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EFFECT OF SOME PLANT EXTRACTS AND ITS BINARY MIXTURES WITH CHLORPYRIFOS AGAINST 2ND INSTAR LARVAE OF COTTON LEAFWORM *Spodoptera littoralis* (BOISD) UNDER LABORATORY CONDITIONS.

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ABSTRACT

The phytochemical screening of active constituents were studied in the aerial parts of *Euphorbia retusa* and *Calotropis procera* wild plants.

Data indicated the occurrence of sterols and triterpens, phenolic glycosides, anthraquinon glycocides, saponin, flavonoides, cardiac glycosides, alkaloids and carbohydrates and /or glycosides,with variable degrees of occurrence due to the plant species. The two plant materials were extracted with chloroform and methanol, then evaluate their potency against 2nd instar larve of cotton leafworm *S.littoralis* as well as the conventional insecticide chlorpyrifos either alone or in combination with each other.

Data revealed that LC_{50} values were [(0.32 and 1.55) , (2.92 and 5.59) and 7×10^{-5} %] for methanolic and chloroformic crude extractes of the two tested plants and chlorpyrifos respectively. The co-toxicity values of chlorpyrifos + *Euphorbia retusa* methanolic extract at 1:1 , 2 : 1 and 1:2 mixing ratio were [(-50, + 60.17 and – 25.93) and (- 49.24 , - 84.92 and – 70.46)] respectively. While in case of chlorophormic crude plant extracts in the same manner were [(+2.31, - 43.33 and 76.47) and (-34.84 , - 50 and – 37.04)] respectively.

INTRODUCTION

Pesticide research in the agricultural business generally continuous to emphasize studies on the development and use of synthetic broadly toxic compounds. Although synthetic organic pesticides appear to provide a solution to the problems of pest control, it has become apparent that repeated application and excessive reliance on synthetic pesticides can be an in-adequate method of control. Health and environmental problems and increasing pest resistance to many of these synthetic pesticides clearly indicated that basic research must be directed to discover a new safe types of pest control agents in order to sure high production and preservation of agricultural products.

Natural products affecting pests, therefore provide continual inspiration to the agricultural chemicals in their research for new products to control pests and improve field. Pesticide mixture are usually used in the field to enhance the spectrum of control when multiple pests are attacking simultaneously. They are also recommended to increase the efficacy of control against a single pest to delay the development of insecticide resistance or to compact current resistance in a pest species.(Asher *et al.*

1986, Mushtaq (2004) and Swelam, S. Eman & Makram (2006). Also, a mixture may give better control of a complex of insect pests.

Many researchers reported that many compounds having insecticidal activity were isolated from plants, belonging to different families. Some of naturally occurring compounds provided to be synergize the toxicity of certain commercially synthetic insecticides. Rasmi *et al.* (1984). Therefore the aims of the present study are to investigate the occurrence of various phytochemical active constituents in some wild plants and study their activity either alone or in combination with the conventional insecticide chlorpyrifos against 2nd instar larvae of *S.littoralis* under laboratory conditions.

MATERIALS AND METHODS

1- Plant materials

The two experimental plants for this study are listed in Table (1) which includes English , lateen, family names in addition to parts used and source of collection.

Table (1): Tested plant materials.

English name	Latin name	Family name	Tested part	Source
Oshar	*Calotropis procera*	Asclepiadace	Aerial parts	El-Arish
Saponelghet	*Euphorbia retusa*	Euphorbiacea	Aerial parts	El-Arish

The tested plant samples were collected during 2006 spring. Identification were bases mainly on the taxonomic characters detailed by Tachholm (1956).

2- Preliminary phytochemical screening of tested plants.

The following biochemical constituents were determind.

2-1. Steroles and triterpenes were determined according to well *et al.*(1964) method.

2-2 Phenolic glycosides were determined by the method adopted by Balbaa (1961).

2-3 Tanins were determined by the methods described by Claus (1961).

2-4 Anthraquinone glycosides were calculated according to Balbaa (1981).

2-5 Saponin glycosides were calculated according to the method mentioned by well *et al.* (1964).

2-6 Flavonoids were determined by Claus (1961) method.

2-7 Cardic glycosides were idintifide according to Baljet and Schwiez-Aphoth-Ztg (1981).

2-8 Carbohydrates and / or glycosides were determined by the method of Karawya and El-Wahab (1975).

3- Preparation of the crude plant extracts.

