New Environmental and Ecological Aspects in Controlling Pests

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Abstract: Laboratory experiments were conducted under controlled conditions to test the insecticidal activity of aqueous and organic extracts of six plants collected from North–Sinia (Tree Tobacco, *Nicotianaglauca* G; Syrian Rue, *Peganumharmala*; Calotrpis, *Calotrpisprocera*; Chinaberry, *Meliaazedarach* L; Egyptianhenbane, *Hyoscyamusmuticus* and *Artemisia monosperma*) against 4th instar larvae of Cotton leaf worm, *Spodopteralittoralis* (Boisd).After extraction with Methyl alcohol and Ethyl alcohol, in case of using Methyl alcohol in extraction, the results showed that the aqueous extract of *Hyoscyamusmuticus* was the highest pesticidal activity then the other aqueous extracts with LD_{50} equal to 0.066 $x10^5$ppm. Also the organic extract of *Hyoscyamusmuticus* has the lowest LD_{50} value and equal to 0.0112 $x10^5$ ppm. Also in case of 2-nd part of experiment, i.e. by using Ethyl alcohol in extraction the results proved that the aqueous extract of *Peganumharmala* achieved the highest value of pesticidal activity with LD_{50} equal to $0.06x10^5$ ppm. More over the organic extract of Peganumharmala recorded the highest pesticidal activity with LD50equal to ($0.00008x10^5$) ppm.

Keywords: Ecological Aspects, Cotton leaf worm, pesticidal activity

INTRODUCTION

TheCotton leafworm,*Spodopteralittoralis* is one of the major pests attacking Cotton and wide varieties of other host crops in Egypt. This pest is partly controlled by chemical efficient pesticides, but because of high resistance to several compounds,new possible alternatives for using safer methods of control have been explored. One of these approaches is the use of natural plant extracts and their secondary products which have recently received considerable attention.(Amer,1984);(AboEl-Ghara*etal.*,1986); (Dimetry *et al.*,1988); (El-Halawanyetal.,1989); (Amer *et al.*,1990); (Darwish, 1990); (Sawires *et al.*, 1990); (Hough,Goldstein and Hahn 1992); (Swidan,1994) and (Sawires *et al.*, 1995).(Taman *et al.*, 1996) also pointed out that the contents of alkaloids, flavonoids, saponins and triterpenes are suggested to be responsible for controlling repelling andantifeeding of Cotton leafworm.

Recently and due to the many difficulties in discovering new pesticides via the lab-synthesis work, a high attention began to be attracted to the naturally occurring chemicals which biosynthesized by plants.

Therefore it was planned to start that work in an attempt to contribute new information about some North Sinai plants, to be applied and used in the future as a main source of Natural pesticides.

MATERIALS AND METHODES

Rearing method of the tested insect:

The cultured of Cotton leaf worm, *Spodopteralittoralis* (Boisd) used in this study originated from egg masses obtained from susceptible strain established in the laboratory of Environment Protection Department Faculty of Environmental Agricultural Sciences, Suez Canal University, Al-Arish, North Sinai Egypt. The progency of the insects together with occasional fresh supplies of egg formed the basis of culture designed to provide insects used in the present investigation.

Five Replicates per each conc. were used and 50 larvae/Replicate. The 4-thinstar larvae were used in the bioassay tests. Under laboratory conditions of 25 ± 2c°(Temperature) and 60±5% RH (Relative Humidity) (El–Defrawy *et al.*, 1964)

Collection and identification of tested plants:

The following (6) plants used in the present study were *Nicotianaglauca* G.; *Peganumharmala; Calotropisprocera*; *Meliaazedarach* L.; *Hyoscyamus-muticus* and *Artemisia monosperma.*

Plant samples were collected from the area surrounding Arish Airport Table (1) Identification of the tested plants was based mainly on the taxonomic characters described by (Boulos and El–Hadidi,1984) and revised through personal communication with Dr. HamedaBedir (An associated Professor of Botany Faculty of Science Suez Canal University). Plant samples Table (1) were air dried for 2-4 weeksuntil complete dryness. Then these plants were milled in an electric grinder into fine powder and stored until used.

