



INSECTICIDAL PROPERTIES OF SOME PLANT EXTRACTS AGAINST COTTON LEAFWORM *Spodoptera littoralis* (BOISD)

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ABSTRACT

Laboratory experiments were conducted under controlled conditions to test the insecticidal activity of aqueous and organic extracts of different solvents (ethanol and acetone) of nine plants collected from North-Sinai (Mint, *Mentha piperita* L.; Clove, *Syzygium aromaticum* L.; Camphor, *Cinnamomum camphora* L.; Tree tobacco, *Nicotiana glauca* G.; Syrian Rue, *Peganum harmala* L.; *Artemisia monosperma* L.; Chinaberry, *Melia azedarach* L.; Egyptian henbane, *Hyoscyamus muticus* L. and Calotropis, *Calotropis procera* L.) against 4th instar larvae of cotton leafworm (*Spodoptera littoralis* Bois). The results showed that the aqueous extracts *C. camphora* L. gave the highest toxicity among all tested plants with LD₅₀ equal to 152 ppm and *M.piperita* L. recorded the lowest toxicity with LD₅₀ equal to 500 ppm. The toxicity index (T.I) was the highest value (100) in case of *C.camphora* L. and the lowest value was (30.6) in case of *M. piperita* L. The relative potency (R.P) showed the highest value equal to 327.2 with *C.camphora* L, while the *M.piperita* L. showed the lowest value equal to (100). In case of using organic (ethanol alcohol) extracts the results showed that the ethanolic extracts of *S.aromaticum* L. has the highest toxicity with LD₅₀ equal to 76.9 ppm and that of *C.procera* L. has the lowest toxicity with LD₅₀ equal to 205.6 ppm . The toxicity index (T.I) of *S.aromaticum* L. have the highest value (T.I) (100) and the *C.procera* have the lowest value T.I (37.4). The relative potency (R.P) was the highest in *S.aromaticum* L. (267.4) and the lowest (R.P) was in *C.procera* (100) .Finally by applying the organic (acetic) extracts, the results, showed that acetic extracts of *S.aromaticum* L. recorded the highest toxicity with LD₅₀ equal to 44.4 ppm while acetic extracts of *C.procera* L. showed the lowest toxicity with LD₅₀ equal to 100.6 ppm. The highest (T.I) was with *S.aromaticum* L. value (100) and the lowest (T.I) was observed in case of *C.procera* (44.1) recorded. The relative potency (R.P). Was the highest in case of *S.aromaticum* L.(226.6) ,while the lowest (R.P) was in case *C.procera* (100).

Key words: Insecticidal activity, natural plants, 4th instar and larva cotton leafworm.

INTRODUCTION

In a number of African countries the cotton is an important economic crop including; Egypt – Sudan – Chad – Mali – Tanzania – Zimbabwe and South Africa. On average, a cotton farmer loose at least one bale out of eleven bales/year due to insects damage and plant diseases. The development of chemical pesticides urge

farmers to abandon most of conventional control strategies, mean while an over reliance on the use of chemical show problems of resistance and or environmental pollution. (Mesbah *et al.* 2007). Currently the use of natural products instead of synthetic insecticides is of interest to overcome the pest resistance, reduce cost of pest control and minimize the environmental risk of different chemical

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groups of pesticides. The present study was conducted to evaluate the efficiency of using aqueous and organic extracts of some promising plants in controlling cotton leafworm *Spodoptera littoralis* (Boisd) in a laboratory study in an attempt to avoid the excess use of synthetic insecticides in environment.