The tested plants were extracted according to freedman *et al.* (1979), Procedure with minor modifications: the aerial parts of the tested plants were air dried at room temperature, then ground into fine powder. After wards 200 grams of each plant powder were extracted three times successively with one litter chloroform and methanol. The ground plant parts were macerated and the homogenated was allowed to stand for three days. The extracts were filtered through anhydrous sodium sulphate. All filtrates were combined and rotary evaporated at temperature not exceeding 50 °C. the crude extract was then weighed and adjusted to 25 ml. With the used solvent and kept in a refrigerator until testing.

4- Tested insecticide

chlorpyrifos (Dursban 48 % EC) o,o diethyl o-)3,5,6 trichloro-2 pyridyl) phosphothioate.
Rate : 1 Litter / Feddan.

5- Tested insects

laboratory strain of cotton leafworm *S.littoralis* was maintained under constant condition of 25 ± 1 °C and 70 ± 5 % RH value, which reared on castor been leaves according to the method described by El-Defrawi *et al.*(1964).

6- Toxicity testes

Series concentration of the tested materials (in water) as (0.25, 0.50, 1.0 , 2.0 and 3.0 %) for crude plant extracts and (0.05, 0.1 , 0.2, 2.0, 4.0, 6.0 ppm) for chloropyrifos were prepared. Castor leaves were dipped for 15 seconds in each concentration then left to dry. The 2nd instar larvae of cotton leafworm were confined with the treated leaves in glass jars covered with muslin for (72 hrs) in case of crude plant extracts and (24 hrs) in chlorpyrifos treatment, then treated leaves were replenished with untreated leaves. Three replicates (10 larvae each) were used in addition to control.

Mortality were recorded for five days in case of crude plant extracts and three days post treatment for chlorpyrifos treatment. Mortality percentages were calculated and corrected using Abbott (1925) formula and statistically analyzed according to Finny (1971) to estimate LC_{25}, LC_{50}, LC_{90} and slope values.

Relative potency of tested compounds was determined according to Sun (1950) using the following equation:

a- Toxicity index = LC_{50} of the most effective compound / LC_{50} of the tested compound × 100

b- to evaluate the joint effect of the different pairs of the used the co- toxicity factor equation toxicants of Mansour *et al.* (1966).

$$\text{Co-toxicity factor} = \frac{\text{Observed \% mortality} - \text{Expected \% M}}{\text{Expected \% mortality}} \times 100$$

The resulted values could be differentiation into three categories as follow :

Potentiation effect : + 20

Antagonism effect : - 20

Additive effect : between – 20 and + 20

RESULTS AND DISCUSSION

1- Phytochemical screening

Data tabulated in table (2) revealed the existence of nine active components in the aerial parts of the two tested plants *Euphorbia retusa* (Sapon elghat) and *Calotropis procera* (Oshar).

Table (2): Preliminary phytochemical screening of the tested plants.

Tested plants / Constituents	*Euphorbia retusa*	*Calotropis procera*
Sterols and triterpens	++	±
Phenolic glycosids	±	++
Tannins	+	+
Anthraquinone glycosides	+	±
Saponin	+++	+
Flavonoids	++	±
Cardiac glycosides	+++	+++
Alkaloids	++	+++
Carbohydrates and / or glycosides	+++	++

- **Traces : ±**
- **Present : +**
- **present in large amount : ++**
- **present in relatively large amount : +++**

Saponin, cardiac glycosides were found in relatively large amount in *Euphorbia retusa* (+++) followed by sterols and triterpens, flavonoides and alkaloids are present in large amount (++), while tannine, anthrax quinone glycoside were present (+), finely the phenolic glycosides were found in traces (±). On the other hand cardiac glycosides, alkaloids and carbohydrates and / or glycosides were found in relatively large amount (+++) followed by phenolic glycosides (++), tannins and saponin (+), while (sterols and triterpens), anthraquinone glycosides and flavonoides were presented in traces (±) in case of *Calotropis procera* (Oshar). These data indicated that saponines, cardiac glycosides, alkaloids, flavonoides and glycosides may be responsible for bioactivity on the studied pests.

2- Insecticidal activity of various crude plant extracts against 2nd instar larvae of cotton leafworm *S.littoralis* :

The tested plants were extracted with two solvent aried in their polarity (Chloroform and methanol). The activity of the crude plant extracts were evaluated against 2nd instar larvae of *S.littoralis*, comparing with the conventional insecticide Clorpyrifos. Data are summarized in tables (3and 4). Table (3) shows the activity of the methanolic crude extract of the two tested plants, while Table (4) presents those of the Chloroformic crude plant extracts.