Aqueous and organic extraction:

Ten grams of each dried plant part Table (1) was soaked in a dark flask containing 100 ml of organic solvents used (Methyl alcohol and Ethyl alcohol) for the organic extraction of each sample and allowed to stand for 24h. The mixture was filtered by a Büchner funnel and that filtrate represents the organic extract for each sample. Simultaneously, the solid deposit on the Buckner funnel was washed with 100 ml of Redist. Water for each. The obtained water wash resembles the water extract for each plant sample, both organic and water extracts were freshly prepared and used for the bioassay purposes. These original crud extracts (organic and aqueous) were considered as stoke solution to be used as it is and by a series of successive dilutions to gain the tested concentration to be applied in bioassay.

Table (1): The list of six plant species and their extract parts studied in this investigation from the vicinity Al -Arish

NO.	Plant	English name	الاسم العربي	Extract part
1	*Nicotianaglauca*G.	Tree Tobacco	مصاص الدخان	All Plant
2	*Peganumharmala*	Syrian Rue	الحرمل	Seeds
3	*Calotrpisprocera*	Calotrpis	العشـار	Seeds
4	*Meliaazedarach*L.	Chinaberry	النيـم	Seeds
5	*Hyoscyamusmuticus*	Egyptian henbane	السكران المصري	All Plant
6	*Artemisiamonosperma*		العادر	All Plant

Bioassay tests for each organic or aqueous extracts:

Series of dilutions with Redist. Water for water extract and Methyl alcohol and Ethyl alcohol for the organic extracts were prepared for each stock solution. The dilutions were 10,100,1000,10000 times of the original stock solution. For the bioassay treatments, five jars each containing (20) 4-thinstar larvae of the tested insect, and each larva was topically treated with 1- ul with the micro-applicator (McCloud *et al*, 1988). Five replicates were used for each treatment or concentration including the control. Average percentage mortality was recorded for each treatment 24h. for 120h. LD_{50} values and the corresponding slopes were obtained from the regression lines (Finney, 1952), and the confidence limits were computed using the normal equivalent deviate programmed.

RESULTS

Screening the toxicity and insecticidal activity of aqueous extracts of the tested plants against 4-th instar larvae of Cotton leaf worm, *Spodopteralittoralis* (Boisd). after extracting with Methyl Alcohol.

The insecticidal activity of aqueous plant extractives of the tested plants on 4-thinstar larvae of *Spodopteralittoralis*, are summarized in Table (2) The aqueous extract of *Hyoscyamusmuticus*was the highest in pesticidal activity than the aqueous extract with LD50 ($0.066x10^5$ppm) followed by ($0.1x10^5$ppm), ($0.2x10^5$ppm), ($0.26x10^5$ppm), ($0.28x10^5$ppm) and ($0.46x10^5$ppm) in *Artemisia monosperma* L.; *Nicotianaglauca* G.; *Calotropisprocera*; *Peganumharmala* and *Meliaazedarach* respectively.

Screening the toxicity and insecticidal activity of organic extracts of the tested plants against 4-th instar larvae of Cotton leaf worm, *Spodopteralittoralis* (Boisd). after extracting with Methyl Alcohol.

The toxicity and insecticide activity of organic extracts of tested plants on 4-thinstar larvae of *Spodopteralittoralis* are tabulated in Table (3) The organic extract of *Hyoscyamusmuticus* has the lowest value with LD_{50} ($0.0112x10^5$ppm) and followed by ($0.02x10^5$ppm), ($0.04x10^5$ppm), ($0.09x10^5$ppm), ($0.09x10^5$ppm) and ($0.1x10^5$ppm) in plants *Artemisia monosperma*L.; *Meliaazedarach*; *Nicotianaglauca* G.; *Peganumharmala*and *Calotropisprocera*, respectively.

