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Effect of four botanical extract oils on biological aspects of cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) under laboratory conditions

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Abstract

The objective of this study was conducted to evaluate the effect of four botanical extract oils (clove oil, mint oil, garlic oil and Neemazal- T/S formulation [1% *Azadirachtin*]) on the biological aspects of the 4th instar larvae of Egyptian cotton leaf worm, *Spodoptera littoralis*. The results cleared that the larval duration were 9.37, 9.17, 8.78, 8.89 and 10.00 days for clove oil, mint oil, garlic oil, Neemazal- T/S formulation and control, respectively. Clove oil caused the highest percent of larval mortality (92.67%), while the least one (59.55%) was recorded in case of mint oil. The pupation and emergence percentages were (7.33, 40.45, 32.44, 17.33 and 88.00 %) and (85.93, 82.67, 82.80, 75.53 and 97.00 %) for the abovementioned oils, successively. Neemazal -T/S formulation caused the highest significant reduction in fecundity percentage recording 224.44 eggs/ female, regardless of the concentration, while control female laid an average of 1703.33 eggs / female. All tested botanical extract oils caused positive effect on investigated biological aspects, especially both of clove oil or Neemazal- T/S formulation.

Keywords: *Spodoptera littoralis*, larvae, botanical extract oils, biological aspects

1. Introduction

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) was and still until now considered one of the most serious polyphagous pests of Egyptian cotton, not only to cotton plants but also to other field crops and vegetables Rawi *et al.* [1] Larvae feed on greater than 90 economically important crop species belong to 40 families Ellis [2]. On cotton, the pest may cause considerable damage by feeding on the leaves, fruiting points, and flower buds and occasionally on bolls. In Egypt, it is one of the most serious cotton pests and also attack about 112 host plants from different families Kandi *et al.* [3]; El-Sinary *et al.* [4] and El-Zohby *et al.* [5] In addition to its direct damage, reducing photosynthetic area and reduce the market ability of vegetables and ornamentals Sluschkell *et al.* [6] The control practices of cotton leaf worm primarily depend upon using chemical insecticides which considered one of the most effective control measures against it. Over the past 40 years, the intensive use of broad spectrum insecticides against the cotton leaf worm had led to the development of resistance to many of them Smagghe and Degheele [7]; Aydin and Gurkan [8] and Rizk *et al.* [9] In the recent time much attention had been given to control and suppress the population of *S. littoralis* by using new types of pest control agents such as plant extracts that would be cheap, used safely, economically and environmentally acceptable. Also, the previous researchers attended to use plant extracts as toxicants, repellents, synergists, growth regulators and antifeedant for cotton leaf worm Hashem *et al.* [10]; Mohamed and El-Gengaihi [11]; Arivudainambi [12]; El-Kholy and Shaheen [13]; Selvamuthukumar and Arivudainambi [14]; Abd El-Aziz [15]. The objective of the present work is to study the biological activity of four types of some botanical extract oils (clove oil, mint oil, garlic oil and Neemzal- T/S formulation) (1% *Azadirachtin*) on 4th larval instar of cotton leaf worm, *S. littoralis*.

2. Materials and Methods

The present study was carried out in Plant Protection Department, Faculty of Agriculture, Zagazig University, Egypt under the laboratory conditions of $28 \pm 2^{\circ}\text{C}$ and $65 \pm 5\%$ R.H.

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2.1 Rearing techniques

The tested insect of *Spodoptera littoralis* (Boisduval) was obtained from the Department of cotton leaf worm, Plant Protection Research Institute, Agriculture Research Center, Ministry of Agriculture, Dokki, Giza, Egypt. The standard laboratory culture of *S. littoralis* was reared on castor leaves, *Ricinus communis* L. in glass jars at a temperature of $28 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ R.H. until reached to the fourth instar larvae which used in the present experiment.