From Table (3) it is clear that the synthetic insecticides Clorpyrifos exhibited the highest toxic action followed by *Euphorbia retusa* crude methanolic extract while *Calotropis procera* extract was the least in toxicity. Values of LC_{50} were (7×10^{-5} , 0.32 and 1.55 %) respectively. In contrast, the same trend of toxicity was observed in case of chloroformic crude plant extracts, where LC_{50} values were (7×10^{-5}, 2.92 and 5.59 % for Chlorpyrifos, *Euphorbia retusa* and *Calotropis procera* crude extractes respectively. It is obvious that methanolic crude plant extracts were more toxic than the chloroformic extracts.

Table (3): Effect of certain methanolic plant extract and Chlorpyrifos insecticides against 2nd instar larvae of cotton leafworm *Spodoptera littoralis*.

Tested materials	LC_{50} %	LC_{90} %	Slope	Toxicity index
Calotropis procera	0.32	24.88	2.61	0.02
Euphorbia retusa	1.55	66.68	1.20	4.51
Chlorpyrifos	7×10^{-5}	31×10^{-5}	1.93	100

Table (4): Effect of certain Chloroformic plant extracts against 2nd instar larvae of cotton leafworm *Spodoptera littoralis*.

Tested materials	LC_{50} %	LC_{90} %	Slope	Toxicity index
Euphorbia retusa	2.92	38.67	1.14	2.4
Calotropis procera	5.59	26.35	1.90	1.2
Chlorpyrifos	7×10^{-5}	31×10^{-5}	1.93	100

3- Binary mixtures toxicity of the tested compounds against 2nd instar larvae of *S.littoralis*.

Table (5) show the toxicity of the binary mixture of the methanolic crude plant extracts and Chlorpyrifos while Table (5) presents those of chloroformic extract.

From Table (4) it is clear that the co-toxicity factor and the joint action categories of Chlorpyrifos + *Calotropis procera* and *Euphorbia retusa*

methanolic extracts binary mixtures at mixing ratio of 1:1, 2:1 and 1:2 were [(-50, +60.17 and – 25.93) and (antagonism, Potentation and antagonism)] and [(-49.24, -84.92 and –70.46) and (antagonism)] respectively.

Data in Table (6) revealed that the co-toxicity factor and the joint action categories of Chlorpyrifos and the chloroformic crude extracts binary mixtures at the same mixing ratio were [(+ 2.31, - 43.33 and + 76.47) and (potentaition, antagonism and potentaition)] and [(-34.84, -50 and –37.04) and (antagonism) respectively. It is importance to stated that the binary mixtures of Chloroyrifos and *Calotropis procera* methanolic extract gave an potentaition effect at 2:1 mixing ratio only (56.67 % observed mortality and + 60.17 co-toxicity factor) while the test of the binary mixtures ratio exihibited an antagonismic effect either at *Calotropis procera* or *Euphorbia retusa* extracts.

Table (5): Toxicity of the binary mixtures of methanolic crude plant extracts against 2nd instar larvae of cotton leafworm *S.littoralis*.

combination	Mixing ratio	% Observed Mortality	Co-toxicity Factor	Joint action category
Chlorpyrifos + *Calotropis procera*	1:1	10	- 50	Antagonism
	2:1	56.67	+ 60.17	Potentiation
	1:2	20	-25.93	Antagonism
Chlorpyrifos + *Euphorbia retusa*	1:1	10	- 49.24	Antagonism
	2:1	10	- 84.92	"
	1:2	7	- 70.46	"

Regarding the chloroformic crude extract and Chlorpyrifos binary mixtures, it is clear that Chlorpyrifos when mixed with *Calotropis procera* at 1:1 and 1:2 ratio exhibited a potentiation effects, co-toxicity factors were (+2.31 and + 76.47). On the other hand the binary mixtures of clorpyrifos and the two tested chloroformic plant extracts at the rest of the mixing ratio gave antagonism effects. Osman, M. Soad (1999) investigated 12 different plant extracts against 4th instar larvae of *Agrotis ipsilon* after 7 days using four different solvents and feeding methods. Out of the 12 plants 6 showed promising results against the target insect. LC_{50} values were *T.vulgares* pet / ether (LC_{50} = 4.22), *A.maritime* / acetone (LC_{50} = 4.22) and *C. longus* / ethyl (LC_{50} = 4.69).