Screening the toxicity and insecticidal activity of aqueous extracts of the tested plants against 4-th instar larvae of Cotton leaf worm, *Spodopteralittoralis* (Boisd). after extracting with Ethyl Alcohol.

The toxicity of the aqueous extracts of the tested plants on 4-thinstar larvae of*Spodopteralittoralis*were tabulated in Table (4). The aqueous extract of *Peganumharmala* achieved the highest value ofpesticidal activitywith LD_{50} ($0.06x10^5$ppm) and followed by ($0.07x10^5$ppm), ($0.074x10^5$ppm), ($0.02x10^5$ppm), ($0.22x10^5$ppm) and ($0.24x10^5$ppm) in the following plants *Calotropisprocera*; *Meliaazedarach*; *Nicotianaglauca* G.; *Hyoscyamusmuticus* and *Artemisia monosperma* L. respectively.

Screening the toxicity and insecticidal activity of organic extracts of the tested plants against 4-th instar larvae of Cotton leafworm, *Spodopteralittoralis* (Boisd). after extracting with Ethyl Alcohol.

The toxicity of the organic extracts of the tested plants against 4-thinstar larvae of *Spodopteralittoralis* were tabulated in Table (5). Postulating that The *Peganumharmala* recorded the highest toxicity with LD50 ($0.00008x10^5$ppm) and followed by ($0.000112x10^5$ppm),($0.003x10^5$ppm), ($0.0104x10^5$ppm), ($0.02x10^5$ppm) and ($0.28x10^5$ppm) inthe following plants *Artemisia monosperma*; *Hyoscyamusmuticus*; *Meliaazedarach* L.; *Nicotianaglauca* G.; *Calotropisprocera*respectively.

DISCUSSION

Many authors and literatures discussed these of crude plant extracts (Zaputa *et al.*, 2009) and crude extracts from leaves (Wellson*etal.*, 2006) essential oils of flowers and leaves (ColomaandSoria, 2006) seed extracts (Ntonifor *et al.*, 2006).According from the previous studies, the present work was conducted to evaluate the efficiency of aqueous and organic extracts of the following 6 plants: *Nicotianaglauca* G.; *Peganumharmala*; *Calotropisprocera*; *Meliaazedarach* L; *Hyoscyamusmuticus* and *Artemisia monosperma* against 4-thinstar larvae of *Spodopteralittoralis*. These plant extracts were made by Methyl alcohol and Ethyl alcohol, to have in each solvent an aqueous phase and an organic phase.

So in the 1-th part of experiment i.e. extracting by Methyl alcohol of the 6 previous plants, we have 6

aqueous extracts, and 6 organic extracts.

