P < 0.05$).
** Nombers followed by the same letter in the same column are significantly different at the 5% level ($P < 0.05$).

Results And Discussion

Toxicity of extracts against adults:

Data on the effectiveness of *S. sesban* seed extracts against *S. granarius* adults (Table 1) showed that all extracts elicited mortality, which ranged between 20-23% within 1 day after treatment in the highest tested concentrations. No mortality was noticed in the lowest tested concentrations of all extracts within the same period after treatment. Nevertheless, all extracts showed high activity after 3 days of treatment, so the efficacy of extracts on adults was significantly different (F=15.385). the efficacy of the extracts was highly significant at higher concentrations. The 4 ml/kg concentration produced 40 and 45% mortality at petroleum ether and acetone extracts respectively, while the same chloroform extract concentration exhibited 95% mortality within 3 days of application. For petroleum ether and acetone extracts a longer exposure time (7 days) was needed to obtain 100 and 94% mortality, respectively.

After 5 days from application, 49-57% mortality was observed at the lowest concentrations of all extracts, but mortality reached 100% at the highest concentrations of all extracts.

After 7 days from application, all tested concentrations of all extracts, except the lowest, killed mostly all the insects.

Table (2) indicates that chloroform extract was the most effective extract. The lethal concentrations (LC) calculated by probit method were LC_{50}=1.9, 3.8 and 4.2 ml/kg while LC_{95} was 3.7, 15.0 and 7.4 ml/kg for chloroform, acetone and petroleum ether respectively.

The three extracts of *S. sesban* seeds demonstrated significant toxic effect against *S. granarius* adults, in spite of the fact that LC_{50} value of the chloroform extract was about half that of petroleum ether and acetone extracts. Also, the LC_{95} value of chloroform extract was about half that of petroleum ether extract and one fourth that of acetone extract.

The toxicity of a number of plant extracts has been evaluated against stored products insects (Ho, *et al*., 1994; Talukder and Howse, 1995; Huang *et al*., 1997, 2000; Lale and Yusuf, 2001; Al-Moajel, 2003). This means that many plant materials can be used as insect toxicants but these investigators gave no details about the active components. Results reported in this study show that *S. sesban* has an insecticidal effect on *S. granarius*.

Effect of plant extracts on egg laying and F1 adults emergence:

It is evident from Table (3) that egg laying in grains treated with the tested extracts at LC_{50} values was reduced by 89-94% as compared with the solvents control, while no eggs were laid in all extracts at LC_{95} values. 24-27 eggs per 5 females where laid in the solvents control.

Consequently, complete suppression of progeny production was observed at LC_{95} concentrations. At LC_{50} concentrations, progeny development was significantly reduced by petroleum ether and acetone extracts, whereas no progeny emerged in chloroform extract.

Eggs laid in the LC_{50} of chloroform extract (1.3 egg/5 pairs) did not produce further progeny, perhaps indicating an ovicidal activity of this extract.

From the above results, it is evident that all extracts tested at both concentrations levels tested (LC_{50} and LC_{95}) were found very effective in suppress oviposition and adult emergence of *S. granarius* adults as compared

to the control. The results suggest that *S. sesban* seed extracts may be a good protectant for wheat grains against *S. granarius*.

Different plant extracts was also tested against *S. granarius* (Schmidt *et al*., 1991; Ahmed and Kassis, 2000; Al-Moajel, 2000; Ahmed *et al*., 2002a), and the results indicated that wheat grains were well protected by some plant extracts.

Effect on weight loss:

Data presented in Table (4) show a decrease in grain weight with increase in concentration in each plant extract tested. The control gave the highest weight loss (12.16%), while the wheat grains treated with LC_{95} concentrations in all extracts gave the lowest weight loss (0.11 – 1.45%). Acetone extract produced the lowest loss of weight (0.11%). All treatments significantly reduced the grain weight, but the higher concentration gave more reduction in weight loss (88.07 to 99.1%).

The maximum reduction in weight loss was recorded in the acetone extract (99.1%), which gave almost complete protection of grains after storage.

Many investigators (Parsai *et al*., 1990; Niber, 1994; Singal, 1995; Keita *et al*., 2001, Abdel-Latif, 2003) working with different plant materials concluded that the percent of grain weight loss decreased with increase in concentration the plant materials used.