2.2 Tested botanical materials

Four commercially available botanical extract oils were tested in this study. They were clove oil (*Syzygium aromaticum* L.), mint oil (*Mentha viridis* L.), garlic oil (*Allium sativum* L.) and Neemazal- T/S formulation (1% *Azadirachtin*) (*Azadirachta indica*). *S. aromaticum* oil was obtained from the pharmacy; *M. viridis* and *A. sativum* were obtained from the Chem Trade Company, Nasr City, Cairo, Egypt and Neemazal - T/S was supplied by Prof. Dr. Ibrahim Mohamed Kelany, Plant Protection Department, Faculty of Agriculture, Zagazig University. All these tested botanical materials were tested against the 4th instar larvae of *S. littoralis*.

Three dilutions of each botanical material (1, 2 and 4% were prepared in acetone. Castor leaves were dipped in the tested concentration for 15 min., then left to dry for 30 min. The 4th larval instars were allowed to feed on the treated leaves for 24 hrs, then the larvae were fed on untreated leaves until the termination of the experiment. Each treatment replicated 3 times with 25 larvae for replicate. After application each replicate was placed in a plastic box (8 cm height, 13 cm top diameter and 8 cm bottom diameter) and tightly covered with muslin, held in place by rubber bands and kept under laboratory conditions at $28 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ R.H. The same technique was used with acetone instead of tested botanical materials as a control. Biological aspects such as mortality percentage, larval duration, 6th larval weight, pupal duration, percentage of pupation, pupal weight, adult emergence, adult weight, male and female longevity, percentage of malformed

adults, fecundity and hatchability were recorded.

3. Results and Discussion

3.1 Effect of some botanical extract oils in controlling *Spodoptera littoralis* (Boisduval)

The results in Table 1 show that the effect of four botanical extract oils (clove oil, mint oil, garlic oil and Neemazal- T/S formulation [1% *Azadirachtin*]) on the total larval duration. Treating 4th instar larvae with garlic oil 4% or Neemazal - T/S formulation 2% resulted in the shortest larval duration, recording 8.83 days for the two treatments, whereas untreated larvae (control) lasted 10.00 days, with insignificant differences between means.

As shown in Table 1 and Figure 1 the highest percent larval mortality (94.00%) was detected when larvae fed on castor leaves treated with clove oil 4%, whereas the lowest percent (53.33%) was noticed with treating by mint oil 1%. Regardless of the concentrations of the tested oils, clove oil caused the highest percent of larval mortality (92.67%), while the least mean (59.55%) was recorded in case of mint oil. Other tested botanical extract oils showed intermediate values. For control larvae the percentage of mortality was 12.00 %. Analysis of data revealed that the differences between means are highly significant. These results are nearly similar with those of Abd El-Aziz and Ezz El-Din [15] and El-Kholy *et al.* [16] who used other plant extracts (datura, camphor and damsissa) which affected positively on the larval mortality of *S. littoralis* than the control recording 7.15 to 81.19 %.

Our results agreed with Yassin [17] who stated that the percentages of mortality ranged between 98.0-42.5%.

Regarding to 6th instar larval weight, all the tested botanical extract oils reduced the average weight, recording 0.745, 0.518 and 0.576 g for clove oil; 0.668, 0.620 and 0.530 g for mint oil; 0.618, 0.590 and 0.530 g for garlic oil and 0.570, 0.563 and 0.535 g for Neemazal formulation for concentrations of 1, 2 and 4%, respectively.

Table 1: Effect of botanical extract oils on biological aspects of *Spodoptera littoralis* (Boisduval) under laboratory conditions of $28 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH.