MATERIALS AND METHODS

Rearing the Tested Insect

The cultured of cotton leafworm, *Spodoptera littoralis* (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, Arish University, Al-Arish, North Sinai, Egypt. The progeny 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/ Treatment. The 4th instar larvae were used in the bioassay tests. Under laboratory conditions of $25 \pm 2C^{\circ}$ (temperature) and $60 \pm 5\%$ RH (Relative Humidity) **El-Defrawi *et al.* (1964).**

Collection and identification of tested plants

The following plants were used in the present study; *Menth piperita* L, *Syzygium aromaticum* L., *Cinnamomum camphora* L. *Nicotiana glauca* G.; *Peganum harmala*; *Calotropis procera*; *Melia azedarach* L.; *Hyoscyamus muticus* and *Artemisia monosperma*. Plant samples were collected from the area surrounding Arish Airport. 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. Hamed Bedir (Professor of Botany Faculty of Science Arish University). Plant samples (Table1) were air dried for 2-4 weeks until 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 aqueous and or organic extracts *i.e* Solvents used (Dist. H₂O – ethyl alcohol and acetone) for the aqueous and organic extraction of each sample and allowed to stand for 24hr. The extract was filtered by a Büchner funnel and that filtrate represents the aqueous and organic extract for each sample. These original crud extracts (organic and aqueous) were freshly prepared and considered as stock solution to be used as it is and by a series of successive dilutions to gain the tested concentration to be applied in bioassay.

Bioassay tests for each organic or aqueous extracts

A series of concentrations prepared from aqueous and organic extracts (ethanol alcohol and acetone) as following: 25, 50, 75, 100, 125, and 250, 500, 750 and 1000 ppm to be used in the bioassay. For the bioassay treatments, five jars each containing (10) 4th instar larvae of the tested insect, and each larva was topically treated with 1- μ l with the micro-applicator **Mc.Cloud *et al.* (1988).** Five replicates were used for each treatment or concentration including the control. Average percentage mortality was recorded for each treatment 24 h. for 120 hr. LD₅₀ values and the corresponding slopes were obtained from the regression lines (**Finney, 1971**) and the confidence limits were computed using the normal equivalent deviate programmed.

Table (1): The list of plant species and their extract parts studied of tested plants.

NO.	E. Name	Arabic name	Scientific name	Family	Part used
1	Mint	النعناع	<i>Menth piperita</i> L.	Lamiaceae	Leaves
2	Clove	قرنفل	<i>Syzygiuma romaticum</i> L.	Myrtaceae	Flowers
3	Camphor	كافور	<i>Cinnamomum camphora</i> L.	Lauraceae	Leaves
4	Tree Tobacco	مصاص الدخان	<i>Nicotiana glauca</i> G.	Solanaceae	All plant
5	Syrian Rue	الحرمل	<i>Peganum harmala</i>	Zygophyllaceae	Seeds
6	Ader	العذار	<i>Artemisia monosperma</i>	Compositae	All plant
7	Chinaberry	النييم	<i>Melia azedarach</i> L.	Meliaceae	Seeds
8	Egyptian henbane	السكران المصري	<i>Hyoscyamus muticus</i>	Solanaceae	All plant
9	Calotrpis	العشار	<i>Calotropis procera</i>	Asclpiadaceae	Seeds

RESULTS

Acute toxicity of tested (aqueous) plant extracts against 4th instar larvae of Cotton leafworm, *Spodoptera littoralis* (Boisd)

Results presented in Table 2 assure that the tested plants were arrayed in a descending order as following: (*M. piperita*, *A. monosperma*, *H. muticus*, *N.glauca*, *M. azedarach*, *C. procera* *S. aromaticum*, *P. harmala* and *C. camphora*), eliciting LD₅₀ values arranged as following: (500, 361.1, 308.3, 397.3, 286.1, 280.6, 277.8, 258.3 and 152.8ppm) respectively. The toxicity index of tested plants were arranged in a descending order as following: *C.camphora* L(100), *P. harmala* (59.2), *S.aromaticum* L.(55), *C. procera* (54.5), *M. azedarach* L. (53.9), *N. glauca* G. (51.4), *H. muticus* (49.6), *A. monosperma* (42.3) and *M. piperita* L. (30.6). The toxicity index (T.I) was the highest value (100) in case of *C. camphora* L. and the lowest value was (30.6) in case of *M. piperita* L. In the same attitude the relative potency (R.P) of the tested plants were arranged in a descending order as following: *C.camphora* L (327.2),

