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بسم الله الرحمن الرحيم

EVALUATION OF *EUCALYPTUS GLOBULUS* EXTRACTS FOR THE CONTROL OF RICE WEEVIL, *SITOPHILUS ORYZAE*

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ABSTRACT

Botanical materials derived from flowers, leaves and branches of *Eucalyptus globulus* were extracted consecutively through five solvents varied in their polarities namely; hexane, petroleum ether 40-60°C, chloroform, ethanol and acetonitrile to study their biocide activity against *Sitophilus oryzae*.

Preliminary studies indicated that 20 mg/ml ethanol flowers and leaves had significant deterrent effects on insect grain damage as well as increased adult insect mortality. Also, there are significant differences on adult emerged between all chloroform extracts and control. Activity of 20 mg/ml plant extracts against adult and feeding deterrence to *S. oryzae* suggest promising extracts. According to correlation coefficient values of promising extracts a significant succession linear relationships were found between tested concentrations and antifeedant effect with chloroform leaf and branch extracts, and percentage adult mortality with chloroform branch and ethanol flower extracts. Significant reversal linear relationship was recorded between tested concentrations and percentage of grain damage with ethanol leaves extract.

According to phytochemical investigation, chloroform flowers, leaves and branches extracts contained sterols and/or triterpens, cardic, alkaloids and flavonoids. Whereas ethanol flowers and leaves extracts contained pyrogallol tannins, phenolic glycosides, saponine glycosides and alkaloids. On the other hand, catecol tannins was found in chloroform leaves and branches extracts only.

Laboratory studies showed that the promising extracts have potential as stored-product insect, *S. oryzae* control agent, because it significantly deters feeding as well as emergence to F_1 adult of *S. oryzae*. And also have slight toxic action against insect. It is suggested that these biologically active natural products may serve as suitable prototypes for the development of commercial insecticides.

INTRODUCTION

Grains of different kinds and many stored products are considered important food sources for people, animals and birds. Insect infestation of stored grains causes a serious problem all over the world since they reduce crop yields, cause contamination for stored products, damage seed germs, carry diseases that affect plants, people and animals and change the quality of stored products. For example, the granary weevil, *Sitophilus granarius* (L.) causes over 10-80 % of the grain damage according to the varieties of grain geographical locality, also the rice weevil *S. oryzae* (L.) causes serious problems for rice and grains (Farag, 1996).

Chemical control of insects in storage has been used for along time, but has serious drawbacks (Sharaby, 1988). The indiscriminate use of

chemical pesticides have given rise to many serious problems, including genetic resistance of pest species, toxic residues, increasing costs of application, environmental pollution, hazards from handling, ... etc. (Ahmed *et al*., 1981; Khanam *et al*., 1990).

There is an urgent need for safe but effective biodegradable pesticides with no toxic effects on non- target organisms. This has created a world-wide interest in the development of alternative strategies, including the search for new types of insecticides, and re-evaluation and use of age-old traditional botanical pest control agents (Heyde *et al*., 1983).

Natural products isolated from plants may have pesticidal properties, growth regulator, insect repellent and attractant activities (Schearmann *et al*., 1977).

Tasmanian blue gum plants "*Eucalyptus globulus*" are well known and widely grown as ornamental and wind shield plants (El-Kadi *et al*., 2000). The potential effect of the secondary products of these plants were previously studied as bioactive agents against insects. Eucalyptus extracts was the most repellent against the pyralid *Corcyra cephalonica* followed by cymbopogon, mustard, neem and datura extracts (Devaraj and Stllatha, 1993). Ethanol extracts from *Eucalyptus globulus* having great effect on the *Tecia solanivora* pupation rate (Castillo *et al*., 1998).

Therefore, the present investigation was undertaken to evaluate the insecticidal activity of the natural products of *E. globulus* against *Sitophilus oryzae*.

MATERIALS AND METHODS

1- Plant materials :

Fresh flowers, leaves, branches of *E. globulus* were collected from trees on Nile bank near Agouza, Giza Governorate, in June 1997. The collected parts were air dried, then ground in mill (Glen Creston Stammore, England), and kept in closed amber glass containers away from light and moisture.

