



TOXICOLOGICAL STUDIES ON SOME NATURAL PLANTS IN NORTH SINAI AGAINST SITOTROGA CEREALELLA LARVAE

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ABSTRACT

Laboratory experiments were conducted under controlled conditions ($28\pm 2^{\circ}\text{C}$ and RH. 65 ± 5) to test the efficiency and toxicity of aqueous and organic extracts of nine natural plants collected from North Sinai (oleander, *Nerium oleander* L.; lemon, *Citrus limon* L.; rue, *Peganum harmala* L.; colocynth, *Citrullus colocynthis*; yarrow, *Achillea santolina* L.; artemisia, *Artemisia monosperma* L.; buckthorn, *Anabasis articulata* L.; camphor, *Cinnamomum camphora* L.; and tree tobacco, *Nicotina glauca* G.), against the 1st –instar larvae of the Angoumois grain moth *Sitotroga cerealella* (olivier). The results showed that the aqueous extract of *Citrus limon* L. gave the highest toxicity with LC_{50} (29×10^{-4} ppm) and *Achillea santolina* L. gave the lowest value of LC_{50} (4033×10^{-4} ppm). Methyl alcohol extract of *C. limon* gave the highest value of LC_{50} (2×10^{-4} ppm) and *A. santolina* gave the lowest value of LC_{50} (607×10^{-4} ppm). The same trend was found in acetone extract of *C. limon* where it gave the highest value of LC_{50} (35×10^{-6} ppm), while *A. articulata* gave the lowest LC_{50} (4033×10^{-4} ppm) against the tested insect.

Key words: *Sitotroga cerealella*, toxicity, mortality, oleander, lemon, rue, colocynth, yarrow, artemisia, buckthorn, camphor, tree tobacco, LC_{50} .

INTRODUCTION

Wheat is one of most strategic crops in Egypt and all-over the world, it is used in production of different types of bread and pasta. Egypt achieved self-sufficiency of the wheat crop by 65-70% during the 2011 harvest season. During the storage periods of wheat and its products they are faced by many sources of damage such as different insects, especially stored seed pests. Insect infestation of stored grains and their products is a serious problem throughout the world. Chemical insecticides are currently the method of choice to protect stored grains from insect damage (Bell *et al.*, 2003; Drinkall *et al.*, 2005) however,

their widespread use has led to the development of pest strains resistant to insecticides (Subramanyam and Hagstrum, 1995). As a result, there is a demand for safer insecticides because of concern about insecticide residues on grain and health hazards to grain handlers. Hence, an alternative to synthetic insecticides especially methyl bromide which depletes the stratospheric ozone layer is of almost importance (Drinkall *et al.*, 2005). Many natural products are used exclusively as stored-product protectants. Such products have been used to control stored-product insect pests since the dawn of agricultural (Levinson and Levinson, 1998).

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In addition to being toxic, many natural products are also repellent or attractant to stored-product insects. Growing public concern for the environment has contributed to the change in attitude towards the use of botanical products in pest control. The use of natural products of plant origin is a new trend that preserves the environment from pollution with harmful toxicants. Several studies have suggested the use of plant extracts (Azadbakht *et al.*, 2004; Nagahban *et al.*, 2007).

The Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) is a major pest of cereals, especially rice, wheat, barley, maize and sorghum during storage condition (Dhaliwal *et al.*, 1989). Although, its infestation may start from the field, severe damage is caused under storage conditions (Visalakshi and Ramulu, 2005) leading to extensive qualitative as well as quantitative loss (Sedlasek *et al.*, 2001). The management of this pest is basically dependent on use of organophosphates (Kao and Tzeng, 1992).

Pyrethroids or fumigation with aluminium phosphide and methyl bromide are often applied (Hashem *et al.*, 2012). However, frequent use of insecticides or fumigants have resulted in the development of resistance, environmental and food contamination and toxicity to non-target organisms (Tavares *et al.*, 2013) and in many countries they are not permitted to be used without technical supervision. Among the available options, plant extracts and their chemical constituents have been found to have potential for replacing the synthetic chemicals in short, medium and long scale storage (Lee *et al.*, 2001).

The aim of this study was planned to contribute some new information about the validity of using these plants of Egyptian deserts to be an applicable and useful tool for future applications in the field and avoiding the harmful effects in silos of grain storage.

