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Control of Rice Weevil, *Sitophilus oryzae* (L.), in Stored Wheat Grains with Mesquite Plant, *Prosopis juliflora* (SW), D.C. Seed Extracts

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مكافحة حشرة سوسة الأرز في القمح المخزن باستخدام مستخلصات بذور نبات الغاف

نادرة المعجل

خلاصة: تم تقييم فعالية مستخلصات بذور نبات الغاف على سوسة الأرز المرباة على حبوب القمح. كان للمستخلصات الثلاثة : الايثر البترولي والكلوروفورم والأسيتون سمية عالية على الحشرات البالغة ، حيث تدرجت فعالية هذه المستخلصات بالتركيزات القاتلة لـ ٩٥% من الحشرات على التوالي كالآتي: الأسيتون (١٢ و ٥,٨ مل /كجم) اقل من الايثر البترولي (٨ و ٤,١ مل / كجم) اقل من الكلوروفورم (٦,٣ و ٢,٢ مل / كجم). كما انخفض معدل وضع البيض انخفاضاً معنوياً عاليا في جميع المستخلصات عند المعاملة بالتركيزات القاتلة لـ ٥٠% من الحشرات، بينما لم يكن هناك أي بيض تقريباً عند المعاملة بالتركيزات القاتلة لـ ٩٥% من الحشرات ، وعلى هذا لم يظهر أي نسل عند هذا التركيز، وكذلك عند المعاملة بمستخلص الأسيتون عند التركيز القاتل لـ ٥٠% من الحشرات .أما عند تخزين الحبوب المعاملة بالمستخلصات الثلاثة فقد كان مستخلص الكلوروفورم أكثر بقاءً، حيث بلغت نسبة الموت ٩٠% بعد شهر من التخزين. كما كانت جميع المستخلصات ذات فعالية عالية في خفض الفقد في الوزن بعد ٤٥ يوماً من المعاملة إلا أن مستخلص الكلوروفورم أكثرها فعالية. لم يكن لغالبية المعاملات تأثير معنوي على معدل امتصاص حبوب القمح للماء إلا أنها أثرت معنويا على معدل الإنبات. كان لمستخلص الايثر البترولي والكلوروفورم تأثير مثبط لإنزيم الكولين استريز بينما كان لمستخلص الأسيتون تأثير منشط لهذا الإنزيم ، أما إنزيم الفوسفاتيز الحمضي فقد كان لمستخلصي الكلوروفورم والأسيتون تأثير مثبط له، إلا أن مستخلص الايثر البترولي كان له تأثير منشط كما كان للمستخلصات الثلاثة تأثير منشط للفوسفاتيز القلوي. كما قلت كمية البروتين والكربوهيدرات في أجسام الحشرات المعاملة معنويا ما عدا مستخلص الأسيتون فقط زاد كمية الكربوهيدرات في أجسام الحشرات المعاملة معنوياً، بينما زادت معنويا كمية الدهون في أجسام الحشرات المعاملة بجميع المستخلصات. لذا ينصح باستخدام مستخلص بذور نبات الغاف في حماية حبوب القمح من الإصابة بسوسة الأرز.

ABSTRACT: The effectiveness of mesquite plant, *Prosopis juliflora* (S.W) D.C. (Family: Mimosaceae), seed extracts against rice weevil, *Sitophilus oryzae* (L.), reared on wheat grains was investigated in the laboratory. The tested plant extracts of *P. juliflora* in petroleum ether, chloroform, and acetone, effectively controlled adults and their toxicity based on LC_{95} and LC_{50} values respectively was in order: acetone (12.0, 5.8ml/kg) $<$ pet ether (8.0, 4.1ml/kg) $<$ chloroform (6.3, 2.2ml/kg). A highly significant oviposition deterency effect ($P < 0.05$) was found for all extracts at LC_{50} levels, while at LC_{95} levels, oviposition was nearly completely inhibited. Thus, progeny emergence was completely suppressed at LC_{95} levels, also at LC_{50} of acetone extract. Chloroform extract indicated a slow rate of degradation after one month of storage (90% mortality). All tested plant extracts reduced weight loss in wheat grains after 45 days of storage, but chloroform extract was the most effective. Most treatments did not significantly affect water absorption but viability was significantly reduced. Petroleum ether and chloroform extracts caused a significant inhibition effect on acetyl choline esterase (AchE) in adults while acetone extract caused a significant activation effect. All three different extracts, caused a significant activation effect on phosphases (AcP and AlkP), except for chloroform and acetone extract treatments which caused significant inhibition of AcP in adults. All extracts caused a significant decrease in protein and carbohydrate contents of adults, except the carbohydrate content of adults treated with acetone extract. There was a significant increase in lipid content in adults treated with all three extracts and significant increase of carbohydrate content only in adults treated with acetone extract.