2- Preparation of the crude extracts :

Each of these ground materials was separately macerated consecutively in five solvents varied in their polarity (hexane, petroleum ether 40-60°C, chloroform, ethanol and acetonitrile).

Each solvent was used at a rate of 500 ml/100 g plant material in one liter brown coloured bottles provided with light stoppers. After 48 hours, each bottle was intermittently agitated by electric shaker for one hour. The solvent was separated from the insoluble plant material and the later was re-extracted with another 250 ml of the same solvent. The obtained extract (750 ml) was filtered over anhydrous sodium sulfate through Buchner funnel to remove debris. Filtrates were concentrated by rotary evaporator under vacuum at 40°C. The crude extracts were then weighed and adjusted to 10 ml with the extraction solvent and placed in a 20°C freezer in refrigerator until testing.

3- Bioassay :

a) Tested insects : *Sitophilus oryzae* (L.), were reared for several years on wheat grains under rearing room conditions away from insecticidal contamination at 27±1°C with 60±10 % R.H. Also, experiments were conducted under the same conditions.

b) Thin-film technique : One ml from each crude extract was solved in 5 ml of the same solvent extraction to obtain 20 mg/ml, one ml of each solution was evenly distribution on the Petri-dish surface (9 cm in diameter). The solvent was completely evaporated under laboratory condition until thin film of plant extracts was formed. Controls treated with extraction solvents only. A group of ten adult insects were exposed to the thin film of each test plant extract for 24 hr. Mortality counts were estimated. Each treatment was replicated three times.

Feeding test :

This test was carried out to determined the promising extracts with 20 mg/ml and study the linear relationship between serial concentrations (20, 40, 80 and 160 mg/ml) of promising extracts and their effectiveness.

The tested concentrations of each extract were prepared by dilution with the same solvent used in extraction except chloroform which changed by ethanol (chloroform effected on *S. oryzae*).

One ml from each extract was pipetted on seven grams of wheat grains in the glass container and agitated to coating seed surface, in addition to controls (solvent only).

Each container was left to solvent evaporation. Ten insects of two week old *S. oryzae* adults were transferred to each glass container. After seven days, these adults were removed and the following data were recorded : Number of dead parent adults, number of damaged seeds by parent adults feeding, lost in grains weight by parent adult feeding, and change in parent adult weight as result to treatments. Total number of emerged of spring was recorded after seven weeks. Each treatment replicated four times.

Preliminary qualitative screening for phytochemical constituents of promising extracts :

The preliminary phytochemical screening tests were carried out on promising extracts which exhibited bioactive agent against *S. oryzae* as follows :

Sterols and or triterpenes were detected according to the method adopted by Wall *et al*. (1964), tannins were estimated by the method described by Claus (1961), phenolic glycosides was detected according to Balbaa (1981), cardic glycosides were estimated according to El-Kadi (1997), saponins glycosides was detected according to Karawya and Abd El-Wahab (1975), alkaloides were estimated by the method described by Romo (1966), and flavonoids was detected according to Vankatarman (1962).

Statistical analysis :

Analysis of variance (ANOVA) for completely randomized block design was conducted on available preliminary data. Significant differences in

inhibition effects among extracts determined using least significant difference (L.S.D.) method at P = 0.05.

Percentage of antifeedant effect, percentage of grains damaged, percentage of dead parent adults, percentage of change in insect growth and percentage of emerged inhibition were determined using the following formula :

$$1\text{- Percentage of antifeedant effect : } \frac{AC - AT}{AC} \times 100$$

A = weight of grains after exposed to insects - weight of grains before exposed to insect.
C = control
T = treatment

$$2\text{- Percentage of grain damaged} = \frac{\text{Number of grain damaged in testing unit}}{\text{Number of grain in testing unit}} \times 100$$

$$3\text{- Percentage of dead parent adults by feeding} = \frac{BC - BT}{BC} \times 100$$

B = number of live adult parent by feeding
C = control
T = treatment

$$4\text{- Percentage of change in insect growth} = \frac{DC - DT}{DC} \times 100$$

D = a - b
a = weight of insects after exposed to treatment grains
b = weight of insects before exposed to treatment grains
C = control
T = treatment

Positive values (+) expressed inhibition and negative values (-) activation.

$$5\text{- Percentage of emerged inhibition} = \frac{EC - ET}{EC} \times 100$$

E = number of emerged insects
C = control
T = treatment

Correlation coefficient was used to determine the linear relationship between tested concentration of promising extracts and effectiveness.