Accordingly the results revealed that *Meliaazedarach*; *Hyoscyamusmuticus* and *Artemisia monosperma* were more effective than the other 3 plants possessed a very high degree of significant discrimination and ranged between ($0.06x10^5$ppm) to ($0.46x10^5$ ppm) i.e. the difference was 7.5 times. By taking all these factors into account, it could be concluded that there are different contents of the active gradients and its concentrations showing a promising plants, even it was in an aqueous form. On the other side, where the organic phase was applied, the range of LD_{50} was ($0.01x10^5$ ppm) and ($0.1x10^5$ ppm) i.e. the difference between lower and upper LD_{50} was 10 times. More over by comparing the values of LD_{50} of both aqueous and organic phase for each plant was in the following order ((2.2 - >2.6 - >3.0 ->5.0 - >6.0 - >11.5)) in the plants *Nicotianaglauca* G.; *Calotropisprocera*; *Peganumharmala*; *Artemisia monosperma*; *Hyoscyamusmuticus* and *Meliaazedarach* respectively. Subsequently, these results are reflecting the effect of multi physiochemical, biochemical and toxicological properties of each plant and its chemical components and concentrations in both aqueous and organic phase in the case of extraction by Methyl alcohol. Also, in the 2-nd part of experiment i.e. by extraction with Ethyl alcohol of the same 6 previous plants, we have, 6 aqueous extracts and 6 organic extracts Accordingly and by calculating the LD_{50} values, the picture was more or less similar to the previous results i.e. it was ranged between ($0.06x10^5$ ppm) and ($0.22x10^5$ ppm), i.e. the difference was 3.66. But in the case of organic phase the LD_{50} values ranged between ($0.000084x10^5$ ppm) and ($0.028x10^5$ ppm), i.e. more than 333 times. More over by comparing the values of LD_{50} of both aqueous and organic phase for each plant was as in the following order. ((>2.5 - 6.7 –>10.0 - >.73 - >.714 - >.2142)) in the plants *Meliaazedarach; Nicotianaglauca* G.; *Hyoscyamusmuticus*; *Peganumharmala* and *Artemisia monosperma* respectively. So virtually these results are offering grossly a unique possibility of the effect of multi-functional physiochemical, biochemical and toxicological properties of the phytochemicals and its concentrations in both aqueous and organic phase due to extraction by Ethyl alcohol. All these results were in agreement with the results of different authors, such as (El-Doksh *et al.*, 1984). where found that LD_{50} values of organic extract was more toxic than LD_{50} of aqueous extract. Also the present data was confirmed by the finding of (Schmidt *et al.*, 1997), by testing different concentrations of the metabolic extract of *Meliaazedarach* L. fruits against *Spodopteralittoralis* and *Agrotisipsilon*, finding that the percentage of mortality increased with higher concentrations of the methanolic extract of *Meliaazedarach* against *Spodopteralittoralis*. and *Agrotisipsilon* (Conyers and Bell, 1996). (Bolter and Chefurka, 1990) and (Bond *et al.*, 1967).

There is a great deal in the literatures on the pesticidal effects of *Calotropisprocera* against different kinds of pests (Al-Rajhy *et al.*, 2003). Also it can be mentioned that there are certain concentrations of aqueous or organic extracts of each plant, which could be named by the optimum and suitableconcentrations causing the best effect, Besides the variations between each plant and its response and insect target sensitivity presence of polar and apolar compounds in the media of testing. So that it is offering a kind of physiological selectivity which occurred due to differences in mode of action showing a variability in type of toxic materials, its concentration and its response. Also the role of genetic factor in elucidating differences in responses and reactions (Upitis *et al.*, 1973). And (Arnaud *et al.*, 2005).Meanwhile and by throwing more light, (Bell *et al.*,1990). reported that the presence of so-called secondary metabolite compounds, which give no know function in photosynthesis, growth or other aspects of plant physiology, give plant materials or their extracts their anti-insect activity. Secondary metabolite compounds include alkaloids, terpenoids, phenolics, flavonoids, chromenes and other minor chemicals can affect insects in several different ways, they may disrupt major metabolite pathways and cause rapid death, act as attractants, deterrents, phagostimulant andantifeedant or modify oviposition. They may retard or accelerate development or interfere with the life cycle of the insect in other ways. So that it can explain the high mortality by using such plants as potent insecticides (Lioyed, 1973), (Huang *et al.*, 1997). (Asgary *et al.*, 2000). and (wink *et al.*, 2004). So subsequently and by more focusing, the high mortality percentages and toxicity effects of the previous tested plants may be due to variations in the type of active ingredients and its chemical structure and their mode of action that recorded in their aqueous or organic extracts.(Bell *et al.*, 1990), (Liu and HO, 1999). and (Sukumar *et al.*, 1991).