Keywords: Grain protectants, *Sitophilus oryzae*, acetylcholinesterase, phosphatase, metabolites.

Currently the measures to control pest infestation in stored grain products rely heavily upon the use of conventional insecticides which can lead to problems of toxic residues and environmental contamination (Zettler and Cuperus, 1990; White, 1995).

Indigenous materials of botanical origin are an important source of grain protectants, because they have been found to exhibit toxic effects against insects (Arroyo, 1995). Research on the evaluation of available local plant protection is necessary to help farmers to use these plants, grown locally, to limit post-harvest losses of their products to different insects.

The mesquite plant, *Prosopis juliflora,* is common and widely spread in most parts of Saudi Arabia (Collenette, 1998, 1999; Chaudhary and Al-Jowaid, 1999). The aqueous extracts of the leaves were previously considered to have antibacterial (Satish *et al.*, 1999) and antifungal (Ahmed *et al.*, 1997; Kurucheve *et al.*, 1997; Gomathi and Kannabiran, 2000) properties. The efficacy of *P. juliflora* leaf extracts was reported against *Callosobruchus analis* (Tabassum *et al.*, 1994) and *Plutella xylostella* (Torres *et al.*, 2001). The efficacy of the plant powder and seed extracts against *C. maculatus* were studied by Al-Moajel and Al-Dosary (2002, 2003). Further studies are needed to know the effectiveness of *P. juliflora* on other insect pests. Keeping this in view, our present experiment was undertaken to evaluate the effectiveness of *P. juliflora* seed extracts as a protectant against the rice weevil, by testing effects on adult mortality, egg laying, adult emergence, grain weight loss, residual effects, grain viability, grain water absorption, enzymes and main metabolites of *S. oryzae* adults.

Materials and Methods

REARING TECHNIQUE: *S. oryzae* were cultured in glass jars containing with wheat grains under controlled temperature and relative humidity (27°C and 70% R.H.). The new cultures were prepared by adding 200-300 adults (unsexed) from a stock culture to about 500g of wheat grain in a glass jar. After 3 weeks of the oviposition period, the parent adults were removed, and one week old insects subsequently emerging were used for the experiments. All experiments were conducted under the same condition using wheat grains.

EXTRACTION TECHNIQUE: Mesquite seeds obtained from the local market were washed, dried and ground in an electric grinding machine. A sufficient quantity of powder was extracted with organic solvents (of increasing polarity), petroleum ether, chloroform and acetone as described by Su (1985). The solvents were sequentially used to extract the active ingredients for a period of 48h each at room temperature, then filtered through anhydrous sodium sulfate. A rotary evaporator was used to remove the solvents. The oils obtained in each case were stored in labeled plastic cap bottles at 5°C until required for use (Islam, 1983). The diagrammatic presentation of the whole extraction process is given in Figure 1.

MIXING TECHNIQUE: For all experiments, extracts were added to wheat grains in glass jars using three solvents as a carrier, shaken thoroughly and then solvent was allowed to evaporate in a stream of air.

All treatments were replicated at least three times. In all cases, the experiments were performed in incubators at constant temperature 27°C and 70% R.H. The grains in the control treatments were treated with 0.2 ml of each solvent. All treated jars were covered with pieces of cloth, fastened with rubber bands to prevent contamination and the escape of insects.

ADULT MORTALITY: Four different concentrations were prepared from each extract after preliminary tests. Twenty unsexed adults of *S. oryaze*, were introduced into each jar containing 10g of wheat grains. The effects of *P. juliflora* seed extracts on the survival of adults were assessed by recording mortality at 1, 3, 5, 7 and 14 days after release. Adults were considered as dead when no response was obtained after probing the abdomen with forceps.

Insect mortality was calculated for each concentration using the formula proposed by Abbott (1987). LC_{50} and LC_{95} values during the first three days were calculated by probit analysis (SPSS, 1999). The data were subjected to analysis of variance (ANOVA). Significant differences between treatment means were separated at the P = 0.05 by Duncan's (1951) Multiple Range Test. Standard errors of means were computed.

