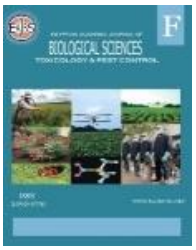
Egypt. Acad. J. Biolog. Sci., 12(1): 1-7 (2020)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest Control
ISSN: 2090 - 0791
http://eajbsf.journals.ekb.eg/



Toxicity of Some Essential Plant Oils Against Cotton Leaf Worm, *Spodoptera littoralis* (BOISD.)

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ARTICLE INFO
Article History
Received:1/1/2020
Accepted:26/1/2020
Key words: *Spodoptera littoralis*, Neem, Garlic, Marjoram, menthol, and biological effects.

ABSTRACT

The study aims to evaluate the bioactivity of plant essential oils (neem oil, garlic oil, marjoram oil, and menthol oil) on 2nd instar larvae of the cotton leafworm, *Spodoptera littoralis* in the laboratory. Also, LC_{50} of each treatment was established against 2nd instar larvae of *S. littoralis* under semi-field conditions. The obtained results reveal that neem oil was more effective than the other oils followed by, marjoram oil then garlic oil and then, menthol oil.LC_{50} was3022.50, 3803.41, 4630.13and5323.38ppm for neem oil, marjoram oil, garlic oil, and menthol oil, respectively, for 2nd instar larvae. While, when $LC_{50}$of these oils applied on the 2nd instar larvae in the semi-field, the results indicated that, garlic oil was the most effective essential oil followed by, menthol oil, marjoram oil, and neem oil.

INTRODUCTION

Egyptian cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) is one of the key pests that cause great damage to cotton plants as well as other fields and vegetable crops. The widespread and continuously increasing use of different types of nonselective pesticides in cotton fields in Egypt and elsewhere disturb the biological balance and cause outbreaks of insect and mite pests (Abdel Rahman *et al.*, 2007).

Pesticides caused many environmental problems. Thus, on the one hand, one needs to search the new highly selective and biodegradable to solve the problem of long term toxicity to mammals and, on the other hand, one must study the environmental friendly pesticides and develop techniques that can be used to reduce pesticide use while maintaining crop yields. Natural products are an excellent alternative to synthetic pesticides as a means to reduce negative impacts on human health and the environment (Opender *et al.*, 2008).

Terpenoids constitute the largest and most heterogeneous class of secondary metabolites and include monoterpenes and sesquiterpenes as volatile constituents (Dudareva *et al.*, 2013). These volatile terpenoids can act both as constitutive and herbivory-induced (Herbivore-Induced Plant Volatiles, HIPVs) defense compounds.

Neem oil is derived from neem seed which is a part of the neem tree that has a high concentration of oil which is widely used as insecticides, lubricant, drugs for diseases like diabetes and tuberculosis. There are several methods to obtain neem oil

from the seeds like mechanical pressing, supercritical fluid extraction, and solvent extraction.

Garlic (*Allium sativum* L.) is considered one of the twenty most important vegetables, with various uses throughout the world, either as a raw vegetable for culinary purposes or as an ingredient of traditional and modern medicine. Furthermore, it has also been proposed as one of the richest sources of total phenolic compounds.

The emulsified oil of marjoram was reported to possess strong anti-parasitic activity. Marjoram was initially used by Hippocrates as an antiseptic agent. Itis a well-liked home remedy for a chest infection, rheumatic pain, nervous disorders, cardiovascular diseases, epilepsy, insomnia, and stomach disorders**.**

Menthol oil is derived from the peppermint plant, *Mentha piperita* L. and has repellent activity against various stored product pests and changes in biological aspects of the two-spotted spider mite, *Tetranychus urticae***.**

The objective of this research was to assess the effect of some essential plant oils against 2nd instar larvae of cotton leafworm, *Spodoptera littoralis*.

MATERIALS AND METHODS

Test Insects:

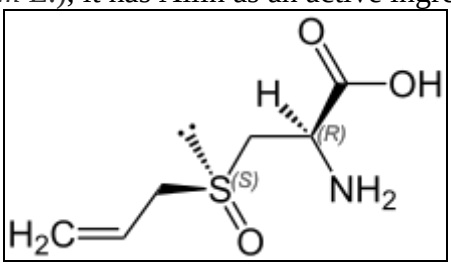
A laboratory strain of cotton leafworm, *S. littoralis* (Lepidoptera: Noctuidae) (maintained on above 30 generations) which was initiated from freshly collected egg-masses supplied from the division of cotton leafworm of Plant Protection Research Institute (PPRI), Dokki, Egypt. Larval stages were reared on castor leaves, which were provided daily, in laboratory under constant conditions of 27±2°C, photoperiod of 14 h light and 10 h dark and 65±5% R.H. The adults were kept separately and mated on the third day of emergence in clean jars (4 lb.) adults were fed on 10% honey solution, fresh green leaves of tafla, *Nerium oleander* (L.) were provided for egg-laying.