MATERIALS AND METHODS

Rearing method of tested insect

These experiments were carried out in the laboratory of Environment Protection Department, Faculty of Environmental Agricultural Sciences, Arish University, North Sinai, Egypt. The work was designed to determine the effect of different concentrations of aqueous and organic (methyl alcohol "acetone") extracts of *Nerium oleander* L., *Citrus limon* L., *Peganum harmala* L., *Citrullus colocynthis*, *Achillea santolina* L., *Artemisia monosperma* L., *Anabasis articulata* L., *Cinnamomum camphora* L., and *Nicotina glauca* G. against the (1st -instar larvae) of *Sitotroga cerealella* (oliv.) under controlled conditions of 28±2 C° and RH. 65±5. Wheat is the culture of tested insect was used in this study for the bioassay method Table 1, the insect was the 1st -instar larvae of *Sitotroga cerealella*.

Collection and identification of tested plants

Plant samples were collected from area surrounding Arish Airport during the experimental period. Identification of tested plants was based mainly on the taxonomic characters detailed by Boulos and El-Hadidi (1984). Samples were air dried for 2-4 weeks until a complete dryness. Samples were milled in an electric grinder into a fine powder and stored until to be used.

Aqueous and Organic extracts

Plant powder was soaked in a dark flasks as follows: Powder and acetone; powder and methyl alcohol; powder and distilled water in the ratio of 1g: 10 ml as an aqueous and organic phase or extract. The mixtures (1g: 10 ml) were allowed to stand for 24 hours, and finally filtered using filter paper (Whatmman No.4). The previous filtered mixtures of aqueous and organic phase or extract were kept in a deepfreeze at -200°C immediately. The series of concentrations of each extract were freshly prepared to be used according to Mc-Cloud *et al.* (1988).

Table (1). Tested insect.

No.	English name	Arabic name	Scientific name	Order	Family
1	Butterfly grain	فراشة الحبوب	<i>Sitotroga cerealella</i>	Lepidoptra	Gelechiidae

Bioassay of the tested plants extractives against the tested insect

For evaluating the effect of the tested plant extracts against the 1st instar larvae of *Sitotroga cerealella* larvae, 100 larvae were carefully transferred on the surface of petri dishes which treated with residue film. Larvae of the tested insect were treated for 72 hours (3 days) with 500 micro liter of each extract (aqueous, methyl alcohol and acetone).

Statistical analysis

Different concentrations of plant extracts were used for bioassay test to determine the mortality percentages, slopes, LC₅₀ and confidence limits using Ldp line program.

RESULTS

The insecticidal activity of each the aqueous and organic extracts of the tested plants against the 1st-instar larvae of *S. cerealella* are summarized in Tables (2-5). The results in Table 3 exhibit that the values of LC₅₀'s of the tested plants were arranged in an ascending order *i.e.* the LC₅₀ of *Citrus limon* L. was 29×10^{-4} ppm and increased to be 4033×10^{-4} ppm in case of *Achillea santolina* L. By another words, the toxicity of the tested plants was very high $29 \times 10^{-4}\%$ in *C limon* and gradually decreased to be $403 \times 10^{-3}\%$ in *A santolina*. Also by the same trend the toxicity of the tested plants were arranged in a descending order as in the following: *C limon*, *P harmala*, *N oleander*, *N glauca*, *A monosperma*, *C colocynthis*, *A articulata*, *C camphora* and *A santolina* L.

The results in Table 4 show that the values of LC₅₀'s of tested plants were

arranged in an ascending order, where it began with LC₅₀ value equal to 2.0×10 ppm in case of *Citrus limon* L. and increased to the value of LC₅₀ equal to be 607×10^{-4} ppm in case of *Achillea santolina* L.

But by showing the degree of toxicity of these species of tested plants, it was noticed that the toxicity degree of these plants were arranged in a descending order as in the following: *C limon*, *N oleander*, *P harmala*, *A monosperma*, *N glauca*, *C colocynthis*, *C camphora*, *A articulata* and *A santolina i.e.* the highest toxicity was recorded with *C limon* and decreased gradually till it was the lowest in case of *A santolina*. The data in the Table 5 illustrated, that the values of LC₅₀'s were arranged in an ascending order to be 35×10 ppm as in case of *C limon* and increased gradually to be 36.0×10^{-4} ppm as in case of *A articulata*.

But by expressing about the toxicity of these tested plants, the plants were arranged in a descending order as in the following: *C limon*, *A monosperma*, *N oleander*, *P harmala*, *N glauca*, *C colocynthis*, *A santolina*, *C camphora* and *A articulata i.e.* the highest toxic plant extract was the *C limon* and the toxicity decreased gradually to be the lowest toxic in plant extract, *A articulate*.