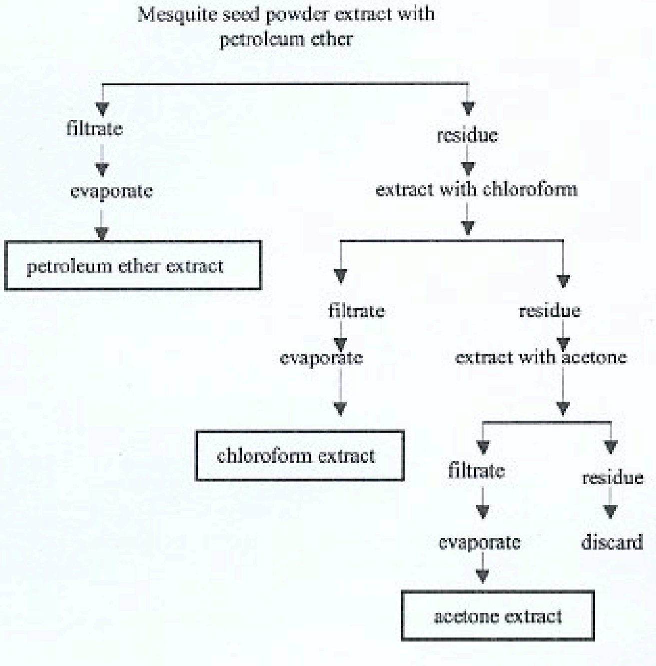

Figure 1. Schematic presentation of mesquite seed extraction.

OVIPOSITION AND ADULT EMERGENCE: Concentrations corresponding to LC_{50}, LC_{95} and controls of either pet-ether, chloroform and acetone extracts were applied to each of three replicates consisting of 5g wheat grains in a glass jar. Ten pairs (1-7 day old) of *S. oryzae* adults were added to each jar. They were sexed following the method described by Halstead (1963). Each jar was covered with a muslin cloth held in place with rubber bands. Observations on egg laying capacity of weevils in each treatment were taken after 10 days.

Acid fucshin stain was employed for the detection of the eggs (Frankenfeld, 1950). The eggs were counted from the stained grain samples. Similar treatments were made to determine total emergence.

After 10 days, the insects were removed, and adults of first generation were removed daily and counted. The following formula was used to determine the percentage reduction in the number of eggs and offspring

$$\% = \frac{X}{Y} x\ 100$$

where X is the number of eggs or adults in the treatment and Y is the number of eggs or adults in the control. Standard errors of the means were computed. For comparisons between treatment and control, t-test was used.

GRAIN WEIGHT LOSS: Percent loss in grain weight was calculated following Khare and Johari (1984). The data were analyzed statistically by using t-test at the 5% significant level. Standard error of means was calculated.

RESIDUAL EFFECT: To assess the persistence of the treatments, each test extract – LC_{95} concentrations including control – was mixed with 1kg samples of wheat grains and stirred continuously for 30 min to ensure even spread of the material over the surface of the grains. Treated grains and controls were allowed to dry for 2 hours before storage.

The grains of each treatment were infested every three days with adult weevils of mixed sex. For each 10 g sample, 20 adults of *S. oryzae* were introduced in each replicate (three replicates from each concentration and the control). Mortality counts were recorded after 3 days, and the data were analyzed to calculate LT_{50} and LT_{95} values.

GRAIN VIABILITY AND WATER ABSORPTION: Viability and water absorption tests of the treated wheat grains were conducted 1 and 30 days after treatment. From each treatment 30 grains with LC_{95} concentrations of all extracts (initial and after storage periods and controls) were kept in three Petri dishes (3 replicates) on two layers of moistened Whatman filter paper. Treatments were moistened daily. Germination was recorded after 10 days (Anonymous, 1966).

Water absorption tests were carried out in small jars three quarters full of distilled water. Two grams of treated grains and controls were submerged in water for 1, 5 and 24 hr (Tikka *et al.*, 1981; Qi and Burkholder, 1981; and Sighamony *et al.*, 1986). At the end of each period, the grains were dried with filter paper and re-weighed to estimate the water absorbed.

Means were recorded and reduction in germination and water absorption were determined. Results were analyzed by ANOVA, and means were compared using Duncan's Multiple Range Test.

ENZYMES ASSAY:

Esterases

Acetylcholine esterase (AchE) - Acetylcholine esterase (AchE) was measured according to the method described by Simpson *et al.* (1964).

Phosphatases

Phosohatases enzymes (AcP and AlkP) -_Acid phosphatase (AcP) and alkaline phosphatase (AlkP) were determined according to the method described by Powell and Smith (1954).

Main metabolites

Total proteins: - Total proteins were determined by the method of Bradford (1976).

Total carbohydrates: - Total carbohydrates were determined by the method described by Singh and Sinha (1977).

Total lipids: -_Total lipids were estimated according to Knight *et al.* (1972).

Results were analyzed using t-test at 5% significant level.