Preparation and Isolation of Essential Plant Oils:

Essential oils (volatile oils) of each of the tested plants were extracted by steam distillation apparatus found in Plant Protection Institute, Mansoura, Egypt. The oils were separated dried over anhydrous sodium sulfate and stored in dark glass bottles at 4° C in the refrigerator until used.

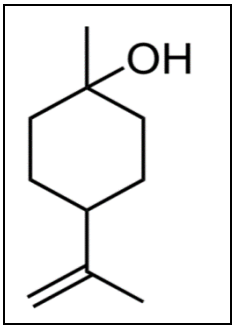
Tested Essential Oils:

Garlic (*Allium sativum* L.), it has Allin as an active ingredient, $C_6H_{11}NO_3S$.



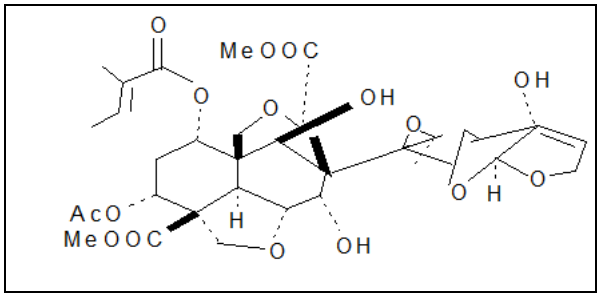
Allin formula (Iberl, 1990)

- Marjoram oil (*Origanummajorana* L.) has terpinen-4-ol (38.4%) as an active ingredient, $C_{10}H_{18}O$.



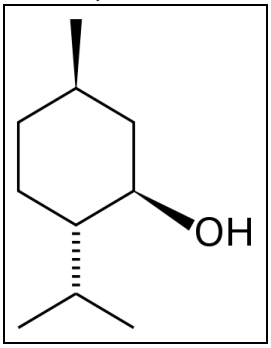
Yuasa (2006)

Neem oil which contain azadirachtin, $C_{35}H_{44}O_{16}$:



(Kausik *et al.* 2002)

- Menthol oil contains menthol, $C_{10}H_{20}O$.



Menthol formula (Opender *et al.*, 2008)

Preparing the Stock Solution The Tested Essential Oil:

Convenient stock concentrations of each essential oil were prepared on basis of the tested plant weight and the volume of the distilled water (w/v) in the presence of tween 80 (0.1%) as an emulsifier. The stock concentrations were kept in glass stoppered bottles and stored under refrigeration. Such stock solutions were prepared periodically. Four diluted concentrations for each plant extract were used to draw the LC-P lines. Three replicates were used for each concentration.

Bioassays:

1. Leaf Dipping Method:

2^{nd} instar larvae were used to determine the toxicity action of the tested essential oils. Castor bean leaf discs were cut and dipped into the treatments for 20 seconds, then left for air dryness, 10 larvae for each replicate were released to each leaf disc placed. Four concentrations and three replicates were used to estimate each concentration-mortality line. The concentrations used were 1000, 5000, 10000 and 15000 ppm (for each

essential oil). The same number of leaf discs per treatment was dipped into dis. water as an untreated check. Before and after treatment, larvae were maintained under laboratory conditions (constant temperature 25 ±2 °C and 70± 5 % R.H. After 24 h of treatment. The percentage of mortality was recorded after one, three, five and seven days. The data were corrected relatively to control mortality (Abbott, 1925). LC_{50} values were determined using the probit analysis statistical method of Finney, 1971:

Equation: Sun, 1950 **(**to determine LC_{50} index**)**

$$\text{Toxicity index for } LC_{50} = \frac{LC_{50} \text{ of the most effective compound}}{LC_{50} \text{ of the least effective compound}} \times 100$$

2 Semi Field Experiments:

LC_{50} of every treatment was applied on green potato leaves and the leaves were collected after zero time and transferred directly to the laboratory for feeding the second larval instars of cotton leafworm to estimate the mortality percent.