Biological aspects/ Botanical extract oils	Larval duration (days)	Larval mortality (%)	6 th larval weight (g)	Pupal duration (days)	Pupation %	Pupal weight (g)	Adult emergence %	
Clove oil	1 %	9.17 ab	92.00 a	0.745 ab	10.50 abc	8.00 f	0.365 ab	87.78
	2 %	9.17 ab	92.00 a	0.518 d	10.17 abc	8.00 f	0.360 ab	89.18
	4 %	9.67 ab	94.00 a	0.576 cd	10.83 ab	6.00 f	0.328 abc	80.83
Mean	9.37	92.67	0.613	10.50	7.33	0.351	85.93	
Mint oil	1 %	9.67 ab	53.33 e	0.668 bc	10.00 bc	46.67 b	0.333 abc	83.11
	2 %	9.17 ab	60.00 de	0.620 cd	10.67 ab	40.00 bc	0.328 abc	84.72
	4 %	8.67 b	65.33 cde	0.530 d	10.33 abc	34.67 bcd	0.295 bc	80.18
Mean	9.17	59.55	0.606	10.33	40.45	0.319	82.67	
Garlic oil	1 %	8.50 b	60.00 de	0.618 cd	10.33 abc	40.00 bc	0.318 abc	95.41
	2 %	9.00 ab	68.00 cd	0.590 cd	9.50 bc	32.00 cd	0.328 abc	80.68
	4 %	8.83 ab	74.67 bc	0.530 d	10.17 abc	25.33 de	0.313 abc	72.30
Mean	8.78	67.56	0.579	10.00	32.44	0.319	82.80	
Neemazal formulation	1 %	8.67 b	73.33 bcd	0.570 cd	10.67 ab	26.67 cde	0.313 abc	82.14
	2 %	8.83 ab	84.00 ab	0.563 d	10.33 abc	16.00 ef	0.288 c	72.22
	4 %	9.17 ab	90.67 a	0.535 d	11.50 a	9.33 f	0.298 bc	72.22
Mean	8.89	82.67	0.556	10.83	17.33	0.300	75.53	
Control		10.00 a	12.00 f	0.790 a	9.17 c	88.00 a	0.373 a	97.00
L.S.D.		N.S	**	**	N.S	**	N.S	N.S

NS indicates that the differences between treatments are not significant.

** indicates that the differences between treatments are highly significant at 0.01

Means followed by similar letters are not significantly different at 0.05 level of probability.

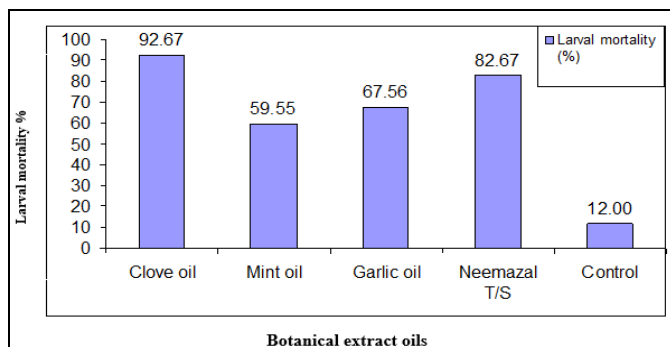


Fig 1: Effect of botanical extract oils on the larval mortality percentage of *Spodoptera littoralis* (Boisduval) under laboratory conditions of $28\pm 2^\circ\text{C}$ and $65\pm 5\%$ R.H.

For control larvae, the mean weight of 6th larval instar reached 0.790 g (Table 1). The differences between means are highly significant. These results are in agreement with those of El-Kholy *et al.* [16] who stated that the three tested crude plant extracts (datura, camphor and dampsissa) decreased the larval weight of *S. littoralis* than the control which ranged between 0.26 to 0.95g.

Data in Table 1 indicate that all the tested botanical extract oils caused an insignificant elongation in the pupal duration as compared to the control. The longest period (11.50 days) was observed when larvae treated with Neemazal -T/S formulation 4%, while the shortest mean recorded for control larvae showing (9.17 days). Regardless of the concentration, the tested materials can be arranged in descending order according to their effect on *S. littoralis* pupal duration as follow: clove oil (10.50 days), mint oil (10.33 days), garlic oil (10.00 days) and Neemazal -T/S formulation (10.83 days). These results are in agreement with those of El-Kholy *et al.*