Effectiveness of plant extracts after storage:

After 5 weeks of storage all extracts inflicted as high as 94 – 95% mortality on *S. granarius* (Table 5), thus they were giving almost complete protection to grains for more than 5 weeks of storage period. After 8 weeks of storage, all extracts, were only partially effective, the percentage mortality found being only 59, 70 and 42% in grain treated with petroleum ether, chloroform and acetone extracts, respectively.

The present results (5-8 weeks protection) agree with those of other workers on *S. granarius* and other insect species with different plant extracts. *Brassica napus* seed extracts have been found to protect stored wheat grain against *S. granarius* (Al-Moajel, 2000). *B. rapa* seed extracts have been reported to be very effective in protection cowpea seeds against *C. maculatus* (Ahmed *et al*., 2001). *Capparis spinosa* seed extracts gave complete protection to cowpea seeds against *C. maculatus* (Ahmed *et al*., 2002b).

Effect on grain germination and water absorption:

It is quite obvious from Table (6) that wheat grains treated with *S. sesban* seed extracts showed significant reduction viability initially (1-24hrs) and after 8 weeks of storage. The reduction of germination in treated grains after storage (9-16%) was more than observed initially (4-6%). The highest reduction in germination was 6 and 16% in grains treated with acetone extract in initial and after storage time respectively.

There was increase in water absorption due to treatments. Most of treatments at initial time and 8 weeks after storage were significantly different from the control.

These findings in reduction germination and water absorption are in agreement with Ahmed and Mahgoub (1996); Mahgoub *et al*., (1998); Ahmed and Kassis (2000); Mahgoub *et al*., (2000), who observed a reduction in the viability of wheat grains and cowpea seeds treated with plant extracts, as well as that no adverse effects of these plant extracts on water absorption.

Conclusion :

The results obtained in this study suggest good potential for the use of *S. sesban* seed extracts as an insect mortality factor. Concentrations at LC_{95} of petroleum ether, chloroform and acetone extracts (7.4, 3.7, and 15.0 ml/kg) are active as a toxicants against *S. granarius* attacking grain and oviposition deterrents as well. They can give good protection to wheat grain for nearly 2 months, and reduce loss in wheat grain weight.

Considering the above results, *S. sesban* seed extracts have great potentiality in the management of an important stored grain pest such as *S. granarius.*

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استعمال مستخلصات بذور السيسبان *Sesbania sesban* في وقاية حبوب القمح من الإصابة بسوسة القمح *(Coleoptera: Curculionidae) Sitophilus granarius (L.)*

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كلية التربية / الأقسام العلمية

الرياض – المملكة العربية السعودية

المخلص :

أظهرت دراسة استخدام مستخلصات الايثر البترولي والكلوروفورم والأسيتون لبذور السيسبان في وقاية حبوب القمح من الإصابة بحشرة سوسة القمح تأثيراً سميا معنويا لجميع هذه المستخلصات على الحشرات الكاملة. وبحساب التركيزات القاتلة لـ ٩٥٪ من الحشرات تدرجت فعاليتها تنازليا: بمستخلص الكلوروفورم (٣,٧ مل/كجم) ومستخلص الايثر البترولي (٧,٤ مل/كجم) ومستخلص الأسيتون (١٥ مل/ كجم)

منعت جميع المستخلصات الحشرات الكاملة من وضع البيض وبالتالي لم يظهر أي نسل وذلك عند المعاملة بالتركيز القاتل لـ ٩٥٪ من الحشرات ، بينما انخفض وضع البيض بنسبة تراوحت بين ٨٩- ٩٤٪ ، وانخفض النسل بنسبة تراوحت بين ٨٨ – ٩٧٪ وذلك عند استعمال التركيز القاتل لـ ٥٠٪ من الحشرات ، ما عدا مستخلص الكلوروفورم الذي منع ظهور نسل تماماً عند نفس التركيز.

انخفض معدل الفقد في الوزن في جميع المعاملات معنويا عن المقارنة كما عملت المستخلصات على حماية حبوب القمح لمدة أكثر من ٦ أسابيع.

زاد معدل امتصاص حبوب القمح للماء ، وانخفض معدل الإنبات معنوياً وذلك في جميع المعاملات.

لذا ينصح باستعمال مستخلصات بذور السيسبان في وقاية حبوب القمح من الإصابة بسوسة القمح ، إلا أنه لا ينصح باستعمالها في تقاوي الحبوب.