RESULTS

Preliminary studies on effectiveness of tested plant extracts against *S. oryzae* were carried out, through exposure tested insects to 20 mg/ml of each

plant extract (Table, 1). Ethanol flower and leaf extracts showed significant differences between the treatments and their controls in the number of grains damage and the number of dead adults with feeding (Table, 1). The L.S.D. values were 3.2 and 1.4, respectively. There was a significant effect in the number of dead parent adults with contact between acetonitrile flower extract and its control; whereas, the mean dead number increased from 0.50 in control to 3.30 with acetonitrile flower extract and L.S.D. was 1.45.

Table (1) : The effectiveness of *Eucalyptus globulus* extracts against *S. oryzae* exposed to 20 mg/ml concentration.

Treatment	Mean lost in grains weight	Mean no. of grains damaged by adult feeding	Mean no. of dead parent adults with feeding	Mean no. of dead parent adults with contact	Mean of change in insect weight/g	Mean of adults emerged
H	0.097	5.50	0.00	0.000	-0.016	33.30
HFX	0.091	4.00	0.00	0.00	-0.021	49.00
HLX	0.092	6.25	0.00	0.00	-0.023	52.30
HBX	0.095	8.50	0.00	0.00	-0.022	29.00
L.S.D.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
P	0.106	6.25	0.25	0.00	-0.022	46.00
PBX	0.104	6.75	1.00	0.00	-0.022	28.00
PLX	0.114	5.25	0.00	0.00	-0.021	41.80
PFX	0.120	4.00	0.50	0.00	-0.025	49.30
L.S.D.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
E	0.096	5.75	0.50	0.00	-0.015	50.50
ClFX	0.106	5.25	1.00	0.00	-0.021	29.50
ClLX	0.116	4.00	0.75	0.00	-0.022	31.30
ClBX	0.115	4.40	1.75	0.00	-0.023	15.80
L.S.D.	N.S.	N.S.	N.S.	N.S.	N.S.	1.7
E	0.106	9.50	0.00	0.00	-0.016	46.30
EBX	0.114	6.75	0.75	0.00	-0.022	25.00
EFX	0.110	5.00	2.00	0.00	-0.021	27.00
ELX	0.100	5.50	3.30	0.00	-0.017	30.00
L.S.D.	N.S.	3.2	1.4	N.S.	N.S.	N.S.
A	0.154	4.25	0.00	0.50	-0.019	35.5
ABX	0.161	3.75	1.00	0.00	-0.028	46.00
AFX	0.164	5.25	0.50	3.30	-0.022	33.30
ALX	0.160	4.75	0.00	0.75	-0.024	32.25
L.S.D.	N.S.	N.S.	N.S.	1.45	N.S.	N.S.

H : Hexane **cl : Chloroform** **A : Acetonitrile**
P : Petroleum ether **E : Ethanol** **F : Flowers**
L : Leaves **B : Branches** **X : extract**

All of the chloroform extract tests gave significant difference in the number of adult emerged between the respective treatments and their controls. The mean number of adults emerged reduced from 50.50 with the control to 15.8, 29.5 and 31.3 with chloroform extracts of branches, flowers and leaves, respectively. And the L.S.D. value was 1.7.

All extracts of *E. globulus* had no significant effect ($P > 0.05$) on the growth of *S. oryzae* adults.

Extracts which showed significant effect in preliminary studies taht considered as promising extracts and their insecticidal activity rescreened

through study the linear relationship between concentrations of these extracts and their effectiveness.