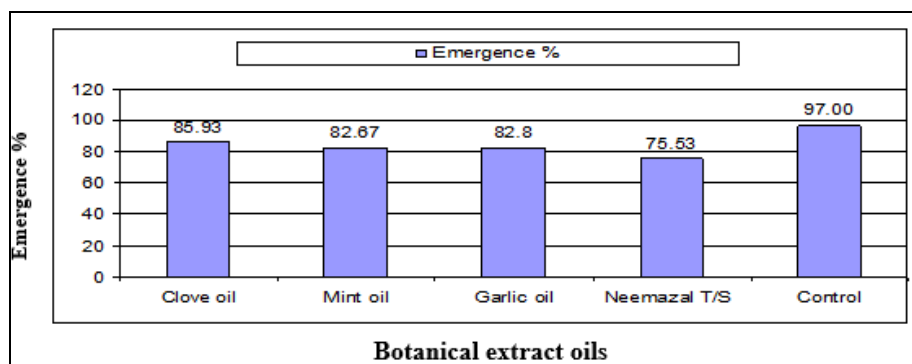


Fig 2: Effect of botanical extract oils on the emergence percentage of *Spodoptera littoralis* (Boisduval) under laboratory conditions of $28\pm 2^\circ\text{C}$ and $65\pm 5\%$ R.H.

Data in Table 2 indicated that the larvae were fed on leaves treated with the tested concentrations of mint oil 1% resulted in the highest weight of male moth recording (0.173g), meanwhile the lowest mean (0.123g) was noticed for Neemazal -T/S formulation 4%. Generally, all tested botanical extract oils affected negatively on male moth weight. In case of female moth weight, the same trend was nearly noticed, the highest mean weight (0.220g) was observed for control larvae meanwhile, the lowest mean (0.163 g) was attained by treated larvae with Neemazal -T/S formulation 2%. Other tested botanical extract oils with different concentration showed intermediate values. The differences between means are insignificant.

With respect of male moth longevity, all the tested botanical extract oils with different concentrations caused a highly

[16] who mentioned that the three tested crude plant extracts (*Datura*, camphor and dampsissa) increased the pupal duration of *S. littoralis*.

As shown in Table 1 the pupation percentages of larvae fed on leaves treated with tested botanical extract oils were lower than the control. The highest percent was recorded for mint oil 1% concentration showing (46.67%), meanwhile the lowest mean (6.00 %) was observed by clove oil 4% concentration. For untreated larvae, the percent pupation recorded (88.00%). Analysis of data revealed that the differences between means are highly significant. These results are in accordance with those of El-Morshedy *et al.* [18].

The obtained data showed that all the tested botanical extract oils reduced the average weight of pupa developed from larvae fed on leaves treated with tested materials than the control. The mean weight of pupa was 0.351, 0.319, 0.319, 0.300 and 0.373 g for larva treated with clove, mint, garlic oils, Neemazal -T/S formulation and control, respectively, regardless of the concentration (Table1). The differences between means are insignificant. The reduction in pupal weight may be due to that the larvae were less able to convert ingested and digested food into body substances Radwan *et al.* [19] and El-Ghar [20].

As shown in Table 1 and Figure 2 the emergence percentages of adult recorded (87.78, 89.18 and 80.83%) for clove oil, (83.11, 84.72 and 80.18%) for mint oil, (95.41, 80.68 and 72.30%) for garlic oil and (82.14, 72.22 and 72.22%) for Neemzal -T/S formulation for concentrations of 1, 2 and 4 %, respectively. For control larvae the emergence percentages attained 97.00%. The differences between means are insignificant. These results are in agreement with Abd El-Aziz and Ezz El-Din [15] and El-Morshedy *et al.* [18].

significant elongation in this character (Table 2).The mean male longevity reached (11.78, 12.11, 12.67 and 11.78 days) for tested botanicals materials i.e. clove, mint, garlic and Neemazal formulation, respectively, regardless of the concentration. For control male moth the mean longevity was 9.67 days. Longevity of adult females involved pre-oviposition, oviposition and post-oviposition periods were tabulated in some of the tested botanical materials increased longevity of females Table 2. While others decreased the female longevity than control. Clove oil with 1,2 and 4% concentrations increased adult female longevity, recording (11.67, 11.67 and 13.33 days), respectively. For mint oil 1% concentration increased female longevity, meanwhile 2 and 4% concentrations showing (10.00 and 9.33 days), respectively. Garlic oil and Neemazal -T/S formulation with

tested concentrations shorting adult female longevity. Females of control lived 10.66 days. The prolongation in adult longevity may be due to the reduction in food consumption, considerable decrease in growth rate and reduction in converting ingested and digested food to body tissues which may be cause rejection of feeding.

