J. Agric. Sci. Mansoura Univ., 30 (1): 623 - 634, 2005

EFFICIENCY OF *Eichhornia crassipes* AS PROTECTANT AGAINST SOME STORED PRODUCT INSECTS

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ABSTRACT

Powder and 3 solvent crude extracts obtained from dry ground leaves of *Eichhornia crassipes* (Mart) Solm. (Pontederaceae) using acetone, methanol and petroleum ether were evaluated under laboratory conditions (30 ±1°C, 65±5% rh) for their bioinsecticidal activity against two strains (laboratory and resistant) of *Triboleum castaneum* (Herbst.), *Sitophilus oryzae* (L.) and *Callosobruchus maculatus* (F.). All levels of the dry ground leaf concentrations inhibited F_1 progeny production and adult emergence of the tested insects. The dosage of 1% of petroleum ether extract killed 100% of *S. oryze* and *C. maculatus* after 9 days of application, while caused 26% mortality of *T. castaneum* at the same exposure time. Also, all levels of tested extracts have deterioration effect on biology of both *S. oryzae* and *C. maculatus*. *C. maculatus* adults were more tolerant than other tested insects at 2 days posttreatment with LC_{50} of 345.97 μg/cm^2 except for *T. castaneum* (RS) which had LC_{50} of 503.22 μg/cm^2, however it exhibited no resistance to petroleum ether extract. Acetone and methanolic extracts exhibited low toxicity against the tested insects.

INTRODUCTION

Annual post harvest losses caused by insect damage, microbial deterioration, and other factors are estimated to be in the order of 10-25% world-wide Matthews 1993. Research on insecticide from plants has been conducted for many years by numerous workers. Excellent reviews of the literature in this area have been provided by Mclndoo 1945, Jacobson (1958, 1975); Su (1977, 1990); Malik and Nagvi 1984; Delobel and Malonga 1987; Weaver *et al*. 1991; Khaire *et al*. 1992; Hu *et al*. 1993, Xie *et al*. 1995; Miyazaki 1996; Mansour 1996; Rajapakse and Senanayake 1997; El-Aidy and Helal 1997; Dwivedi *et al*. 1998; Mahgoub *et al*. 1998; El-Lakwah and El-Kashlan 1999; Keita *et al*. 2000 and Raja *et al*. 2001. With the advent of synthetic pesticides, research on plant derived pesticides diminished. The synthetic pesticides, however, have come under increasing attack in recent years due to persistence in the environment, insect resistance, and high mammalian toxicity.

On the other hand, plant-derived pesticides are more readily biodegraded therefore, they are less likely to contaminate the environment and may be less toxic to mammals (Freedman *et al*. 1979. Water hyacinth, *Eichhornia crassipes* (Mart) Solm. (Pontederaceae) is one of the most troublesome and prolific aquatic weeds. It grows abundantly in lakes and ponds and a high growth rate coupled with ability to absorb several nutrients and water pollutants, has led interesting proposals for its utilization (Gopal and Sharma, 1981). Studies have shown that the acetone extract of water hyacinth petiole is capable of inhibiting reproduction as well as inducing

morphological abnormalities in *Dysdercus cingulatus* (F.) and *Tribolium castaneum*, besides producing ovicidal and toxic effects in *D. cingulatus* (Jamil *et al*., 1984). Sita and Thakur (1984) determined the effects of water hyacinth paper on *Dysdercus similis*. They concluded that *E. Crassipes* has an active growth regulating factor for *D. similis*.

Siddiqui and Alam (1989) found that tested plant parts extracts of water hyacinth against *Meloidogyne incongita* and *Rotylenchulus reniforms* attacking Tomato and eggplant effectively controlled the tested insects. Also, the extracts exhibited nematocidal, nemato-static properties, retarded nematode development and plant damage.

Begum *et al*. (1993) showed that leaf extracts of the aquatic weed *E. crassipes* had an antifeedant effect on larvae of the noctuid, *Spodoptera litura*, reducing the area of leaf consumed. Water hyacinth is unknown as a toxic to mammals. The objectives of study were to evaluate toxicity and reproduction inhibiting effects of powder and 3 water hyacinth extracts of different solvents against three stored-product insect pests.