P.harmala (309.7), *A.monosperma* (221.5), *S.aromaticum* L.(180), *C.procera* (178.2), *M.azedarach* L. (174.8), *N.glauca* G.(168.2) and *M. piperita* L. (100). *i.e* (R.P) have the highest value equal to 327.2 with *C.camphora* L while the *M. piperita* L. showed the lowest value equal to (100).

Acute toxicity of tested organic (ethanolic) plant extracts against 4th instar larvae of Cotton leafworm, *Spodoptera littoralis* (Boisd).

Results presented in Table 3 affirm that the tested plants were arranged in a descending order as following: (*C. procera*, *N.glauca*, *M. azedarach*, *H.muticus*, *M. piperita*, *P.harmala*, *A.monosperma*, *C. camphora* and *S. aromaticum*) anticipating that LD₅₀ values arranged as in the following: (205.6, 143.1, 131.9, 125, 113.9, 103.1, 103.1, 88.9 and 76.9 ppm) respectively. The toxicity index (T.I) values were arranged in a descending order of the tested plants as following: *S.aromaticum* L.(100), *C. camphora* L. (86.6), *P. harmala* (74.6), *A.monosperma* (74.6), *M. piperita* L. (67.5), *H. muticus* (61.5), *M. azedarach* L. (58.3), *N. glauca*

Table (2): Acute toxicity of tested (aqueous) plant extracts against 4th instar larvae of Cotton leafworm, *Spodoptera littoralis* (Boisd).

No.	Treatments	LD ₅₀ (ppm)	Slope	Confidence limits of LD ₅₀	Toxicity index	Relative Potency at(fold)
1	<i>Menth piperita</i> L.	500	0.421	357 - 706	30.60	100
2	<i>Syzygium aromaticum</i> L.	277.8	0.547	205 – 374	55	180
3	<i>Cinnamomum camphora</i> L.	152.8	0.507	114 – 203	100	327.20
4	<i>Nicotiana glauca</i> G.	297.3	0.516	212 – 420	51.40	168.20
5	<i>Peganum harmala</i>	258.3	0.598	190 – 353	59.20	309.70
6	<i>Artemisia monosperma</i>	361.1	0.467	178 – 350	42.30	221.50
7	<i>Melia azedarach</i> L.	286.1	0.533	201 – 406	53.40	174.80
8	<i>Hyoscy muticus</i>	308.3	0.503	220 – 434	49.60	162.20
9	<i>Calotropis procera</i>	280.6	0.542	206 – 382	54.50	178.20

LD₅₀ values were calculated from the regression lines using method of **Finney(1971)**.

Toxicity index according to **Sun's (1950)**

Relative Potency at fold according to **Zidan and Abd El-Megeed (1988)**.

Table (3). Acute toxicity of tested organic (ethanolic) plant extracts against 4th instar larvae of cotton leafworm, *Spodoptera littoralis* (Boisd).

No.	Treatment	LD ₅₀ (ppm)	Slope	Confidence limits of LD ₅₀	Toxicity index	Relative potency at (fold)
1	<i>Menth piperita</i> L.	113.9	0.711	87.6 –148.5	67.5	180.5
2	<i>Syzygium aromaticum</i> L.	76.9	0.566	64.98 – 91.13	100	267.9
3	<i>Cinnamomum camphora</i> L.	88.9	0.711	75.38 –104.79	86.6	231.3
4	<i>Nicotiana glauca</i> G.	143.1	0.551	112.99 –181.18	53.7	143.7
5	<i>Peganum harmala</i>	103.1	0.542	80.50– 131.94	74.6	199.4
6	<i>Artemisia monosperma</i>	103.1	0.547	85.78 – 123.94	74.6	199.4
7	<i>Melia azedarach</i> L.	131.9	0.397	85.12 –205.14	58.3	155.9
8	<i>Hyoscyamus muticus</i>	125	0.627	99.12–157.7	61.5	164.5
9	<i>Calotropis procera</i>	205.6	0.688	154.89 – 272.91	37.4	100

LD₅₀ values were calculated from the regression lines using method of **Finney(1971)**.