As shown in Table 2 and Figure 3 the total number of eggs laid/ female descended from the 4th instar larvae fed on caster leaves treated with tested botanicals extract oils was reduced in comparison with the control. Neemazal -T/S formulation caused the highest significant reduction recording 224.44 eggs/ female, regardless of the concentration, while control female laid an average of 1703.33 eggs / female. The decrease in the fecundity may be due to the inhibition of the protein contents and its synthesis which is necessary for the nutrition of eggs.

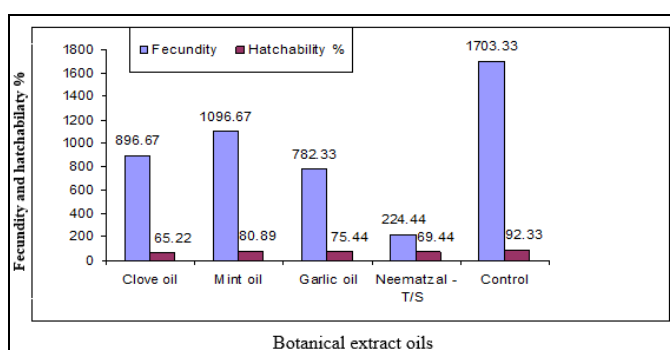
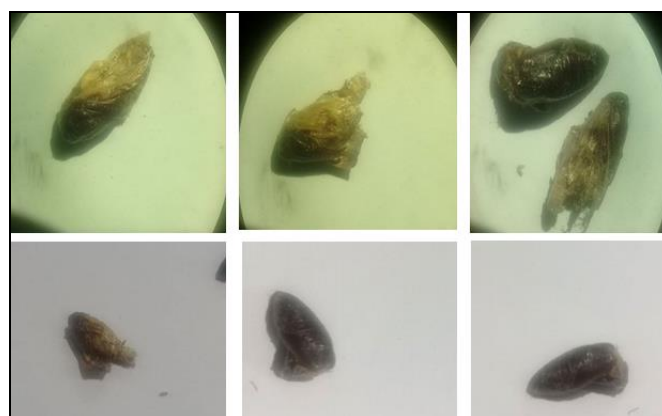


Fig 3: Effect of botanical extract oils on the fecundity and hatchability percentage of *Spodoptera littoralis* (Boisduval) under laboratory conditions of 28±2°C and 65±5% R.H.

For hatching percentage, data presented in Table 2 indicated that there was insignificant reduction in this parameter compared to control. The highest percentage 92.33 % was recorded for the control, meanwhile the lowest one (69.44 %) was noticed for Neemazal -T/S formulation, regardless of the concentration. Other tested botanical extract oils manifested intermediate values.

Data presented in Table 2 showed that the incubation period of eggs laid per female lasted 3.22, 3.33, 3.44 and 3.67 days for clove, mint, garlic oils and Neemazal -T/S formulation, successively, regardless of the concentration. Analysis of data revealed that the differences between means are insignificant. These results are similar with those of El-Morshedy *et al.* [18] who using bio insecticides.

As presented in Table 2 and Plate 1 all tested botanical extract oils with investigated concentrations didn't result any malphormed moths except both of clove oil 4 % concentration and Neematzal - T/S formulation showing 3.33 and 3.67% malphormed moth, respectively. The differences between means are highly significant.



Pupae malformations of *S. littoralis*



Adult malformations of *S. littoralis*

Plate 1: Pupae and adult malformations of *Spodoptera littoralis* (Boisduval) resulted from treatment with some botanical extract oils.