DISCUSSION

The present results are in agreement with the results found by some such as **Eissa et al. (1989)**, who found that the crude extract of *A monosperma*, exerted excellent nematocidal activity due to some water soluble compounds having high nematocidal activity effect such as alkaloids and polyphenols.

Table (2): The list of the tested nine plant species and their extracted parts studied in this investigation from the vicinity of Al-Arish.

English name	Arabic name	Scientific name	Family	Part used*
Oleander	الدفلة	<i>Nerium oleander</i> L.	Apocyanaceae	L
Lemon	الليمون	<i>Citrus limon</i> L.	Rutaceae	L
Rue	حرمل	<i>Peganum harmala</i> L.	Zygophyllaceae	H
Colocynth	حنظل	<i>Citrullus colocynthis</i>	Gourd	H
Yarrow	شبح خرساني	<i>Achillea santolina</i> L.	Compositae	H
Artemisia	عادر	<i>Artemisia monosperma</i> L.	Compositae	H
Buckthorn	عجرم	<i>Anabasis articulata</i> L.	Chenopodioideae	H
Camphor	كافور	<i>Cinnamomum camphora</i> L.	Myrtaceae	L
Tree tobacco	مصاص الدخان	<i>Nicotina glauca</i> G.	Solanaceae	H

*Part used (L= leaves, H= whole plants)

Table (3): LC₅₀, slope values and confidence limits of aqueous extracts of the tested plants against the 1st instar larvae of *Sitotroga cerealella*.

Plant	LC ₅₀ (ppm)	Slope	Confidence limits of LC ₅₀	Toxicity Index
<i>Citrus limon</i> L.	29×10 ⁻⁴	0.3531 ± 0.0279	15×10 ⁻⁴ - 58×10 ⁻⁴	100
<i>Peganum harmala</i> L.	58×10 ⁻⁴	0.3828 ± 0.0286	31×10 ⁻⁴ - 108×10 ⁻⁴	50
<i>Nerium oleander</i> L.	110×10 ⁻⁴	0.3499 ± 0.0277	19×10 ⁻⁴ - 733×10 ⁻⁴	26.3
<i>Nicotina glauca</i> G.	113×10 ⁻⁴	0.4013 ± 0.0352	53×10 ⁻⁴ - 228×10 ⁻⁴	25.6
<i>Artemisia monosperma</i> L.	163×10 ⁻⁴	0.4070 ± 0.0350	85×10 ⁻⁴ - 302×10 ⁻⁴	17.7
<i>Citrullus colocynthis</i>	1280×10 ⁻⁴	0.5156 ± 0.0390	765×10 ⁻⁴ - 2223×10 ⁻⁴	2.2
<i>Anabasis articulata</i> L.	2080×10 ⁻⁴	0.4726 ± 0.0379	1185×10 ⁻⁴ - 3816×10 ⁻⁴	1.3
<i>Cinnamomum camphora</i> L.	2950×10 ⁻⁴	0.3244 ± 0.0337	1341×10 ⁻⁴ - 7558×10 ⁻⁴	0.98
<i>Achillea santolina</i> L.	4033×10 ⁻⁴	0.3560 ± 0.0347	1920×10 ⁻⁴ - 9790×10 ⁻⁴	0.71

*LC₅₀ values were calculated from the regression lines using Ldp Line program.

Table (4): LC₅₀, slope and confidence limits of organic extract (methyl alcohol) of the tested plants against the 1st instar larvae of *Sitotroga cerealella*.

Plant	LC ₅₀ (ppm)	Slope	Confidence limits of LC ₅₀	Toxicity Index
<i>Citrus limon</i> L.	2×10 ⁻⁴	0.3882 ± 0.0290	1×10 ⁻⁴ - 4×10 ⁻⁴	100
<i>Nerium oleander</i> L.	15×10 ⁻⁴	0.3122 ± 0.0272	2×10 ⁻⁴ - 64×10 ⁻⁴	13.3
<i>Peganum harmala</i> L.	16×10 ⁻⁴	0.3752 ± 0.0245	8×10 ⁻⁴ - 29×10 ⁻⁴	12.5
<i>Artemisia monosperma</i> L.	16×10 ⁻⁴	0.3847 ± 0.0291	8×10 ⁻⁴ - 29×10 ⁻⁴	12.5
<i>Nicotina glauca</i> G.	16×10 ⁻⁴	0.3722 ± 0.0288	8×10 ⁻⁴ - 30×10 ⁻⁴	12.5
<i>Citrullus colocynthis</i>	163×10 ⁻⁴	0.4707 ± 0.0368	92×10 ⁻⁴ - 283×10 ⁻⁴	1.22
<i>Cinnamomum camphora</i> L.	241×10 ⁻⁴	0.3392 ± 0.0275	121×10 ⁻⁴ - 492×10 ⁻⁴	0.829
<i>Anabasis articulata</i> L.	242×10 ⁻⁴	0.4499 ± 0.0361	135×10 ⁻⁴ - 480×10 ⁻⁴	0.826
<i>Achillea santolina</i> L.	607×10 ⁻⁴	0.3410 ± 0.0334	295×10 ⁻⁴ - 1298×10 ⁻⁴	0.329

*LC₅₀ values were calculated from the regression lines using Ldp Line program.