RESULTS AND DISCUSSION

The Efficiency of The Essential Plant Oils On 2nd Instar Larvae of Cotton Leafworm, *Spodoptera Littoralis* Under Laboratory Conditions:

The data in **Table (1)** indicated that neem oil caused a high mortality proportion on the 2nd instar larvae than the other essential oils.

Table 1: Mortality % of 2nd instar larvae of the cotton leaf worm, *Spodoptera littoralis* treated with some essential oils under laboratory conditions

No.	Treatments	Conc. (ppm)	Mortality after treatments %				Total Mortality %
			One day	Three days	Five days	Seven days	
1	Neem oil	1000	------	6.67	10	13.33	30.00
		5000	6.67	13.33	13.33	16.67	50.00
		10000	13.33	13.33	20	26.67	73.33
		15000	6.67	53.33	20	13.33	93.33
2	Marjoram oil	1000	-----	10	6.67	10	26.67
		5000	6.67	20	6.67	13.33	46.67
		10000	13.33	26.67	13.33	13.33	66.67
		15000	13.33	26.67	26.67	20	86.67
3	Garlic oil	1000	-----	6.67	13.33	6.67	26.67
		5000	6.67	3.33	20	13.33	43.33
		10000	10	20	13.33	13.33	56.67
		15000	33.33	20	16.67	13.33	83.33
4	Mentha oil	1000	6.67	6.67	6.67	6.67	26.67
		5000	3.33	13.33	13.33	13.33	40.00
		10000	6.67	20	13.33	13.33	53.33
		15000	13.33	20	30	16.67	80

However, Table (2) and Figure (1) demonstrated that, neem oil was the most effective one of the tested oils with Lc_{50}:3022.50ppm and the toxicity index was 100%, followed by marjoram with Lc_{50}:3803.41ppm, then garlic oil with LC_{50}: 4630.13 ppm. Menthol oil had the highest LC_{50}which was 5323.38 ppm. The slope values indicated that

the neem oil had the highest value was 1.42 followed by 1.30, 1.137 and 1.05 for marjoram, garlic oil, and menthol oil, respectively Hanan *et al.* (2012). Proved that neem oil had a significant effect on larval mortality of cotton leafworm.

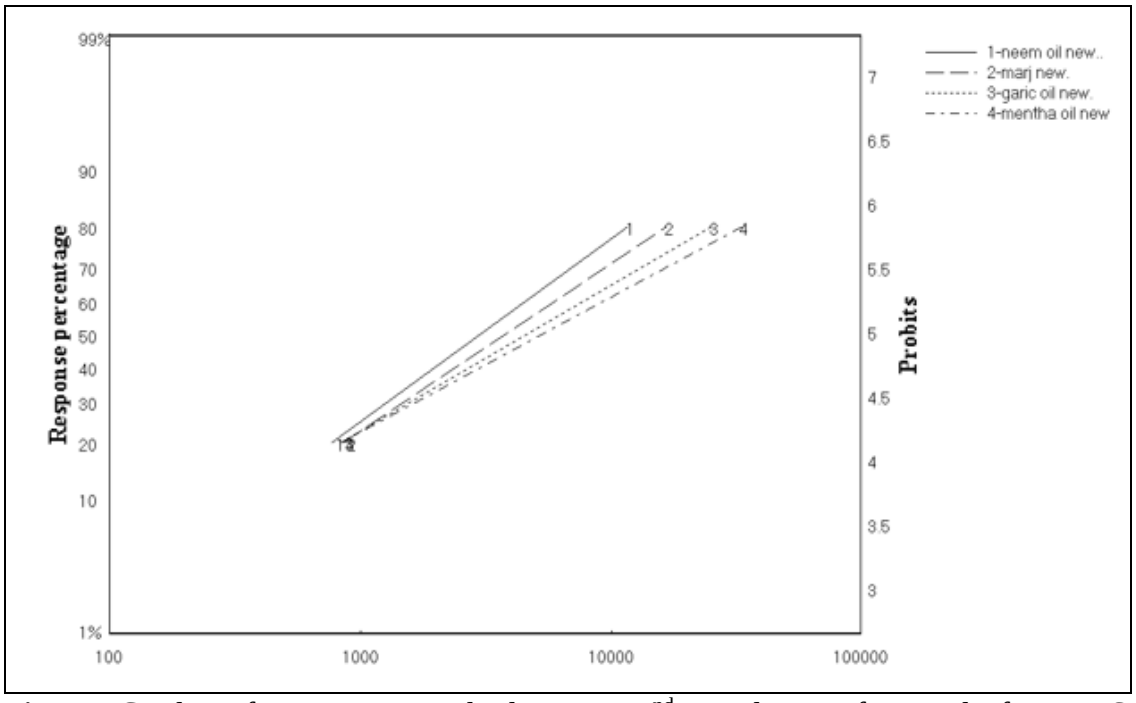


Fig. 1: LC-P lines for some essential oils against 2nd instar larvae of cotton leaf worm, *S. littoralis*