Toxicity index according to **Sun's (1950)**

Relative Potency at fold according to **Zidan and Abd El-Megeed (1988)**.

G. (53.7) and *C. procera* (37.4). *i.e.* the *S.aromaticum* L. have the highest (T.I) was (100) and the *C.procera* have the lowest value T.I was (37.4). Also the relative potency (R.P) values were arranged in a descending order of the tested plants as in the following: *S.aromaticum* L. (267.4), *C.camphora* L (231.3), *P. harmala* (199.4), *A.monosperma* (199.4), *M. piperita* L. (180.5), *H.muticus* (164.5), *M. azedarach* L. (155.9), *N.glauca* G. (143.7) and *C. procera* (100). *i.e* the highest R.P was in *S.aromaticum* L.(267.4) and the lowest (R.P) was in *C. procera* (100).

Acute toxicity of tested organic (acetonic) plant extracts against 4th instar larvae of cotton leafworm, *Spodoptera littoralis* (Boisd)

Results presented in Table 4 illustrate that the tested plants were arranged in a descending order as: (*C. procera*, *N. glauca*, *M. azedarach*, *H.muticus*, *M. piperita*, *A. monosperma*, *P. harmala*,

C.camphora and *S.aromaticum*) possessing that LD₅₀ values arranged as following: (100.6, 87.7, 84.3, 83.3, 82.5, 77.8, 62.2, 51.9 and 44.4 ppm) respectively. The toxicity index (T.I) showed an arrangement in a descending order as following: *S. aromaticum* L.(100), *C.camphora* L (85.5), *P.harmala* (71.4), *A.monosperma* (57.1), *M. piperita* L. (53.8), *H.muticus* (53.3), *M.azedarach* L. (52.4), *N.glauca* G. (50.6) and *C.procera* (44.1). *i.e.* the highest (T.I) was with *S.aromaticum* L. (100) and the lowest (T.I) was in case of *C.procera* value (44.1). The relative potency (R.P) also showed a similar trend *i.e* the arranged in a descending order as in the following: *S.aromaticum* L.(226.6), *C.camphora* L (193.8), *P. harmala* (161.7), *A.monosperma* (139.3), *M. piperita* L. (122), *H. muticus* (120.8), *M. azedarach* L. (118.8), *N. glauca* G. (114.6) and *C.procera* (100). *i.e* the highest value of R.P was in case of *S.aromaticum* L. (226.6), also the lowest (R.P) was in case *C. procera* (100).

Table (4): Acute toxicity of tested organic (acetonic) plant extracts against 4th instar larvae of cotton leafworm, *Spodoptera littoralis* (Boisd).

No.	Treatment	LD ₅₀ (ppm)	Slope	Confidence limits of LD ₅₀	Toxicity Index	Relative potency at (fold)
1	<i>Menth piperita</i> L.	82.5	0.488	67.9 -103.98	53.8	122
2	<i>Syzygium aromaticum</i> L.	44.4	0.666	24 – 98	100	226.6
3	<i>Cinnamomum camphora</i> L.	51.9	0.566	36 – 83	85.5	193.8
4	<i>Nicotiana glauca</i> G.	87.8	0.719	74.82 - 102.96	50.6	114.6
5	<i>Peganum harmala</i>	62.2	0.727	49.93 - 77.56	71.4	161.7
6	<i>Artemisia monosperma</i>	77.8	0.547	60.90 - 99.33	57.1	139.3
7	<i>Melia azedarach</i> L.	84.7	0.474	70.48 - 101.14	52.4	118.8
8	<i>Hyoscyamus muticus</i>	83.3	0.484	64.24 - 108.14	53.3	120.8
9	<i>Calotropis procera</i>	100.6	0.609	77.52 - 130.49	44.1	100

LD₅₀ values were calculated from the regression lines using method of Finney(1971).