Table (5): LC₅₀, slope and confidence limits of organic extract (acetone) of the tested plants against the 1st instar larvae of *Sitotroga cerealella*.

Plant	LC ₅₀ (ppm)	Slope	Confidence limits of LC ₅₀	Toxicity Index
<i>Citrus limon</i> L.	0.035×10 ⁻⁴	0.3640 ± 0.0281	20×10 ⁻⁶ - 10×10 ⁻⁶	100
<i>Artemisia monosperma</i> L.	2×10 ⁻⁴	0.3889 ± 0.0291	1×10 ⁻⁴ - 4×10 ⁻⁴	1.75
<i>Nerium oleander</i> L.	3×10 ⁻⁴	0.2876 ± 0.0265	1×10 ⁻⁴ - 6×10 ⁻⁴	1.16
<i>Peganum harmala</i> L.	3×10 ⁻⁴	0.3720 ± 0.0285	2×10 ⁻⁴ - 6×10 ⁻⁴	1.16
<i>Nicotina glauca</i> G.	10×10 ⁻⁴	0.4049 ± 0.028	5×10 ⁻⁴ - 18×10 ⁻⁴	0.35
<i>Citrullus colocynthis</i>	18×10 ⁻⁴	0.4380 ± 0.0358	10×10 ⁻⁴ - 31×10 ⁻⁴	0.19
<i>Achillea santolina</i> L.	25×10 ⁻⁴	0.4194 ± 0.0352	13×10 ⁻⁴ - 45×10 ⁻⁴	0.14
<i>Cinnamomum camphora</i> L.	27×10 ⁻⁴	0.3512 ± 0.0238	14×10 ⁻⁴ - 52×10 ⁻⁴	0.12
<i>Anabasis articulata</i> L.	36×10 ⁻⁴	0.4835 ± 0.0302	21×10 ⁻⁴ - 59×10 ⁻⁴	0.097

*LC₅₀ values were calculated from the regression lines using Ldp Line program.

Also, recently experiments carried out by **Ali et al. (1997)**, indicated that aqueous and organic extracts of *Artemisia monosperma* L., showed different degrees of lethal effect on egg hatching of *M. hapla*. More recently **Eid (2010)** proved the organic extract was highly effective than the aqueous extract of *A monosperma*, leading to prevent the egg-mass hatching 98.5% of *M. incognita*. Also, **Ali et al. (1997)** reported that the *A monosperma* L., achieved an insecticidal effect of it by aqueous and organic extract against 4th instar larvae of *M. lapla*.

Rahman et al. (2006), revealed the best performance of camphor at a rate of 4.5g/kg of nice grain in suppressing the moth of *S cerealella* in storage. And in more recent studies **Rao et al. (2005)**, reported that camphor (*C camphora*) pellets at 1.4% and 1.2% (W/W) showed absolute control of the population built-up of *Sitotroga cerealella* at all observational periods.

Sathiyamoorthy et al. (1997) evaluated the aqueous plant extracts of *A articulata*, *N. restica* and *A fragrantissima* proved to be highly toxic plant extracts.

Al-Obaedi et al. (1987) proved that one of the four most effective plant extracts was *A santolina*, against *M. javanica* but at high concentrations. Also, **Mc-Cloud, et al. (1988)** investigated the validity of using ethanolic extract and volatile oil of *A santolina*, but in that case as an insecticide against domestic flies.

Recently **Eid (2005)** showed that plant extracts of *A santolina*, were very effective in minimizing the No. of galls, egg-masses and juveniles of *M. incognita*. And more recently **El-Araby (2008)**, proved that aqueous and organic extract of *A santolina*, were effective in controlling *S. granaries* and *T. castaneum*. **Eid (2005)**, showed that aqueous and organic extract of *Citrullus colocynthis*, were effective in reducing the no. of galls egg-mosses and juveniles of *M.*

incognita and recording high percentage of non-hatching 96%-98%.