MATERIALS AND METHODS

Tested insects :

This study was carried out at Stored Products Research Department, Plant Protection Institute-Sakha Research Station Kafr El-Sheikh.

The rust red flour beetle, *Tribolium castaneum* (Herbst.); the rice weevil, *Sitophilus oryzae* L., and the cowpea beetle, *Callosobruchus maculatus* (F.) were reared in the laboratory in jars under 30 ± 1°C and 65 ± 5% R.H. The rearing media contained wheat seeds for *S. oryzae*, wheat seeds mixed with wheat flour plus 5% Brewer's yeast for *T. castaneum*, cowpea seeds for *C. maculatus*. The powder prepared from dry leaves of *E. crassipes* was mixed with the grains at different dosages ranging from 1-20% (wt/wt) for *S. oryzae* and from 5-20% (wt/wt) for *C. maculatus*. An additional strain of *T. castaneum* resistant to malathion was obtained by selection pressure. Two days old of fourth instar larvae of *T. castaneum* were used.

Selection pressure on *T. castaneum* :

Adults of *T. castaneum* reared in the laboratory as susceptible strain (2-3 week old) were exposed to the LC_{25} of malathion for 24 hours by thin film technique using Petri dishes. The surviving insects were transferred to clean glass jars containing sterilized media. After 20 days the tested parent adults were removed and after 60 days, the adults of the new generation were treated in the same manner. Selection pressure was repeated for ten generations. In every generation, LC_{50} of malathion was determined using thin film technique.

2- Plant materials :

The water hyacinth leaves collected from the local canals were washed with tap water and then with distilled water. The leaves were dried in shade, ground, and extracted with three different solvents individually

(acetone, methanol and petroleum ether) according to Freedman *et al.* (1979), 250 g samples each was separately soaked in 750 ml of the solvent in a large conical flask for 72 hrs with shaken for 3 hrs., the contents of the flask were filtered through anhydrous sodium sulphates using filter paper. The extract concentrated by removing the solvent on water bath at 40°C to obtain the crude extract. The obtained extracts were weighed and redissolved in an appropriate volume of pure acetone, and kept in the refrigerator till it were assayed.

3- Chemical used :

Malathion 57% EC. O, O, dimethyl phosphorodithioate ester of diethyl mercapto succinate.

Methods of application

The toxicity of powder and 3 extracts with 3 different solvents of water hyacinth leaves was assayed by thin film residue, mixing with media, and by dipping methods.

Thin film residue method was used with the all tested insects, while mixing with media technique was used against adults of *S. oryzae*, *T. castaneum* and *C. maculatus*. Dipping method was applied on 2-day-old fourth instar larvae of *T. castaneum* only.

Thin film residue (Surface treatment):

A residual film was prepared by dissolving the considerable concentrations of each extract with acetone, 1 ml from each concentration was spread into Petri-dish (9cm in diameter) by moving the dishes gently in circle. The Petri-dishes used as a control were treated only with one ml acetone. The acetone evaporated in a few minutes leaving thin film of the tested extracts on the surface of the Petri-dishes. Ten adults of the tested insects, *S. oryzae*, *T. castaneum* (7-14 days old), *C. maculatus* (0-24 hrs old), and larvae of *T. castaneum* were realesed separately on thin film on each dish. Each concentration was replicated three times. Mortality percentages were recorded after different periods (2, 4 and 6 days) and all obtained results were corrected for natural mortality (control) by using Abbott's formula (Abbott, 1925). LC_{50} values were calculated by the method of Litchfield and Wilcoxon (1949).

Mixing with media :

For seed treatment 20 g (wheat grain and cowpea seeds) for both (*S. oryzae* and *T. castaneum*) and *C. maculatus*, respectively were mixed with certain different concentrations of the tested extracts ranging from 1-20% (wt/wt) for *S. oryzae* and from 5-20% (wt/wt) for *C. maculatus*. Treated samples were placed in small glass jars (11.5 by 6 cm diameter). The jars were shaken by hand to mix the media with the extracts. The jars with extracts were left a convenient time until the solvent evaporated, each concentration was replicated three times. The jars with solvent free of extracts served as control treatment. Five pairs of newly emerged adults of *S. oryzae, T. castaneum,* and *C. maculatus* were transferred to each jar,

covered with muslin cloth and kept under laboratory conditions, death counts were recorded after different intervals, results obtained were corrected with Abbott's formula.

