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Toxicological and Biological effect of *Ocimum basilicum* L. Oil on some Cotton Pests

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ABSTRACT

Egyptian *Ocimum basilicum L.* oil (basil oil) was analyzed with High Performance Liquid Chromatography (HPLC) to give Forty two compounds; characterization and identification of the two major active components were identified as: Linalool (33.9 %) and Eugenol (8.31 %) of the oil constituent. The effect of basil oil on toxicity and biological activity on different cotton pests were studied. The basil oil had toxic effect against newly hatched larvae of spiny bollworm (*Earias insulana*), cotton leafworm (*Spodoptera littoralis*) and red spider mite (*Tetranychus urticae)*. The LC_{50} and LC_{90} values of basil oil against newly hatched larvae of spiny bollworm were calculated after 24 hrs. from treatment 41.34 and 178.76 % ; for 1st instars larvae of the cotton leaf-worm were 14.13 and 93.25 % but for 2nd instars larvae of the cotton leaf-worm were 17.91and 93.38 %, while in the case of 4th instars larvae were 19.26 and 81.74 %. The LC_{50} and LC_{90} values for red spider mite (*T.urticae*) recorded 0.46 and 2.82 %. Basil oil (0.20 %) showed significantly prolonged larval duration, pre oviposition period and prolonged pupal duration of the spiny bollworm larvae. Also, it decreased pupation percentage, adult emergence and hatchability percentages.

Key words: Basil oil, HPLC, *Earias insulana*, *Tetranychus urticae*, *Spodoptera littoralis*, chemical composition, latent effect.

Introduction

In Egypt, cotton plants can be infested by many pests such as the Egyptian cotton leaf worm, *Spodoptera littoralis* (which considered as the most important cotton pest which can be found almost everywhere cotton is grown in Egypt), and cotton bollworms group; pink bollworm, *Pectinophora gossypiella*, spiny bollworm, *E. insulana* and American bollworm, *Helicoverpa armigera* which cause serious damage to cotton bolls. Plant damage resulting from sucking of cell sap, reducing leaves area, fruit quality and quantity of the production (El-Halawany *et al*., 1986 and Butler & Henneberry, 1990). Abbas, *et al*., 1996 reported that, pests resistance is another problem as a result of continuous use of synthetic pesticides. Natural product of plants and plant derivatives are alternative agent to currently use for insect control because they constitute rich sources of bio-active chemicals, they are often biodegradable to non-toxic products. Additionally, plant-derived materials are found to be highly effective against insecticide resistant insect pests (Arnason *et al*., 1989; Kwon *et al*., 1996 and Ahn *et al*., 1997). Essential oil can be inhaled, ingested or skin absorbed by insects (Ozols and Bicevskis, 1979) such as *Ocimum basilicum* (Basil). Essential oils obtained from some other aromatic plants showed toxic effects on insects and mites (Choi *et al.*, 2004 and Tripathi *et al*., 2000). Hussein *et al*., 2014 proved that, Basil oil at 0.5% had toxic effect against *Tuta absoluta* infestation on tomato plants under field condition. Coats *et al*., 1991 suggest its mode of action related to neurotoxic, linalool acts on nervous system which affect on ion transport and release of acetylcholine esterase. Enan, 2005 showed that, eugenol mimicked octopamine in increasing intracellular calcium level in cloned cells from the brain of *Periplaneta americana* and *Drosophila melanogaster* and cellular changed by eugenol responsible for its insecticidal property (Price and Berry, 2006) . Researchers have confirmed the antimicrobial, insecticidal and antifungal properties of the essential oil of basil (Bagamboula *et al*., 2004; Sacchetti *et al*., 2004)

The aim of this study was to find new materials for potential use to control some cotton pests. Thus commercially available plant essential oils were screened against spiny bollworm, cotton leafworm larvae and red spider mite adult female. High Performance Liquid Chromatography (HPLC) analysis was used to identify the main constituents of basil oil.

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Materials and Methods

Analysis technique:

Basil oil was purchased from EL-Captain Company, Egypt. *Ocimum basilicum* L.oil were subjected to further analysis using HPLC (a Shimadzu Class –VP HPLC system with a computer –controlled system containing upgraded Class-VP 5.03 software. Separation were carried out on a reversed phase column LiChroCART RP-18 (Merck, Darmstadt, Germany, 12.5 cm x 0.4 cm, particle size =5 µm), using mobile phase of water/formic acid (19:1,v/v) (solventA) and methanol (solvent B) at a constant solvent flow rate of 1ml/min) ,according to the method of (Yao *et al.* 2003) to support the identification of the differental kind of phenolics flavonoid and saponin compounds. The injection volume was 20 µL and peaks were monitored at λ 280 nm and identified by comparison of their relative retention times with those of authentic standards analyzed in the same conditions.