Generally, according to correlation coefficient values significant succession linear relationships were found among tested concentration and antifeedant effect in case CIIX, CLBX and ELX also the same relationships were found between tested concentrations and percentage of adult dead parent in case of CLBX and EFX and it recorded, also between tested concentrations and percentage of the change in insect growth with CLFX and CLBX. Whereas a significant reversal linear relationship was found only between tested concentration and percentage of grains damaged in case of ELX (Table, 2).

Table (2) : Evaluation of promising extracts against *S. oryzae*.

Treatment	Concen-tration	% Antifeedant effect	% Grains damaged by adult feeding	% Dead parent adults by feeding	% Insect growth change	% Emerged inhibition
ClFX	160	11.5	1.6	8	200	31
	80	8.8	2.1	2.5	80	33.2
	40	8	2.5	2.5	-80	53.7
	20	5.9	3	0.0	-300	66.7
	zero	0.0	2.1	0.0	-	0.0
C.F. "r"		0.786 N.S.	-0.286 N.S.	0.338 N.S.	0.950**	0.656 N.S.
ClLX	160	41.7	2.6	10.5	-140	29.5
	80	17.9	3.3	10.5	-100	35.8
	40	12	3.4	5.3	-20	65
	20	6.6	3.6	0.0	-20	75.3
	zero	0.0	2.1	0.0	-	0.0
C.F. "r"		0.994**	0.02 N.S.	0.666 N.S.	-0.144 N.S.	0.098 N.S.
ClBX	160	38.5	2.1	13	100	28.0
	80	28.8	2.5	7.8	-20	38.5
	40	27.6	2.7	2.5	-240	44.0
	20	14.3	3.0	0.0	80	52.7
	zero	0.0	2.1	0.0	-	0.0
C.F. "r"		0.874**	0.263 N.S.	0.984**	0.964**	0.113 N.S.
EFX	160	0.0	2.1	18.5	-220	7.8
	80	0.0	1.9	16.3	-220	47.5
	40	0.0	1.6	13.3	-280	53.0
	20	0.0	1.4	10.5	-80	59.0
	zero	0.0	2.1	0.0	-	0.0
C.F. "r"		0.0 N.S.	0.430 N.S.	0.987**	-0.036 N.S.	0.245 N.S.
ELX	160	35.2	3.6	18.5	20	17.0
	80	0.0	2.6	16.3	20	37.3
	40	0.0	2.5	13.3	20	69.3
	20	0.0	2.1	2.5	80	75
	zero	0.0	2.1	0.0	-	0.0
C.F. "r"		2.2**	0.978**	0.851 N.S.	0.592 N.S.	-0.431 N.S.

cl : Chloroform **E : Ethanol** **F : Flowers**
L : Leaves **B : Branches** **X : extract**
C.F. : Correlation coefficient at 0.05.

Tested chloroform extracts (flowers, leaves and branches) appears to have antifeedant effect against *S. oryzae* percentage of antifeedant effect were 5.9 to 11.5, 6.6 to 41.7 and 14.3 to 38.5, respectively; in contrary tested ethanol flower extract don't showed any antifeedant effect against the tested insect.

Except EFX all tested extracts increased percentage of grains damaged in treatments from control. On the other hand, relationship between tested concentration and percentage of grains damaged was reversal in all tested extract except EFX and ELX.

Insect growth were affected with tested extracts with three different ways :

a) Extracts active on insect growth in case of CIIX and EFX.
b) Extracts inhibited insect growth in case of ELX.
c) Extracts inhibited insect growth with high concentrations, however, insect growth active with low concentration in case of CLFX.

With all tested extracts, the low concentrations were more effective against insect adult emerged than high concentration, the percentage of emerged inhibition were between 66.7 to 31.0, 78.3 to 29.5, 52.7 to 28.0, 59.0 to 7.8 and 75.0 to 17.0 in case of CLFX, CIIX, CLBX, EFX and ELX, respectively. On the other hand, no significant linear relationship was recorded between tested concentrations and percentage of insect emerged inhibition.