Moustafa *et al.* (2006) isolated two glucosidal compounds (simmondsin(1) and simmondin2- ferulate (21) from the chloroformic extract of *S.chinensis* displayed highest insecticidal and antifeedant activity against the 3rd instar larvae of *S.littoralis* the LD_{50} values as µg / larvae of the two compounds and reference insecticide, Chlorpyrifos were 1.49, 2.58 and 0.12 respectively. Both compounds revealed a pronounced insecticidal activity but were less active than Chlorpyrifos. In the same manner, our results were greatly agreement with the above findings.

Table (6): Toxicity of the binary mixtures of chloroform crude plant extracts against 2nd instar larvae of cotton leafworm *S.littoralis*.

Combination	Mixing ratio	% Observed Mortality	Co-toxicity Factor	Joint action category
Chlorpyrifos	1:1	13.3	+2.31	Potentiation
+	2:1	17	-43.33	Antagonism
Calotropis procera	1:2	30	+76.47	Potentiation
Chlorpyrifos	1:1	13	- 34.84	Antagonism
+	2:1	20	- 50	"
Euphorbia retusa	1:2	17	- 37.04	"

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تأثير بعض المستخلصات النباتية ومخاليطها مع مبيد الكلوربيروفوس على يرقات العمر اليرقى الثانى لدودة ورق القطن تحت الظروف المعملية.

حلمى على زيدان - إيهاب محمد أنور و محمد عبد الوهاب الجندى

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تم استخلاص المكونات الخام لنباتى صابون الغيط والعشار بمذيبى الكلوروفورم والميثانول وذلك لدراسة تأثيرها على العمر اليرقى الثانى لدودة ورق القطن سواء منفردة أو مخلوطة بنسب مختلفة مع مبيد الكلوروبيروفوس وقد أظهرت النتائج مايلى:

١- تم التعرف على تسعة مكونات ذات نشاط حيوى من النباتات تحت الدراسة وقد وجدت هذه المكونات بكميات مختلفة حسب نوع النبات. وقد أظهرت الدراسة وجود الستيرولات ، التربينات الثلاثية ، الفينولات الجليكوسيدية ، التانينات ، الأنثراكينون جليكوسيدات ، الصابونينات ، الفلافونويدات ، الكاردك جليكوسيدات ، القلويدات وأخيرا الكربوهيدرات أو الجليكوسيدات بدرجات مختلفة حسب نوع النبات.

٢- تم استخلاص المكونات الفعالة الخام من كلا النباتين باستخدام مذيبى الكلوروفورم والميثانول.

٣- تم اختبار المستخلصات الخام ضد العمر اليرقى الثانى لدودة ورق القطن كل نبات على حده ثم مخاليط هذه المستخلصات مع مبيد الكلوربيروفوس بنسب خلط ١:١ & ٢:١ ، ١:٢ .

٤- كانت قيم الجرعة النصفية للمستخلص الميثانولى والكلوروفورم من كلا النباتين ومبيد الكلوروبيروفوس كما يلى [(٠,٣٢ & ١,٥٥) ، (٢,٩٢ ، ٥,٥٩) ، (٧ × ١٠ $^{-٥}$) على التوالى.

٥- كانت قيم دليل السمية لمركبات الكلوروبيروفوس + المستخلص الميثانولى لصابون الغيط ، كلوربيروفوس + العشار.

٦- كانت قيم دليل السمية لمركب الكلوربيروفوس + المستخلص الميثانولى لكل من نباتى صابون الغيط والعشار عند نسب خلط ١:١ ، ٢:١ ، ١:٢ كما يلى (- ٥٠ ، ٦٠,١٧ ، ٢٥,٩٣) & (٤٩,٢٤ ، - ٨٤,٩٢ & - ٧٠,٤٦) على التوالى.

٧- كانت قيم دليل السمية لمركب الكلوربيروفوس خلطا مع المستخلص الكلوروفورمى لكلا النباتين (+ ٢٠,٣١ ، - ٤٣,٣٣ ، ٧٦,٤٧) ، (- ٣٤,٨٤ ، - ٥٠ ، ٣٧,٠٤) على التوالى.

٨- أعطى خلط مبيد الكلوربيروفوس + المستخلص الميثانولى لنبات صابون الغيط تأثير تنشيطى عند الخلط بنسبة ٢:١ فقط (+ ٦٠,١٧) بينما أعطى خلط نفس المبيد + المستخلص الكلوروفورمى لنبات صابون الغيط تأثير تنشيطى عند نسب خلط ١:١ (+ ٢٠,٣) ، ١:٢ (+ ٧٦,٤٧ بينما أعطى الخلط مع مستخلص النباتين الميثانولى والكلوروفورمى عند باقى نسب الخلط تأثير تضادى.