Table 2: Effect of botanical extract oils on biological aspects of *Spodoptera littoralis* (Boisduval) under laboratory conditions of 28±2°C and 65±5% RH.

Biological Aspects/ Botanical extract oils		Adult weight (g)		Malphormed moths %	Longevity				Fecundity	Hatchability %	Incubation period	
		♂	♀		♂	Female						
						Pre	Ovi	Post				Total
Clove oil	1 %	0.167 a	0.210 abcd	0.00 b	12.33 abc	3.67 cd	4.00 b	4.00 b	11.67 ab	966.67 c	71.67 bcd	2.67
	2 %	0.147 abc	0.207 abcd	0.00 b	11.67 bc	4.33 bcd	3.67 b	3.67 bc	11.67 ab	913.33 cd	69.33 bcd	3.33
	4 %	0.147 abc	0.217 ab	3.33 a	11.33 cd	4.67 bc	3.33 bc	5.33 a	13.33 a	810.00 de	54.67 d	3.67
Mean		0.154	0.211	1.11	11.78	4.22	3.67	4.33	12.22	896.67	65.22	3.22
Mint oil	1 %	0.173 a	0.213 abc	0.00 b	12.67 ab	3.33 de	4.00 b	4.00 b	11.33 bc	1316.67 b	84.33 ab	3.33
	2 %	0.153 abc	0.213 abc	0.00 b	12.33 abc	4.67 bc	2.00 def	3.33 bcd	10.00 bcd	1050.00 c	82.33 ab	3.00
	4 %	0.150 abc	0.200 abcd	0.00 b	11.33 cd	5.33 ab	1.67 ef	2.33 def	9.33 cd	923.33 cd	76.00 abc	3.67
Mean		0.159	0.209	0.00	12.11	4.44	2.56	3.22	10.22	1096.67	80.89	3.33
Garlic oil	1 %	0.150 abc	0.217 ab	0.00 b	13.67 a	4.00 cd	3.00 bcd	3.33 bcd	10.33 bcd	1023.33 c	81.00 abc	3.33
	2 %	0.157 ab	0.213 abc	0.00 b	12.67 abc	4.67 bc	1.67 ef	2.00 ef	8.43 d	743.33 e	77.33 abc	3.67
	4 %	0.163 a	0.207 abcd	0.00 b	11.67 bc	5.33 ab	1.33 ef	2.33 def	8.99 cd	580.33 f	68.00 bcd	3.33
Mean		0.157	0.212	0.00	12.67	4.67	2.00	2.55	9.25	782.33	75.44	3.44
Neemazal formulation	1 %	0.143 abc	0.170 bcd	0.00 b	13.33 a	4.00 cd	3.00 bcd	2.67 cde	9.67 bcd	406.67 g	70.33 bcd	3.33
	2 %	0.130 bc	0.163 d	0.00 b	11.67 bc	5.33 ab	1.67 ef	2.00 ef	9.00 cd	248.33 h	73.67 bc	3.67
	4 %	0.123 c	0.167 cd	3.67 a	10.33 de	6.33 a	1.00 f	1.33 f	8.66 cd	18.33 i	64.33 cd	4.00
Mean		0.132	0.167	1.22	11.78	5.22	1.89	2.00	9.11	224.44	69.44	3.67
Control		0.173 a	0.220 a	0.00 b	9.67 e	2.33 a	5.33 a	3.00 bcde	10.66 bc	1703.33 a	92.33 a	2.67
L.S.D.		N.S	N.S	**	**	**	**	**	**	**	*	N.S

NS indicates that the differences between treatments are not significant.

** indicates that the differences between treatments are highly significant at 0.01

Means followed by similar letters are not significantly different at 0.05 level of probability

4. Conclusion

The abovementioned results indicate that clove oil and Neemazal-T/S formulation botanical extract oils were the most effective on percentages of larval mortality, malformed moth and hatchability of *S. littoralis*. Generally, all the tested botanical extract oils caused positive effect on investigated biological aspects, so we recommended by using different botanical extract oils for controlling *S. littoralis* instate of insecticides.

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