Results and Discussion

EFFECT ON ADULT MORTALITY: The data in Table 1 show that *P. juliflora* seed extracts were remarkably effective causing mortality after 3 days at almost all concentrations of *S. oryzae*, However, within 1 day of exposure to wheat grains treated with pet-ether and chloroform extracts, 20 and 25% of the weevil adults respectively were killed at 5 ml/kg.

After 5 days of exposure, one hundred percent mortality was achieved by pet-ether, chloroform and acetone extracts at 5, 3 and 10 concentrations, respectively. A similar trend in mortality was observed with most other extracts after 7 days of exposure.

The effects of higher and lower concentrations of extracts on adult mortality was almost significantly different from each other. Mortality was directly proportional to the concentration level and to time. When each treatment was compared with the control, a significant difference was obtained ($P < 0.05$).

TABLE 1

Average mortality of* S. oryzae *adults when exposed to wheat grain treated with different concentrations of* P. juliflora *seed extracts.

Extract	Concentration (ml/kg)	% Cumulative mortality* Days 1	3	5	7	14
Pet-ether	3	0^{ade}	30^{abch}	71^{ab}	73^{ac}	79^{a}
	4	2^{ade}	40^{abi}	82^{abc}	85	100^{b}
	5	20^{bcefg}	60^{cgij}	100^{ce}	-	-
	6	29^{bcfg}	85^{dfg}	100^{ce}	-	-
Control		0	0	0	0	4
Chloroform	1	0^{ade}	20^{afh}	54^{d}	70^{acd}	74^{a}
	2	0^{ade}	30^{abch}	81^{abc}	100^{bc}	-
	3	8^{abdef}	57^{cgij}	100^{ce}	-	-
	5	25^{bcfg}	95^{d}	100^{ce}	-	-
Control		0	0	2	2	4
Acetone	4	0^{ae}	20^{acfh}	52^{d}	62^{cd}	73^{a}
	6	4^{ade}	46^{bcgi}	90^{bce}	100^{bde}	-
	8	16^{bcef}	63^{cg}	94^{bce}	100^{bde}	-
	10	32^{bcg}	95^{df}	100^{ce}	-	-
Control		0	0	0	4	8
F-ratio		7.82	30.42	17.13	18.04	16.93
F-tabulated				2.216		
LSD		21.51	8.25	15.19	15.00	18.36

*Each datum point is a mean of three replicates and was corrected for mortalities in the control (Abbott's formula).
- Mortality means in each column differ significantly at the significance level indicated for each column (ANOVA).
- Means in the same column with the same letters are not significantly different (Duncan's Multiple Range Test P <0.05).

directly proportional to the level concentration and to time. When each treatment was compared with the control, a high significant difference was obtained ($P < 0.05$).

The slopes of the probit lines were steeper as concentration increased (Table 2). On the basis of relative toxicity at both levels (LC_{50} and LC_{95}), the treatment may be summarized as: chloroform extract (2.2, 6.3 ml/kg) > pet-ether extract (4.1, 8.0 ml/kg) > acetone extract (5.8, 12.0) at LC_{50} and LC_{95} values respectively. The data on adult mortality showed a strong relationship between mortalities and extract concentrations and more than 50% of adult mortality occurred at all tested concentrations of all extracts after an exposure period of 5 days. Hence, the ANOVA showed statistically significant differences. Consequently, the tested concentrations of the plant extracts are sufficient to cause significant mortalities. This was reported by Naqvi and Parveen, (1991), Tabassum *et al.* (1994) and Al-Moajel, (2003).

Recently, many researchers have worked on the pesticidal properties of plant extract essential oils against *S. oryzae* (Risha *et al.*, 1990; Chander *et al.*, 1991; Shaaya *et al.*, 1991, 1997; Niber *et al.*, 1992; Chimbe and Galley, 1996; El-Lakwah *et al.*, 1998; Kestenholz and Stevenson, 1998; Owusu, 2001). In respect of *P. juliflora* plants, only three studies have been carried out on the toxicity of leaf extracts or juliflorine on stored-product insect species. Juliflorine was found to be the principal bioactive compound in leaves for use

TABLE 2

***Toxicity of* P. juliflora *seed extracts applied to wheat grains against adults of* S. oryzae.**

Extract	Slope	LC_{50} (ml/kg)	LC_{95} (ml/kg)
Pet-ether	5.70	4.1	8.0
Chloroform	3.52	2.2	6.3
Acetone	5.20	5.8	12.0

against *Musca domestica* larvae (Jahan *et al.*, 1990) and *Calloso-bruchus analis* (Tabassum, *et al.*, 1994). *P. juliflora* leaf extract was also effective on *Plutella xyostella* larvae (Torres *et al.*, 2001). No reference was found on the effect of seeds except for our earlier work (Al-Moajel and Al-Dosary, 2002, 2003) on *C. maculatus* insect. Adults of *C. maculatus* were more susceptible than adults of *S. oryzae.* This is in agreement with Shaaya, *et al.* (1997). They found that out of four major stored-product insects, *S. oryzae* showed the highest tolerance to the oils tested.