Effect on the progeny and infestation :

Samples each of 40 gm from wheat grains or cowpea seeds grains for *S. oryzae* and *C. maculatus*, respectively were thoroughly mixed with different concentrations of the tested extracts or powder of water hyacinth leaves. Treated samples were placed into ¼ liter glass jars. The jars with extracts were left a convenient time until the solvent evaporated, each concentration was replicated three times. The jars with solvent free of extracts or powder served as control treatment. Unsexual ten pairs of *S. oryzae* (0-15 days old) were transferred to each jar. The infested jars were then covered with muslin. Adults of *S. oryzae* were allowed to oviposit for 10 days and then removed and jars were left for 7 weeks, however the grains were kept for development of insects. The emergence adults were counted and removed. For *C. maculatus* 10 adult females and 10 adult males were transferred to a jar containing treated seeds. Exposure continoued for three days then the survival were removed. After six days, the number of deposited eggs and their hatchability were recorded, while after 30 days, the progeny as number of adults were recorded. Kernels which showed exit holes of insects were separated from the samples and counted to estimate the percentage of external infestation according to the following equation :

$$\% \text{ infestation} = \frac{\text{N infested kernels}}{\text{N total kernels (sound and infested)}} \times 100$$

Percent of sterility was calculated using Chamberlain formula (1962) which was modified by Toppozada *et al*. (1966) as follows:

$$\% \text{ sterility} = 100 - \left[\frac{a \times b}{A \times B} \times 100 \right]$$

Where:
a = number of eggs laid/ treatment female.
b = % of hatchability of treatment females.
A= number of eggs laid/untreated female (control).
B= % of hatchability of untreated females (control).

Analysis of data :

Duncan's (1955) multiple range test was used to compare means at 0.05 level of probability, where the analysis of variance was significant.

Dipping method :

In this experiment, 2-days-old fourth instar larvae of *T. castaneum* were used. Larvae were dipped in each concentration for 5 sec. The treated larvae were then transferred to normal food media. Control larvae were dipped at the same manner in acetone. Mortality was recorded after different periods and corrected with Abbott's formula.

RESULTS AND DISCUSSION

Selection pressure for ten successive generations succeded in building up resistance in *T. castaneum* to malathion (Resistant factor : 65.5) (Table 1). Although malathion could be considered as a standard insecticide for stored grain insects (Oudejans 1991), resistance of *T. castaneum* to its action has grown in both scope and intensity Speirs *et al.* (1967); Zettler (1974, 1982); Warui (1976); Bansode & Campbell (1979); Horton (1984); Abo Arab (1989); Zettler & Cuperus (1990); Zattler & Arthur (1994) and Ibrahim (2000).

Table (1): Level of resistance of *T. castaneum* (Herbst.) selected by malathion for ten generations.

Strain	LC_{50} (Mg/cm^2)	RF*
Resistant strain (RS)	20.57	65.5
Laboratory strain (LS)	0.314	-

*** RF : Resistant factor : LC50 of malathion to RS/LC50 of malathion to LS.**

Except for the resistant strain of *T. castaneum*, the results obtained in Table (2) show the toxicity of petroleum ether water hyacinth extract to the tested insects after 2, 4 and 6 days post-treatment using thin film technique. At 2 days cowpea weevil, *C. maculatus* was the most tolerant compared with the remaining tested insects (LC_{50}'s : 345.97, 204.43 and 119.52 μg/cm^2) for *C. maculatus, T. castaneum* (LS) and *S. oryzae*, respectively. After 4 days *T. casteneum* (LC_{50} : 180.85 μg/cm^2) was the least susceptible compared with the other tested insects. *S. oryzae* and *C. maculatus* were more susceptible than the other investigated insects at 6 days post-treatment. Malathion resistant strain of *T. castaneum* showed no cross-resistance to the tested extract where the resistant factor (R.F.: LC_{50} of malathion to RS/LC_{50} of malathion to LS) was 2.46, 1.56 and 0.62 fold, at 2, 4 and 6 days, respectively. In this respect the tested extract had the lowest effect on *T. castaneum* larvae in compared with the nature adult insects tested at 6 days with the exception of *T. castaneum* adults (LS). The different action of the same extract against the tested adults and larvae may due to the behaviour and span life of the stage.