Table 2: Efficiency of some plant essential oils against 2nd instar larvae of the cotton leafworm, *Spodoptera littoralis.*

Treatments	Conc.	Corrected mortality%	LC_{50}	LC_{90}	Slope± S.D.	Toxicity index LC_{50}	LC_{90}/LC_{50}	R	P
Neem oil	1000	30.00	3022.50	24109.76	1.42± 0.156	100	7.98	0.915	0.001
	5000	50.00							
	10000	73.33							
	15000	93.33							
Marjoram	1000	26.67	3803.41	36794.41	1.30± 0.155	79.47	9.67	0.938	0.008
	5000	46.67							
	10000	66.67							
	15000	86.67							
Garlic oil	1000	26.67	4630.13	62108.07	1.137± 0.152	65.28	13.41	0.902	0.001
	5000	43.33							
	10000	56.67							
	15000	83.33							
Mentha oil	1000	26.67	5323.38	88020.57	1.05± 0.152	56.78	16.53	0.888	0.001
	5000	40.00							
	10000	53.33							
	15000	80							

R: Regression P: Propability

Effect of LC_{50} of The Plant Essential Oils on The Larvae of *S. Littoralis* Which Fed on Treated Green Potato Leaves:

The results in Table (3) indicated that garlic oil was the most effective essential oil than the other tested oils. Neem oil was the most effective oil when applied on 2nd

instar larvae in the laboratory, while in this experiment in semi-field, it has a low effect. The cause of this may be due to LC_{50} of neem oil was the lowest among the other essential oils and the difference between the feeding of larvae on castor leaves in the laboratory and feeding on potato leaves in semi-field Ghada and Amal (2015). Proved that also, when larvae of cotton leave worm fed on castor bean in the laboratory while fed on pepper leaves in semi-field, the effect of camphor differs between laboratory and semi-field.

The obtained results were in agreement with and who proved that the *S. littoralis* could be affected by plant extracts and their active ingredients.

The obtained results were in agreement with Ghoneim *et al.*, 2012 and Reda *et al.*, 2013 who proved that the *S. littoralis* could be affected by plant extracts and their active ingredients.

Table 3: Efficacy of LC_{50} of plant essential oils on 2nd instar larvae of *S. littoralis* which fed on treated green potato leaves in semi-field conditions.

Treatments	LC_{50}	Mortality after treatments % (days)				Total Mortality %
		1	3	5	7	
Neem oil	3022.50	3.33	10	10	10	33.33
Marjoram oil	3803.41	13.33	16.67	3.33	6.67	40
Garlic oil	4630.13	33.33	20	6.67	6.67	66.67
Menthol oil	5323.38	23.33	3.33	20	----	46.67

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ARABIC SAMMARY

سمية بعض الزيوت النباتية ضد دودة ورق القطن

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تعكس هذه الدراسة إلي تقدير كفاءة بعض الزيوت النباتية وهي (زيت النيم، زيت الثوم، زيت البردقوش وزيت النعناع) علي العمر اليرقي الثاني لدودة ورق القطن معملياً. وكذلك تم تقدير التركيز النصف المميت لكل معاملة علي العمر اليرقي الثاني لدودة ورق القطن في ظروف نصف حقلية وقد أظهرت النتائج أن زيت النيم هو الأكثر تأثيراً عن باقي الزيوت متبوعاً بزيت البردقوش ثم زيت الثوم ويليه زيت النعناع. وسجل التركيز النصف المميت ٣٠٢٢,٥، ٣٨٠٣,٤١ ٤٦٣٠,١٣، ٥٣٢٣,٣٨ جزء في المليون لكل من زيت النيم، زيت البردقوش، زيت الثوم و زيت النعناع علي التوالي للعمر اليرقي الثاني لدودة ورق القطن. بينما عندما تم تطبيق التركيز النصف المميت للعمر اليرقي الثاني في التجربة النصف حقلية أظهرت النتائج أن زيت الثوم هو الأكثر فاعلية متبوعاً بزيت النعناع، البردقوش ثم النيم.