Additionally, pet-ether extract has been found most toxic against *C. maculatus* (Al-Moajel and Al-Dosary, 2003), whereas in this study, chloroform extract was most toxic to *S. oryzae*.

EFFECT ON OVIPOSITION AND ADULT EMERGENCE BEHAVIOR: Table 3 compares the ovipoitional response of *S. oryzae* exposed to the extracts of *P. juliflora* seeds. The mean number of eggs laid in grain treated with mesquite extracts, both at lower and higher concentrations (LC_{50} and LC_{95}), was observed to be minimum (0.7-17 eggs). Maximum deterrence of oviposition was obtained from all extracts at LC_{95} concentrations (98-99%), while 84-92% ovipositional reduction was observed at LC_{50} concentrations. When adults were fed with wheat grains treated with solvents only (controls: pet-ether, chloroform and acetone), the mean number of eggs laid was 76.4, 94.7, 106.7 eggs per 10 pairs, respectively. It is apparent from the data presented in Table 3 that a significant oviposition deterency effect ($P < 0.05$) was found for all extracts at LC_{50} concentrations, while at LC_{95} concentrations, oviposition was nearly completely inhibited.

A 97-85% reduction in progeny was observed at LC_{50} concentrations of pet- ether and chloroform extracts, respectively. T-test analysis showed that there were significant differences, while progeny emergence was completely suppressed in grains combining LC_{95} concentration of all extracts, and also at LC_{50} of acetone extract.

Complete reduction in progeny of *S. oryzae* reared on wheat grains treated with LC_{95} concentrations could be possible based upon the observed high adult mortality. At LC_{50} concentration of acetone extract, egg laying was reduced by 86%, but progeny emergence was reduced by 100%, thus acetone extract could be considered as an ovicide for *S. oryzae*.

TABLE 3

Efficacy of different seed extracts of* Prosopis juliflora *against* S. oryzae *for ovipositional and progeny deterent properties in wheat grain.

Extract	Concentration (ml/kg)	Average no. of eggs laid/10 pairs ± SE	T-value	Oviposition reduction %	Average no. of emergers ± SE	T-value	Emergence reduction %
Pet-ether	Control	76.4 ± 0.38			29.7 ± 1.2		
	LC_{50} (4.1)	5.2 ± 0.61	-98.68	92*	0.7 ± 0.12	-24.19	97*
	LC_{95} (8.0)	0.7 ± 0.06	-194.82	99*	0.0 ± 0.00	-	100
Test of Significance			S			S	
Chloroform	Control	94.7 ± 2.40			25.7 ± 0.0		
	LC_{50} (2.2)	15.0 ± 1.20	-29.79	84*	3.7 ± 0.00	-65.99	85*
	LC_{95} (6.3)	1.7 ± 0.00	-28.54	98*	0.0 ± 0.00	-	100
Test of Significance			S			S	
Acetone	Control	106.7 ± 3.50			34.7 ± 0.93	-	
	LC_{50} (5.8)	17.0 ± 1.70	-22.79	86*	0.0 ± 0.00	-	100
	LC_{95} (12.0)	0.7 ± 0.15	-29.97	99*	0.0 ± 0.00	-	100
Test of Significance			S			S	

*S = Significant ($\alpha = 0.05$).

Plant extracts are known to significantly reduce progeny emergence of stored grain and pulse insects (Kelany *et al.*, 1991; Seck, *et al.*, 1993; Ho *et al.*, 1994; Talukder and Howse, 1995; Huang, *et al.*, 1997; Dwivedi and Kumar, 1998; Elhag, 2000; Papachristos and Stamopoulos, 2002).

Some workers have observed a reduction in oviposition of *S. oryzae* weevils on grains treated with plant extracts (Risha *et al.*, 1990; Su, 1990, Schmidt *et al.*, 1991; Mostafa *et al.*, 1995, Mahgoub *et al.*, 1998; Ahmed, 2000, 2002). These results were confirmed by Al-Moajel and Al-Dosary (2003), who reported that *P. juliflora* seed extracts were effective in reducing the oviposition and progeny emergence of *C. maculatus*.