All tested insects mentioned before did not any adverse effects after exposure to treated surfaces in Petri dishes with the acetone water hyacinth extract. On the other side methanolic water hyacinth extract produced 20% mortality when applied against adults of *T. castaneum*, *S. oryzae* and *T. castaneum* larvae after 5 days of treatment of the highest concentration used (550.4 μg/cm^2). For *C. maculatus* it caused 10% mortality at 8 days of treatment with the same concentration.

Table (2): Toxicity of petroleum ether water hyacinth extract to adults of certain stored grain insects and larvae of *T. castaneum* by using thin film technique at 2, 4 and 6 days.

Insects	Time	LC_{50} µg/cm²	C.L.(1)	S.V. (2)	R.F
T. castaneum (LS)	2	204.43	228.97 - 182.53	4.35	
	4	180.85	215.21 - 151.97	4.17	
	6	172.98	207.58 - 144.16	3.85	
T. castanum (RS)	2	503.22	618.97 - 409.12	3.40	2.46
	4	283.06	336.85 - 237.87	4.17	1.56
	6	106.94	128.32 - 89.12	4.00	0.62
S. oryzae	2	119.52	148.20 - 96.38	3.03	
	4	69.19	79.57 - 60.17	4.72	
	6	59.76	68.72 - 51.96	4.1	
C. maculatus	2	345.97	603.55 - 268.19	2.53	
	4	97.50	122.85 - 77.37	2.74	
	6	59.76	75.29 - 47.43	2.79	
T. castaneum larvae	2	157.26	196.57 - 125.81	2.94	
	4	119.52	144.61 - 98.77	3.03	
	6	119.52	144.61 - 98.77	3.03	

(1) C.L. = confidence limits (2) S.V. = slope value

Data in (Table 3) show the response of the tested insects exposed to treated diet with different concentrations at certain periods. Results exhibited that petroleum ether extract had the most effectiveness with all tested insects at the all times except for the *T. castaneum* adults on wheat which exhibited no response at 2 days post-treatment. *T. castaneum* adults were the most tolerant compared with both *C. maculatus* and *S. oryzae* which were the most susceptible insects with (100%) mortality after 6 and 9 days of applications. Similary, results showed the same trend mentioned before with thin film method where, low toxicity effect exhibited when acetone and methanolic extracts were used against tested insects.

Table (3): Insecticidal activity of petroleum ether water hyacinth extract on various stored grain insects by food treatment.

Toxicant	insect	1%			0.5%			0.25%		
		days after treatment								
		2	6	9	2	6	9	2	6	9
PE extract	*T. castaneum*(LS)	0	26	26	0	16	21	0	5	21
	C. maculatus	50	100	100	15	100	100	8	70	93
	S. oryzae	70	100	100	10	90	100	0	60	80

P.E. extract = petroleum ether extract.

Data in Table (4) indicated the mean numbers of emerged *S. oryzae* adults were affected by the type of (extract or powder) and concentration. Powder of water hyacinth significantly decreased the number of adults produced at the used concentration, except for that of 1% powder. In general,

the all tested materials in Table (4) significantly reduced the emerged adults compared with that of control and pronounced effect was recorded with high dose where the produced numbers of adults decreased as the concentrate increased.

Table (4): Percent of reduction (F_1) of *S. oryzae* adults exposed to treated wheat grain with powder and 3 extracts of water hyacinth.