Also it can be declared that there are certain concentrations of both aqueous and organic extracts of each plant, and it could be named as an optimum and best concentrations, causing the maximum effect. Also and besides the variations between each plant and its response and insect target sensitivity to the concentrations applied and at each tested phase *i.e.* the presence of polar and a polar compounds in the media of testing. 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 concentration and its response. Also, the role of genetic factors in elucidating differences in responses and reactions (**Upitis et al. 1973, Arnaud et al. 2005**). Also, by going after LC₅₀ values the differences between the results in aqueous and organic (methanolic and acetonetic) of each plant may be due to differences in morphological and physiological and metabolic responses in each pest and in pest species (**Conyers and Bell, 1996**).

By more focusing, the organic extracts of the tested plants, LC₅₀ values, also indicated that organic extracts of most tested plants were better than aqueous extracts. These results were in agreement with **El-Doksh et al. (1984)**, found that the LC₅₀ values of organic extracts were more toxic than LC₅₀ values of aqueous extracts. Also, in the same time all plant concentrations (aqueous and organic) against the tested insect showed that mortality percentages increased by increasing of used concentrations (**Schmidt et al., 1997**), showed that highest concentrations of methanolic extract of neem led to high mortality percentage against *S. littoralis* and *Agrotis ipsilon*. Ultimately, it is appearing that all plant extracts (aqueous and organic) are affecting oxygen arrives inside the experimental insect body and that cause asphyxia and

leading to death. Mortality percentage is different with concentrations, extract types, tested insect and tested plants (**El-Araby, 2008; El-Araby, 2014**).

Meanwhile 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 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 metabolic path way and cause rapid death, act as attractants, deterrents, phagostimulants, antifeedants 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, (**Lioed, 1973; Huang et al., 1997; Asgary et al., 2000; Wink et al., 2004**).

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 effect by the used extracts on these membranes (**Hambuger and Hostellman, 1991**), reporting that the drug affects integrity of membranes and localized these membranes due to its highly lipophilic nature. Moreover that, the chemical characteristics of the effective compounds such as charge

and polarity of natural compound affecting rates of interchange especially across membranes and cuticles to determine whether it reaches that tissue or target at intoxicating concentrations.

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 extract for some plants and have lethal effect, that indicating, these plants have properties of the selectivity and sensitivity. Also, there is a natural selection pressure has often negatively affect the other species (**Keeler and Tu, 1991**).

Finally this 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 concentrations of active ingredients 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 insect. 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.

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الملخص العربي

الخصائص السمية على بعض النباتات الطبيعية في شمال سيناء ضد يرقات فراشة الحبوب

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تم اختبار كفاءة وسمية المستخلصين المائي والعضوي تحت درجة حرارة المختبر خلال فترة التجربة (28 ± 2 درجة مئوية نسبة رطوبة 65 ± 5) وذلك لتسعة نباتات تم جمعها من شمال سيناء وهي: نبات الدفلة وأشجار الليمون ونبات الحرمل ونبات الحنظل ونبات الشيح الخرساني ونبات العاذر ونبات العجرم ونبات الكافور ونبات مصاص الدخان ضد يرقات حديثة الفقس من فراشة الحبوب (الطور الأول من يرقات فراشة الحبوب)، وأظهرت النتائج أن المستخلص المائي من أوراق الليمون أعلى سمية بالمقارنة بباقي النباتات المختبرة بقيمة LC_{50} إلي ($29 \times 10^{-4} ppm$)، وأعطى الشيح الخرساني أدنى قيمة من LC_{50} ($4033 \times 10^{-4} ppm$) ضد يرقات فراشة الحبوب، أعطى مستخلص كحول الميثيل من أوراق الليمون أعلى قيمة من LC_{50} ($2 \times 10^{-4} ppm$)، بينما أعطى الشيح الخرساني أدنى قيمة LC_{50} حيث وصلت ($607 \times 10^{-4} ppm$). وعند استخدام مذيب الاسيتون لقد تبين نفس الاتجاه حيث أعطت أوراق الليمون أعلى قيمة لـ LC_{50} ($0.035 \times 10^{-4} ppm$)، في حين أن العجرم أعطى أدنى قيمة ($4033 \times 10^{-4} ppm$) LC_{50} ضد اليرقات حديثة الفقس من فراشة الحبوب.

الكلمات الاسترشادية: فراشة الحبوب، السمية، نسبة الموت، نبات الدفلة، نبات الليمون، نبات الحرمل، نبات الحنظل، نبات الشيح الخرساني، نبات العاذر، نبات العجرم، نبات الكافور، نبات مصاص الدخان.

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