More recently, (Trombetta *et al.*, 2005). and (Salvelev *et al.*,2003). reported that the requirements of ideal biopesticides (i)-They perform their effects invert short time and disappear from the environment quickly, with no residues to threat the components of the environment. (ii)-They have broad spectrum of pesticide effects, that is, they control many pests of different species and classes. (iii)-They have no or minimum effect on man and target organisms (EPA., 1993). (iv)-They have several modes of action on the target pest, since they contain many compounds with different chemical structures and different chemical groups which prevents or postpones the development of pest resistance.So by more elucidating by focusing on the nature and body composition of the tested insect. (Reynold, 1987). reported that the insect cuticle is a layered structure and the functions of the cuticle that are most vulnerable to insecticidal action are mechanical. These properties of the cuticle stiffness, strength and hardness are largely due to the major part of the cuticle thickness. Cuticle is a composite material made of carbohydrates,proteins, lipids, phenolics and tannins. They confer a chemical and mechanical stability to the cuticle by increasing the hydrophobicity of the cuticle matrix. And by going more after the nature and composition of the membranes and its effects by extracts on these membranes,(Hamburger and Hostellman, 1991). reported that the drug affects

integrity of membranes and localized these membranes due to its highly lipophilic nature. On the other side, chemical characteristics of the effective compounds such as charge and polarity of natural compounds affecting rates of interchange especially across membranes and cuticles to determine whether it reaches that tissue or target at intoxicating concentrations (Gilpy, 1984).

Also these results are indicating that these plants have certain properties of selectivity and sensitivity. Also there is a natural selection pressure that has often negatively affect the other species (Keeler and Tu, 1991).

Ultimately many groups of chemicals having a diverse chemical structure, but that possess common biological effect such as killer, attractants, hormonal stimulation of growth and behavior. And since biological functions are normally very selective processes. so, a group of chemicals having similar biological activities must have same feature of similarity in selectivity (Harborne, 1988). These ecological and physiological selectivity were appearing in all tested plants and insects (Wilkinson, 1976). Also (Suffness and Douros, 1982). defined the selectivity i.e. it may be high to limit the no. of leads for follow up evaluation and expressed about sensitivity i.e. it must be very high in order to detect the low concentrations of active ingredients of compounds. Very Recently,(El-sebae *et al.*, 2008). indicated that there were a significant variations between most of the tested plants, Also the organic extract gave in general higher potencies than the aquatic extract. However, there are other obvious examples of specificity and selective toxicity of the compared plant extracts. Thus it can be concluded that extracts can lead to discover newly alternative plant pesticide molecules which can replace the known hazardous conventional pesticides as much safer, selective and effective, insecticides. Further detailed studies are still needed.

Table (2): LD50, slope and confidence limits values of the aqueous extract after soaking in Methyl Alcohol of tested plants against 4th instar oflarvae*Spodopteralittoralis*

NO.	Plant	LD_{50}(ppm)	Slope	Confidence limits of LD_{50}
1	*Nicotianaglauca*G.	0.2×10^5	0.6274	0.0585×10^5-0.6837×10^5
2	*Peganumharmala*	0.28×10^5	0.5925	0.0722×10^5-1.0857×10^5
3	*Calotropisprocera*	0.26×10^5	0.5981	0.067×10^5-1.0062×10^5
4	*Meliaazedarach*L.	0.46×10^5	0.547	0.1134×10^5-0.8658×10^5
5	*Hyoscyamusmuticus*	0.066×10^5	0.8888	0.277×10^5-0.0157×10^5
6	*Artemisiamonosperma*	0.1×10^5	0.7441	0.0278×10^5-0.359×10^5

Table (3): LD_{50},slope and confidence limits values of organic extractafter soaking in Methyl Alcohol of the tested plants against 4thinstar larvae of*Spodopteralittoralis*

NO.	Plant	LD_{50}(ppm)	Slope	Confidence limits of LD_{50}
1	*Nicotianaglauca*G.	0.09×10^5	0.7619	0.0265×10^5-0.3045×10^5
2	*Peganumharmala*	0.09×10^5	0.7619	0.0266×10^5-0.3043×10^5
3	*Calotropisprocera*	0.1×10^5	0.7441	0.03×10^5-0.3247×10^5
4	*Meliaazedarach*L.	0.04×10^5	0.9696	0.0117×10^5-0.1359×10^5
5	*Hyoscyamusmuticus*	0.0112×10^5	0.7191	0.0027×10^5-0.0454×10^5
6	*Artemisia monosperma*	0.09×10^5	0.7619	0.0265×10^5-0.3045×10^5