RESIDUAL EFFECT: All the seed extracts at LC_{95} concentrations gave almost complete protection until 15 days of storage (Table 4), but gradually the residual toxicities of pet-ether and acetone extracts decreased with length of storage. So, after 30 days of storage, the number of killed insects in the treated wheat grains was only 75 and 40% respectively, while in the grains treated with chloroform extracts 90% mortality of *S. oryzae* was recorded after this period of exposure. Consequently, adult mortality in chloroform extracts over various storage durations seem to have remained almost as the initial activity. The chloroform extract of *P. juliflora* seeds may give a higher degree of protection for stored wheat grains against *S. oryzae*, while the two other extracts were less effective. No mortality was recorded in solvent controls.

It should also be noted that effectiveness of LT_{95} concentrations of chloroform extract lasts longer than those of pet-ether and acetone. It can be concluded that chloroform extract indicated a slow rate of degradation after 1 month of storage.

Similar result on the effectiveness of plant extracts for nearly one month of storage were also obtained by Ahmed and Kassis, (2000) with *Lupinus termis* extracts against *C. maculatus*; Al-Moajel and Abd El-Baki, (2000) with *Brassica rapa* extracts against *R. dominica*; and Ahmed *et al.*, (2002) with *Capparis spinosa* extracts against *C. maculatus*. On the other hand, some plant extracts were effective as adulticide over 2-8 months of storage: Kelany *et al.* (1991) and Mahgoub (1992) with neem extracts against *C. chinensis* and *C. maculatus* respectively; Mostafa *et al.* (1995) with *Nigella sativa* extracts against *S. oryzae* and Al-Moajel (2000) with *Brassica napus* against *S. granarius*. In respect of *P. juliflora* extracts, these results are in agreement with the findings of Al-Moajel and Al-Dosary (2002) on cowpea seeds against *C. maculatus*.

TABLE 4

Susceptibility of* S. oryzae *adults to wheat grains treated with* P. juliflora *seed extracts after different intervals of storage.

Intervals of storage (days)	% Adult mortality Pet-ether	Chloroform	Acetone	Control
Initial	100	98	98	0
3	96	96	95	0
6	96	95	96	0
9	95	96	96	0
12	94	94	94	0
15	95	96	94	0
18	93	95	90	0
21	88	95	85	0
24	85	95	60	2
27	82	90	52	0
30	75	90	40	0
Slope	-3.73	-1.86	-2.21	
LT_{95}	12	14	8	
LT_{50}	33	104	43	

TABLE 5

Effect of* P. juliflora *seed extracts on germination of wheat grains initially and 30 days after treatment (DAT).

Extract and concentration (ml/kg)	Initially		30 days after treatment	
	% Germination ± SEM	% Reduction	% Germination ± SEM	% Reduction
Control	$98 + 1.15^{bcd}$		$96 + 1.00^{cde}$	
Pet-ether (8.0)	$92 + 0.00^{ab}$	6.2	$90 + 0.00^{abc}$	6.3
Chloroform (6.3)	$88 + 1.15^{c}$	10.2	$84 + 2.00^{ad}$	12.5
Acetone (12.0)	$84 + 2.31^{ad}$	14.2	$81 + 0.58^{bc}$	15.6
F-ratio	17.82		33.19	
F-tabulated		4.07		
LSD	4.61		3.79	

Means within column followed by same letter are significantly different at $P < 0.05$.

TABLE 6

Effect of* P. juliflora *seed extracts on water absorption of wheat grains initially and 30 days after treatment.

Extract and concentration (ml/kg)	% Water absorption					
	Initially			30 days after application		
	1 hr	5 hrs	24 hrs	1 hr	5 hrs	24 hrs
Control	18^{b}	32^{b}	51^{a}	14^{a}	28^{a}	50^{a}
Pet-ether (8.0)	21^{ab}	35^{a}	52^{a}	17^{a}	31^{a}	50^{a}
Chloroform (6.3)	23^{a}	38^{a}	55^{a}	14^{a}	29^{a}	50^{a}
Acetone (12.0)	23^{a}	38^{a}	56^{a}	14^{a}	27^{a}	47^{a}
F-ratio	4.47	37.50	2.96	2.25	13.2	1.59
F-tabulated			4.0661			

Means followed by the same letter are not significantly different at $P < 0.05$, comparison made for columns.

EFFECT ON GRAIN VIABILITY: Data in Table (5) show that the germination of grains treated with pet-ether, chloroform and acetone extracts at the rates of 8.0, 6.3, and 12.0 ml/kg respectively was significantly reduced initially or after storage time. All treatments gave 6.2 – 15.6% reduction in germination. The lowest germination was in grains treated with acetone extract (84-81%), at two intervals: initially and after 30 days of storage, respectively.

Our results are in agreement with Khaire *et al.* (1992), Pacheco *et al.* (1995) and Abdel-Latif (2003), who reported adverse effects of plant extract and oil treatments on germination of seeds and grains, significantly reducing percentage germination.