Toxicity index according to Sun`s (1950)

Relative Potency at fold according to Zidan and Abd El-Megeed (1988).

DISCUSSION

Finally, results showed that the mortality occurred, due to the presence of active ingredients found in the utilized plants which have potential insecticidal activities against the tested pest or organism. Also it can be declared that there are certain conc.'s of both aqueous and/or organic extracts of each plant and it could be named as an optimum and best concentrations (conc.), causing, the maximum effect. Also and besides the variation between each plant and its response and insect target sensitivity to the conc. 's applied and at each tested phase *i.e* the presence of polar and apolar compounds. So that it is offering a kind of physiological selectivity which occurred due to the differences in its mode of action, showing a variability in type of toxic materials, its conc. and its response. Also the role of genetic factors in elucidating different responses and reactions (**Upitis *et al.*, 1973**) and **Arnaud *et al.*(2005)**. Also by going after LD₅₀ values, the differences between the resulted in aqueous and organic (ethanolic and acetonc) of each plant may be due to differences in morphological and physiological and metabolic responses in each pest and in pest species (**Conyers By Bell 1996**). By more focusing the organic extracts of the tested plants, LD₅₀ values, also indicated that organic extracts of most tested plants were better than aqueous extracts. These results were in an agreement with **Eldoksch *et al.* (1984)**, who found that LD₅₀ values of organic extracts were more toxic than LD₅₀ values of aqueous extracts. Also in the same time all the plant conc.'s (aqueous and organic) against the tested insect showed that mortality percentages increased by increasing of used conc.'s. **Schmidt *et al.* (1997)**, showed that, high conc.'s of methanolic extrat of neem led to high mortality (%) against *S.littoralis* and *Agrotis ipsilon*. Ultimately, it is appearing that all plant extracts (aqueous and organic)

are affecting oxygen arrives inside the experimental body insect and that cause asphyxia and leading to death. Death (%) is different with conc.'s, extract types, tested insect and tested plants **El-Araby (2008)**, **Sayed (2010)** and **El-Araby (2014)**. Mean while and by throwing more light, **Bell *et al.* (1990)** reported that the presence of so-called secondary metabolite compounds, which have no known function in photosynthesis, growth or other aspects of plant physiology, give plant materials or their extracts or 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 metabolic pathways and cause rapid death, act as attractants, deterrents, phagostimulants, antifeedants or mortality 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 (**Lloyd, 1973; Huang; *et al.* 1997; Asgary *et al.* 2000; Wink *et al.* (2004)**). Also the high mortality% and toxicity effects of the previous tested plants may be due to variation in the type of active ingredients and its chemicals structure and their mode of action which well presented in their aqueous or organic extracts (**Liu and Ho, 1999**). In conclusion and by focusing on the nature and body composition of the tested insect, **Rynolds (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 proteins, lipids, phenolics and tannins. They confer chemical and mechanical stability to the cuticle by increasing the hydrophobicity and of the cuticle matrix. And by more focusing on the