Table (4): LD_{50}, slope and confidence limits values of aqueous extractafter soaking in Ethyl Alcohol of the tested plants against 4th instar oflarvae*Spodopteralittoralis*

NO.	Plant	LD_{50}(ppm)	Slope	Confidence limits of LD_{50}
1	*Nicotianaglauca*G.	0.2×10^5	0.6274	0.0580×10^5-0.6833×10^5
2	*Peganumharmala*	0.06×10^5	0.8533	0.0141×10^5-0.254×10^5
3	*Calotropisprocera*	0.07×10^5	0.81012	0.0202×10^5-0.2415×10^5
4	*Meliaazedarach*L.	0.074×10^5	0.8101	0.0178×10^5-0.306×10^5
5	*Hyoscyamusmuticus*	0.22×10^5	0.6274	0.058×10^5-0.8278×10^5
6	*Artemisiamonosperma*	0.22×10^5	0.6153	0.0906×10^5-0.5339×10^5

Table (5): LD_{50}, slope and confidence limits values of organic extractafter soaking in Ethyl Alcohol of the tested plants against 4th instar oflarvae*Spodopteralittoralis*

NO.	Plant	LD_{50}(ppm)	Slope	Confidence limits of LD_{50}
1	*Nicotianaglauca*G.	0.02×10^5	0.6274	0.0049×10^5-0.081×10^5
2	*Peganumharmala*	0.00008×10^5	0.7804	0.000033×10^5-0.00021×10^5
3	*Calotropisprocera*	0.028×10^5	0.6274	0.0071×10^5-0.1098×10^5
4	*Meliaazedarac*L.	0.0104×10^5	0.7356	0.0025×10^5-0.0424×10^5
5	*Hyoscyamusmuticus*	0.003×10^5	0.5765	0.00066×10^5-0.0135×10^5
6	*Artemisia monosperma*	0.000112×10^5	0.7191	0.00003×10^5-0.00041×10^5

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الأبعاد البيئية والإيكولوجية في مكافحة الآفات

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***مركز البحوث الزراعية- معهد بحوث وقاية النباتات- مصر

تم دراسة تأثير التركيزات المختلفة لكلا من المستخلص المائي والعضوي للنباتات المختبرة ضديرقات العمر الرابع من دودة ورق القطن بعد الاستخلاص بـ كحول الميثيل.

١- المستخلص المائي لنبات السكران كان أفضل المستخلصات المائية وحقق اعلى قيمة للـ LD50وتلي ذلك في النباتات الآتية :العادر، مصاص الدخان، بذور العشار، بذور الحرمل، بذور النيم على التوالي.

٢-أيضا اظهر المستخلص العضوي لنبات السكران اعلي قيمة لـ LD50 وتلي ذلك النباتات الاتية:

العادر، بذور النيم، مصاص الدخان، بذور الحرمل، بذور العشارعلى التوالي.

وقد لوحظ ان تأثير التركيزات المختلفة لكلا من المستخلص المائىوالعضوى للنباتات

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١- المستخلص المائي لنباتبذور الحرمل كان أفضل المستخلصات المائية وحقق اعلى قيمة لـ LD50 وتلي ذلك في النباتات الآتية. بذور العشار،بذور النيم،مصاص الدخان، السكران، العادرعلى التوالي.

٢- أيضا اظهر المستخلص العضويلنبات بذور الحرمل اعلي قيمة لـ LD50 وتلي ذلك النباتات الآتية. العادر،السكران، بذور النيم، مصاص الدخان، بذور العشارعلى التوالي.