Treatment	Concentrates	Mean No. of adult emergence/10 insects	Reduction of progeny %	Deterrent index*
Powder	20%	0 c	100.0	100.0
	10%	1 c	97.3	94.9
	5%	4 c	89.5	81.0
	1%	22 a	42.1	26.7
A. extract	(1%)	2.5 c	93.7	87.7
	(0.5%)	10.0 b	73.7	58.3
	(0.25%)	12.5 b	67.1	50.5
M. extract	(1%)	4.5 c	88.2	78.8
	(0.5%)	13.0 b	65.8	49.0
	(0.25%)	33.0 a	13.2	7.0
PE. extract	(1%)	3.0 c	92.0	85.2
	(0.5%)	5.3 c	86.0	75.4
	(0.25%)	11.0 b	71.0	55.0
Control		38.0 a		

Means followed by the same letter are not significantly different at the level 5% by DMRT (Duncan Multiple Range Test (Duncan 1955)
Where : A. extract = acetone water hyacinth extract

M. extract = methanol water hyacinth extract.

$$* \text{ Deterrent index} = \left[\frac{B - A}{A + B} \times 100 \right]$$

A = n. off spring in treatment
B = n. off spring in control.

Serious reduction in the fecundity of *C. maculatus* was noticed following adult treatment with the powder and the three extracts (Table 5).

Also, the all tested materials in Table (5) exhibited tremendous sterilizing action when applied to adults of *C. maculatus*. The high concentrations 1.0 for extracts and 20% for powder) induced 91.9, 63.9, 100 and 59.13 % sterility with acetone, methanol, petroleum ether extracts and powder of water hyacinth, respectively. Petroleum ether extract achieved the highest effect in all tested concentrations. No emergence of adults was observed in treatment of petroleum ether extract at the higher dose (1%). From the mentioned result above it can be concluded that petroleum ether extract could be successfully used at the all concentrations tested.

Table (5): Effect of adult treatment with the powder and extracts of water hyacinth on the progeny and sterility of *C. maculatus* using mixing with media method.

Parameters	A. extract			M. extract			PE. extract			Powder			Check
	1 %	0.5 %	0.25 %	1 %	0.5 %	0.25 %	1 %	0.5 %	0.25 %	1 %	0.5 %	0.25 %	
Total eggs	157	180	319	240	259	321.3	57	66	108.5	156	165	191	346
No. of eggs/female	31.4	36	39.9	48	52	64.25	11.4	13.2	21.7	31.2	33	38.2	69.2
% hatcability	17.4	54.5	70.6	50.6	61.4	66.92	0	5.3	14.7	88	90.7	59.5	97.1
% Progeny	0.64	27.3	29.6	21.7	27.4	33.86	0	0.76	6.0	47.4	48.4	64.6	83.3
% reduction of progeny	99.6	83.0	67.2	81.92	75.4	62.2	100	99.8	97.7	74.3	72.0	57.2	-
% damage	1.9	3.9	82.8	58.5	66.03	62.7	0.98	1.2	7.3	48	70.6	72.3	94.7
%Reduction of damage	98.0	95.9	12.6	38.2	30.3	2.1	99.0	98.7	92.3	49.3	25.4	23.6	-
% sterility	91.9	70.8	58.1	63.9	52.5	36.0	100	99.0	95.3	59.13	55.5	45.7	-

1- % progeny = $\left[\frac{\text{Emergence } (F_1)}{\text{Total eggs}} \times 100\right]$

2- Reduction of progeny = $\left[\frac{\text{Mean progeny control - mean progeny treatment}}{\text{Mean progeny control}} \times 100\right]$

The LC_{50}'s were 78.63 and 70.77 $\mu g/cm^2$ at 2 and 4 days after treatment with dipping technique (Table 6) while it were 157.26 & 119.52$\mu g/cm^2$ at the same periods when mixing with media method was used. Consequently, the bioassay method plays important role concern the bioinsecticidal activity of the same used toxicant, therefore the dipping technique was the best in this present study with the used immature stage.
These results agree with those obtained by Usha Rani and Jamil (1989) who studied the effect of petroleum ether water hyacinth extract by different methods on some of stored product insect pests. El-Doksh *et al*. (1984b) and Geriguis *et al*. (1991) studied some plant extracts on the fecundity and sterility of *S. littoralis* larvae. Many other research workers (Fraenckel, 1969; Jilani and Malik, 1973; Bhadui *et al*. 1985; Su, (1977, 1990); Khaire *et al*. 1992; Mansour, 1996; El-Aidy and Helal, 1997 and Raja *et al*. 2001) studied the effect of some plant extracts on the toxicity, oviposition and progeny of different insect pests of stored products.