nature and composition of the membranes and its affect by the used 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. More over that the 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 conc.'s **Gilby (1984)**. In the other side, where there is an increase of effective compounds in organic extract of all plants, although there are some compounds can be soluble in aqueous extracts for some plants and lethal effect that indicating these, plants have properties of the selectivity and sensitivity. Also there is a natural selection pressure has often negativity affect the other species, (**Keeler and Tu 1991**). Ultimately many group of chemicals having a diverse chemical structure, but that posses common biological effect such as killer, attractants, hormonal, stimulation of growth and behavior. And since biological function are normally very selective processes so, a group of chemicals having similar biological activities must have some 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 must be high to limit the No. of leads for follow-up evaluation and expressed about sensitivity *i.e* it must be high in order to detect the low conc.'s of active ingrediants of compounds. So from all what mentioned in the literatures and references are in an accommodation and assuming the obtained results in nearly all cases of the study .Appointing the effect of the different plant extractives (aqueous and organic) applied on tested insects. And bearing in mind new more safe and

ecotoxicological attitudes and considerations, facing the pests and unfavorable impacts of traditional insecticides applied in the environment. All these results were in an agreement with a No. of early studies dealing not only with *S.littoralis*, but also with many pests. Also very recently studies by **Sayed et al. (2010)**, **Rawi et al. (2011)**, **Boursier et al. (2011)** and **El-Araby et al. (2014)**, were in an agreement with the obtained results.

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المخلص العربي

الخواص الإبادية لبعض المستخلصات النباتية ضد دودة ورق القطن

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أجريت تجارب معملية تحت ظروف قياسية لاختبار النشاط الابادى للمستخلصات المائية والعضوية لمذيبات مختلفة (كحول ايثايل - الأستون) لتسع (٩) نباتات تم تجميعها من شمال سيناء وهي: (النعناع، القرنفل، كافور، مصاص الدخان، الحرمل، العادر، النيم، السكران، العشار) وتم اختبارها على يرقات العمر الرابع لدودة ورق القطن ولقد أوضحت النتائج ان المستخلصات المائية لنبات الكافور أعطت أعلى سمية من بين جميع النباتات المختبرة وأعطت بقيمة ١٥٢ جزء في المليون (LD_{50})، بينما سجل نبات النعناع أقل سمية وكانت قيمة (LD_{50}) ٥٠٠ جزء في المليون وفي حالة المستخلصات العضوية (كحول الايثايل) أظهرت النتائج أن المستخلصات الكحولية لنبات القرنفل أعلاها سمية وأعطت (LD_{50}) ٧٦،٩ جزء في المليون، بينما أعطى نبات العشار أقل قيمة سمية (LD_{50}) ٢٠٥،٦ جزء في المليون واخبراً وباستخدام المستخلصات الاسيتونية أظهرت النتائج أن مستخلص نبات القرنفل سجل أعلى سمية بقيمة (LD_{50}) ٤٤،٤ جزء في المليون وأعطى مستخلص نبات العشار في هذه الحالة أقل قيمة وكانت قيمة (LD_{50}) ١٠٠،٦ جزء في المليون. سجلت قيمة (T.I) في المستخلص المائي لنبات الكافور أعلى قيمة وكانت ١٠٠ وأقل قيمة كانت لنبات النعناع أعطى قيمة ٣٠،٦. وعند حساب قيمة (R.P) أعطى الكافور أعلى قيمة وكانت ٣٢٧،٢٠ وأقلها كانت في نبات النعناع كانت ١٠٠. وفي المستخلص الايثانولي كانت أعلى قيمة لـ (T.I) في نبات سجل ١٠٠ ونبات العشار أعطى قيمة ٣٧،٤ وقدرت أعلى قيمة لـ (R.P) في نبات القرنفل كانت ٢٦٧،٩ وأقلها لنبات العشار سجلت ١٠٠. وفي المستخلص الاسيتوني سجل (T.I) لنبات القرنفل أعلى قيمة أعطى ١٠٠ وأقلها نبات العشار أعطى ٤٤،١ وكانت قيمة (R.P) لنبات القرنفل أعلاهم سجل ٢٢٦،٦ وأقلهم لنبات العشار سجل ١٠٠.

الكلمات الإسترشادية: المبيدات الفعالة، النباتات الطبيعية، الطور الرابع، دودة ورق القطن.

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