Preliminary screening of phytochemical constituents in the effective extracts were studied (Table, 3). The following groups detected in the tested extracts as follow :

1- Alkaloids were found in all effective extracts.
2- Flavonoids, sterols and or triterpenes were found in chloroform flowers, leaves and branches extracts.
3- Pyrogallol tannins, phenolic glycosides and saponine glycosides were found in ethanol flowers and leaves extracts.
4- Cardic glycosides were found in all effective extracts except ethanol leaves extracts.
5- Tannins catecol detected in chloroform leaves and branches extracts.

Table (3) : Preliminary screening of phytochemical constituents in active extracts.

Solvent	Ethanol		Chloroform		
Part of plant	Flowers	Leaves	Flowers	Leaves	Branches
Effective components					
Sterols and triterpens	-	-	+	+	+
Tannins					
Catecol	-	-	-	+	+
Pyrogallol	+	+	-	-	-
Glycosides					
Phenolic	+	+	-	-	-
Cardic	+	-	+	+	+
Saponine	+	+	-	-	-
Alkaloids	+	+	+	+	+

Flavonoids	-	-	+	+	+

DISCUSSION

With regard to solvent extraction, it exhibited a chloroform extracts of *E. globulus* possessed antifeedant effect compared with ethanolic extracts. This effect may be due to the polarity of antifeedant component in flowers, leaves and branches of the tested plant which compatible with polarity of chloroform.

Therefore, the method of extraction may be play an important role. Burgess *et al*. (1988) indicated that discs of castor were palatable but highly toxic to adult scarabaeids, after extraction with chloroform, castor leaf discs were non-toxic. Also non of the all treatments deterred feeding. The main toxic substance was identified by mass spectrometry as alkaloid ricinine. Roychoudhury (1993) demonstrated that the chloroform of *Podocarpa* sp. appeared to be the most antifeedant and insecticidal chemicals against adults of *S. oryzae*. Non-polar extracts of *Illicium verum* was more effective than polar extracts on eggs, larvae and adult of *S. zezamais* (Ho *et al*., 1995).

The results obtained showed that chloroform extracts increased the percentage of grains damaged in treatment from control. The relationship between tested chloroform extracts concentration and percentage of grains damage were succession. This effect may be due to that chloroform extracts increased insect desire to grains gnaw without feeding.

All tested extracts showed a slight toxic effect on adult stage by feeding. Both CLBX and EFX gave a significant positive linear relationship between tested concentrations and percentage of dead parent adult feeding. The present data appear that *E. globulus* possessed a slight active component act as toxic stomach against *S. oryzae*.

Javaid and Mpotokwane (1997) indicated that *Eucalyptus* sp. was shown to have insecticidal activity against *Callosobruchus maculatus*. All tested extracts inhibited insect emerged. The relationship between the percentage of adult emerged inhibition and tested concentrations were reversal. This effect may be due to bioactive agent in tested extracts, which affected on ovipositional preferences, hatching rate and development of immature stage. Castillo *et al*. (1998) indicated that *E. globulus* having the great effect on the pupation of *Tecia solanivora*.

According to phytochemical investigation; chloroform flowers, leaves and branches extracts partaked in sterols and triterpens, cardic, alkaloids and flavonoids. Whereas, ethanol flowers and leaves extracts partaked in tannins pyrogallol, glycosides phenolic, glycosides saponine and alkaloids; on the other hand, tannins, catecol was found in chloroform leaves and branches extracts only. While cardic glycosides was found in ethanol flower extract.

Antifeedant effect, dead parent adults by feeding and effect on insect growth which caused as result of treatment with effective extracts may be due to present terpenes and alkaloids component in these extracts.

The effect of these component against insects was reported by Bentley *et al*. (1984) showed that six*th* instar larvae of *Choristoneura fumiferana* Clem, were exposed in feeding bioassay to the five solanum alkaloids; *i.e.*,

tomatidine, tomatine, α-solanine, α-chaconine and solanidine. All the five tested alkaloids exhibited significant feeding deterrence.