On the contrary, others found negligible effects of other plants on germination (Singh and Singh, 1990).

EFFECT ON WATER ABSORPTION: Table 6 shows that in initial studies (1 hour after application), chloroform and acetone treatments absorbed significantly more water than the control. Also 5 hours after application all three extract treatments absorbed significantly more water than the controls. Other treatments at initial time and all treatments after the storage period (30 days) recorded negligible (not significant) effects on the amount of water absorbed.

This effect has been reported in previous studies with other plants (Begum and Quiniones, 1990; Mahgoub *et al.* 1998; Shemais and Al-Moajel, 2000), found no effect on water absorption. Tembo and Murfitt (1995), however, reported significant effects of some plant oils on water absorption.

EFFECT ON WEIGHT LOSS: Table 7 indicated that the percentage losses in grain weight in different treatments ranged 1-5%, while the percent weight loss was maximum in controls (11.33%).

Chloroform extract at LC_{95} concentration was found to be the best one preventing the damage, recording only 1% loss. There were significant differences ($P < 0.05$) between the weight loss of treated wheat grains and controls after 2 months. The percentage losses in grain weight were significantly lower in grain treated with acetone extract ($t = 6.01 - 6.29$) than in grain treated with both other extracts, and the percentage weight loss was significantly higher in wheat grains treated with chloroform extract ($t = 8.32 – 11.72$) at LC_{50} and LC_{95} concentrations, respectively.

Consequently, chloroform extract at LC_{95} was most effective in reducing the grain weight loss which was 91%. All plant extracts protect the grains against feeding by *S. oryzae*.

The effectiveness of some plant materials: powders, oils and extracts, on weight loss reduction have been reported in several studies on other insect species (Begum and Quiniones, 1990; Shivanna *et al.*, 1994; Singh, 1995; Keita *et al.*, 2001; Abdel-Latif, 2003) and on *S. oryzae* (Niber, 1994; Chimbe and Galley, 1996).

EFFECT ON ESTERASE AND PHOSPHATASE ENZYMES: Table 8 indicates the acetylcholinesterase (AchE) and phosphatases (AcP and AlkP) enzyme activity of *S. oryzae* adults treated with LC_{50} of *P. julifora* seed extracts after 72 hours of exposure. The data revealed that in the pet-ether and chloroform extract treatments, AchE enzyme decreased significantly (1101.48 and 1057.67

TABLE 7

Grain weight losses caused by* S. oryzae *weevils infesting stored wheat grains treated with* P. juliflora *seed extracts.

Extract	Concentration (ml/kg)	% Loss in weight ± SE	T-value	% Protection
	Control	11.33 ± 0.88		
Pre-ether	LC_{50} (4.1)	3 ± 0.58	7.91	75
	LC_{95} (8.0)	2 ± 0.00	10.58	83
Test of significance		S		
	Control	11.33 ± 0.88		
Chloroform	LC_{50} (2.2)	4 ± 0.00	8.32	66
	LC_{95} (6.3)	1 ± 0.00	11.72	91
Test of significance		S		
	Control	11.33 ± 0.88		
Acetone	LC_{50} (5.8)	5 ± 0.58	6.01	58
	LC_{95} (12.0)	4 ± 0.76	6.29	66
Test of significance		S		

*S = Significant ($\alpha = 0.05$).

TABLE 8

Effect of* P. juliflora *seed extracts (LC_{95}) on the rate of acetylcholinesterase (AchE) and Phosphatases (AcP and AlkP) in* S. oryzae *72 hrs after treatment.

Extract and concentration (ml/kg)	AchE (µg/g/min)				AcP (µg/g/min)				AlkP (µg/g/min)			
	Mean ± SE	95% Confidence interval Lower	Upper	T-value	Mean ± SE	95% Confidence interval Lower	Upper	T-value	Mean ± SE	95% Confidence interval Lower	Upper	T-value
Control	1222.07 ± 16.15	1190.89	1244.92		281.39 ± 4.97	179.11	292.74		4.63 ± 0.16	4.31	4.85	
Pre-ether (4.1)	1101.48 ± 42.82	1027.39	1175.73	23.39	311.63 ± 5.55	303.48	322.23	38.14	6.33 ± 0.23	6.08	6.79	-404.06
Test of significance		S				S				S		
Chloroform (2.2)	1057.67 ± 68.39	950.34	1184.77	14.00	233.65 ± 8.29	221.34	249.43	16.12	5.41 ± 0.31	4.92	5.97	-310.01
Test of significance		S				S				S		
Acetone (5.8)	1230.41 ± 26.89	1199.89	1284.01	42.05	253.85 ± 4.19	247.17	261.56	36.76	10.95 ± 0.39	10.19	11.47	-228.59
Test of significance		S				S				S		

S = significant ($\alpha = 0.05$).