Table (6): Efficiency of petroleum ether water hyacinth extract against *T. castaneum* larvae using dipping method.

Toxicant	Time	LC_{50} $\mu g/cm^2$	C.L.	S.V.
P.E. extract	2 days	78.63	101.43 - 60.95	3.57
	4 days	70.77	87.04 - 57.45	4.35

Petroleum ether extract acted as insect growth regulator might have disrupted the moult cycle by interfering with the regulation and release of the brain hormones that control moulting or interferred with the regulation and release of endogenous JH, which influences the type of cuticle formed. The symptoms appeared on the insects after water hyacinth treatment resembled those appeared by juvenile hormone compounds. Compounds causing death by direct interference with insect growth and metamorphosis allow a selective

and specific control strategy, and in this aspect water hyacinth proves to be a plant with potential promise (Usha Rani and Jamil (1989).

In conclusion, water hyacinth is unknown to be toxic to mammals. Extracts and powder of water hyacinth in this study produced toxic and ovicidal effects, inhibited damage, reproduction and increased sterility of some tested insects, especially *C. maculatus* and *S. oryzae*. Petroleum ether water hyacinth extract was the best for the mentioned parameters, since it completely prevented emergence of *C. maculatus* and reflected the resistant *T. castaneum* strain to susceptible one. Therefore, it has an evident to be a substitute of synthetic insecticides where some stored pests gained resistance against it. Also, it exhibited the highest deterrent effect on progeny of *S. oryzae* adults (F_1), besides producing insecticidal activity to *T. castaneum* larvae. Further studies are needed to assure these findings.

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دراسة على كفاءة نبات ورد النيل كمادة واقية ضد بعض حشرات المواد المخزونة

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تم تقييم النشاط الأبادى الحيوى لأوراق نبات ورد النيل كمسحوق ثلاثة من مستخلصاته الناتجة من استخدام مذيب الأسيتون، والميثانول، والأثير البترولى كل على حدة ضد سلاله مقاومة واخرى حساسة لخنفساء الدقيق الصدئية الحمراء، سوسة الأرز وخنفساء اللوبيا وكذلك يرقات العمر الرابع لخنفساء الدقيق الصدئية الحمراء.

استخدمت حبوب القمح السليمة فى تربية سوسة الأرز *S. oryzae* وحبوب القمح + دقيق القمح لتربية الحشرات الكاملة ويرقات خنفساء الدقيق الصدئية الحمراء وبذور اللوبيا لتربية خنفساء اللوبيا.

تم خلط مسحوق أوراق ورد النيل جيداً مع الحبوب بجرعات تتراوح بين ١-٢٠% (وزن/وزن) بالنسبة لسوسة الأرز، ٥-٢٠% (وزن/وزن) لخنفساء اللوبيا *C. maculatus*.

وكانت النتائج كالآتى :

- أظهرت كل المعدلات المستخدمة من مسحوق أوراق ورد النيل تأثيراً مثبطاً على خروج الذرية للحشرات المختبرة.
- قتل تركيز ١% من مستخلص الأثير البترولى لورق ورد النيل ١٠٠% من الحشرات المختبرة لسوسة الأرز وخنفساء اللوبيا بينما سبب ٢٦% موت لخنفساء الدقيق الصدئية الحمراء عند نفس وقت التعرض.
- أظهرت كل معاملات المستخلصات المختلفة المستخدمة تأثيراً ضاراً على بيولوجى سوسة الأرز وخنفساء اللوبيا.
- أظهرت خنفساء اللوبيا قوة تحمل أعلى من باقى الحشرات المختلفة (التركيز القاتل لنصف الحشرات المختبرة: ٣٤٥,٩٧ ميكروجرام/سم٢) بينما السلالة المقاومة لخنفساء الدقيق لم تظهر أى مقاومة للمستخلص.
- أظهرت مستخلصات الميثانول والأسيتون سمية منخفضة جداً للحشرات المختبرة.