El-Doksh *et al*. (1984) indicated that the crude alkaloids from *Datura stramonium* exhibited toxic action to *Spodoptera* larvae.

Smith *et al*. (1983) mentioned that the crude syrups of *Melampodium americanum* and *M. leucanthum* and their principal sesquiterpene lactones, melampodin A and melampodinin A, significantly inhibited growth and deterred feeding of the full armyworm *Spodoptera frugiperda*.

Gershenzon *et al*. (1985) found that high concentration of sesquiterpene lactones and diterpenes in glandular hairs on several resistant wild species of *Helianthus* were toxic and antifeedants towards some major sunflower insect pests.

The present work has demonstrated that *E. globulus* is an effective protectant for *S. oryzae* and may be useful as a component of pest management in the stored product insect control.

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التقييم الحيوى لمستخلصات نبات كافور الصقيع وامكانيه مكافحة سوسة الارز

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المعمل المركزى للمبيدات، مركز البحوث الزراعية، الدقى - الجيزة، مصر

فى هذه الدراسة تم إستخلاص المستخلصات الخام من أوراق وأزهار وأفرع نبات كافور الصقيع، إستخلاص متتابع بخمس مذيبات مختلفة القطبية وهى الهكسان والبتروليم إيثر ٤٠-٦٠ والكلوروفورم والإثيانول والأسيتونيترل، بغرض دراسة التأثير الحيوى على سوسة الأرز.

وقد أظهرت الإختبارات الأولية والتى أجريت بأستخدام تركيز ٢٠ ملجم/مل. وقد وجد أن المستخلص الايثانولى لكل من الزهرة والورقة له تأثير معنوى كمانع للتغذية، بالاضافة إلى التأثير الابادى ومن ناحية أخرى فقد وجدت فروق معنوية مابين مستخلص الكلورفورم للزهرة والورقة والأفرع وبين المقارنة، من حيث قدرتها على إعاقة خروج الحشرات الكاملة من الحبوب المعاملة ولذلك أعتبرت هذه المستخلصات مبشرة.

وقد أجريت على المستخلصات التى أعتبرت مبشرة دراسات أكثر شمولاً لتقييمها حيوياً بإستخدام سلسلة تركيزات١٦٠،٨٠،٤٠،٢٠ملجم/مل. حيث إتضح بالاعتماد على معامل التلازم أن هناك علاقة خطية طردية مابين التركيزات المستخدمة والتأثير المانع للتغذية لمستخلص الكلورفورم لكل من الورقة والفروع وكذلك لوحظت العلاقة السابقة مابين التركيزات المستخدمة وبين النسبه المئوية لموت الافراد البالغة فى حالة مستخلص الكلورفورم للفروع ومستخلص الإيثانول للزهرة، بينما كان هناك علاقة خطية عكسية معنوية مابين التركيزات المستخدمة ونسبة الحبوب المصابة نتيجة للمعاملة بالمستخلص الإثيانولى للأوراق.

وعند إجراء الاختبارات الفيتوكيمائية على المستخلصات التى أظهرت فاعلية أتضح أن مستخلصات الكلورفورم لكل من الورقة والزهرة والفروع أحتويت على الاستيرولات و التربينات الثلاثية والكارديك والقلويدات والفينولات بينما إحتوت مستخلصات الاثيانول للزهرة والورقة على تانينات البيروجالول وجليكوسيدات الفينول وجليكوسيدات السابونين والقلويدات بينما وجدت تانينات الكاتيكول فقط فى مستخلصات الكلورفوم للأوراق والفروع

من هذه النتائج يتضح أن المستخلصات السابقة تحتوى عوامل مكافحة فعالة، ضد سوسة الأرز حيث أنها أظهرت معنوية من حيث قدرتها على إعاقة التغذية والتأثير على النمو وخروج الأطوار الكاملة وكذلك تأثير إبادى طفيف.

وبذلك يمكن إعتبار هذه المستخلصات مصادر طبيعية يمكن الحصول منها على مجاميع فعاله يمكن الإستفادة منها مستقبلاً.