µm/g/min for the two extracts, respectively) compared with 1222.07 in the control. But in case of acetone extract, AchE activity increased slightly (1230.41 µm/g/min).

AcP activity was significantly inhibited by the use of chloroform and acetone extracts (233.65 and 253.85 µm/g/min respectively) compared with 281.39 in the control, while at pet-ether extract AcP was significantly increased (311.63).

On the other hand, AlkP activity increased at all tested extracts, reaching 6.33, 5.41 and 10.95 µm/g/min with pet-ether, chloroform and acetone extracts treatments, respectively. Acetone extract caused the greatest effect.

So pet-ether and chloroform extracts caused a significant inhibition of AchE, and similar effects on AcP.

On the contrary, all treatments caused a significant activation of AlkP. AcP was activated in the pet-ether treatment. Ahmed (2000) found that after 72 hours of exposure, *Ricinus communis* seed extracts had caused an inhibition effect in *S. oryzae* adults.

EFFECT ON METABOLITES: Data presented in Table 9 shows that after 72 hours of *P. juliflora* seed extract treatments, the total protein content significantly decreased at all extracts. Protein content was the lowest (13.78 mg/g) in adults treated with chloroform extract, and similar in pet-ether and acetone extracts (16.59 and 16.48, respectively) compared with 20.08mg/g in the control treatment.

On the contrary, total lipids were significantly increased by all extracts. Total lipids were the highest (36.95 mg/g) in the chloroform extract treatment, whereas at pet-ether and acetone extracts treatments, total lipids had a significant normal increase (a mean of 22.25 and 21.58 mg/g respectively) compared with 20.02 mg/g in the control. Meanwhile, the carbohydrate content was significantly higher in adults treated with

TABLE 9

Effect of* P. juliflora *seed extracts (LC_{50}) on the rate of main metabolites in* S. oryzae *72 hrs after treatment.

Extract and concentration (ml/kg)	Total proteins (mg/g)				Total lipids (mg/g)				Total carbohydrates (mg/g)			
	Mean ± SE	95% Confidence interval Lower	Upper	T-value	Mean ± SE	95% Confidence interval Lower	Upper	T-value	Mean ± SE	95% Confidence interval Lower	Upper	T-value
Control	20.08 ± 0.67	18.85	21.17		20.02 ± 0.97	18.30	21.65		14.17 ± 0.43	13.41	14.91	
Pre-ether (4.1)	16.59 ± 0.60	15.89	17.79	-138.07	22.25 ± 0.47	21.47	23.09	-165.91	6.20 ± 0.21	5.8	6.50	-404.06
Test of significance		S				S				S		
Chloroform (2.2)	13.78 ± 0.24	13.32	14.12	-362.60	36.95 ± 1.39	34.32	39.03	-45.43	11.24 ± 0.50	10.34	12.08	-176.47
Test of significance		S				S				S		
Acetone (5.8)	16.48 ± 0.14	16.21	16.69	-589.11	21.58 ± 1.34	19.39	23.16	-69.15	15.08 ± 0.44	14.37	15.87	-195.41
Test of significance		S				S				S		

S = significant ($\alpha = 0.05$).

acetone extracts (15.08 mg/g), and significantly lower (6.20 and 11.24 mg/g) under pet-ether and chloroform extracts respectively, compared with 14.17 mg/g in the control.

All extracts caused a significant decrease in protein and carbohydrate contents of adults, except the carbohydrate content of adults treated with acetone extract; meanwhile there was a significant increase of lipid and carbohydrate contents in adults treated with all extracts.

The results showed that the lowest amount of protein and the highest amount of lipid was in the chloroform extract treatments, and the lowest amount of carbohydrate was in pet-ether extract treatment. Reduction in protein and carbohydrate contents and increase of lipid content with *P. juliflora* seed extracts may be due to its prevention action. These results of increasing lipid are in agreement with Mostafa and Sherif (1993) who used different plant powders.

Conclusions

To the best of our knowledge, no study has been reported previously concerning the activity of seeds of *P. juliflora* as protectant. Seeds of *P. juliflora* plant are useful grain protectant. These results indicate that in addition to its toxic effect on *S. oryzae* adults, the fecundity of adults and weight loss of wheat grains were reduced. Chloroform extract was the most effective on adult mortality with more active of residual effects and less gram weight loss than the other tested extracts. Further research into the constituents and bioactivity of *P. juliflora* seed extracts is needed.

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