Pests used:

Spiny bollworm larvae were reared on a modified artificial diet as described by Rashad and Ammar (1985) and Amer *et al*., (2010) under laboratory conditions of 26 ± 1 °C and 70 ± 5 % R. H. , cotton leafworm larvae were reared in the laboratory on castor bean leaves (*Ricinus cannapenus*) as described by(El-Defrawi *et al*., 1964) rearing on 27 ± 2°C and 60 ± 5 % R.H under laboratory conditions and Pure culture of *T. urticae* was initiated by transferring males and females using a fine hair brush to fresh discs of mulberry leaves in Petri-dishes (10 cm in diameter), each leaf was put on a pad of cotton wool saturated with water as a source of moisture, and to prevent mite escaping, under laboratory conditions (27 ± 2°C and 75±5 % R.H.),for rearing adult females of known age, ten groups of twenty five adult females each, were transferred by fine hair brush to ten discs of mulberry leaves (10 cm in diameter) on moist cotton wool pads in Petri-dishes, the females were removed after 24 hours and the eggs were left to hatch. Care was taken to keep the cotton wool pads always wet.

Toxicological studies:

To evaluate the toxic effect of basil oil against newly hatched larvae of *E. insulana* serial aqueous dilution of basil oil concentrations (40, 30 and 20 %) were prepared, cotton leaf-worm serial aqueous dilution of basil oil concentrations (70 ,40 , 20 ,10 and 5 %)for 1st, 2nd and 4th instars larvae, and against adult females of red spider mite serial successive concentrations of each treatment were prepared using distilled water (5, 2.5 ,1.25 , 0.30 %). Larval mortality was recorded after one day from treatment. The LC_{50} and LC_{90} values were calculated according to the method described by (Finney, 1971).and corrected mortality using Abbott's formula (Abbott, 1925).

Latent effect of basil oil on larvae of the spiny bollworm:

To study the latent effects of the basil oil on most biological aspects of *E. insulana* larvae. The newly hatched larvae of the spiny bollworm were fed on artificial diet treated with the concentration of basil oil (20 %) for 24 hrs. The tested concentration was replicated six times (50 / larvae / replicate) as well as the untreated check. The alive larvae were transferred individually into clean glass tubes (2 x 7.5 cm) containing untreated artificial diet and covered with a piece of absorbent cotton and held in incubator under the above mentioned conditions. The larvae were inspected daily till pupation to record larval duration, weight of 4th instars larvae and mortality percentages. Pupae were transferred individually into glass tubes and incubated till moth's emergence. Pupal duration, percent mortality of pupae and pupal weight were recorded. Newly emerged moths of the spiny bollworm were sexed and transferred to glass cages (three pairs / cage) and replicated four times. The moths were feed on 10 % sugar solution. The number of deposited eggs / female, pre- oviposition, oviposition, post- oviposition periods, female and male longevity and hatchability percentages were recorded.

Statistical analysis:

The obtained results of each mortality and biological parameters were subjected to analysis of variance to clear toxicity and latent effect parameters using Costat software computer program Cohort Software. P.O. Box 1149, Berkeley CA 9471 (Costat program methods 2005).

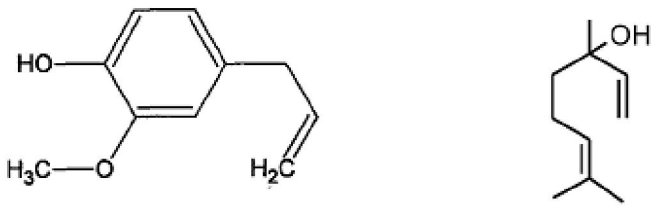
Results and Discussions

Chemical composition of the volatile oil:

As can be seen from Fig. (1) and Table (1) forty two compounds were characterized and identified by using HPLC for the oil of *Ocimum basilicum* cultivated in Egypt. The two major active components were identified as: Linalool (33.9 %) and Eugenol (8.31 %) of the oil constituent (Fig. 2).

Toxicological effect of basil oil on some cotton pests:

Data in table (2) revealed that, the LC_{50} and LC_{90} values of basil oil against newly hatched larvae of spiny bollworm after one days from treatment were 41.34 and 178.76 %, while 1st instars larvae of cotton leafworm recorded 14.13 and 93.25 % and decreased after one day for 2nd instars larvae of cotton leafworm larvae were 17.91 and 93.38 % and decreased after one day for 4th instars larvae of the cotton leafworm recorded 19.26 and 81.74 %. The LC_{50} and LC_{90} values of basil oil against red spider mite were 0.4626 and 2.8214 %. At LC_{50} level basil oil was highly affect on red spider mite, followed by 1st and 2nd instars larvae of cotton leafworm and 4th instars larvae. The least basil oil effect on newly hatched larvae of spiny bollworm was noticed after one day from treatment. At LC_{90} level basil oil was highly affect on red spider mite, followed by 1st, 2nd and 4th instars larvae of cotton leafworm. In case of spiny bollworm result indicated that, basil oil had the least effect on newly hatched larvae one day from treatment.



Eugenol **linalool**

Fig. 2: Chemical structure of Eugenol and linalool

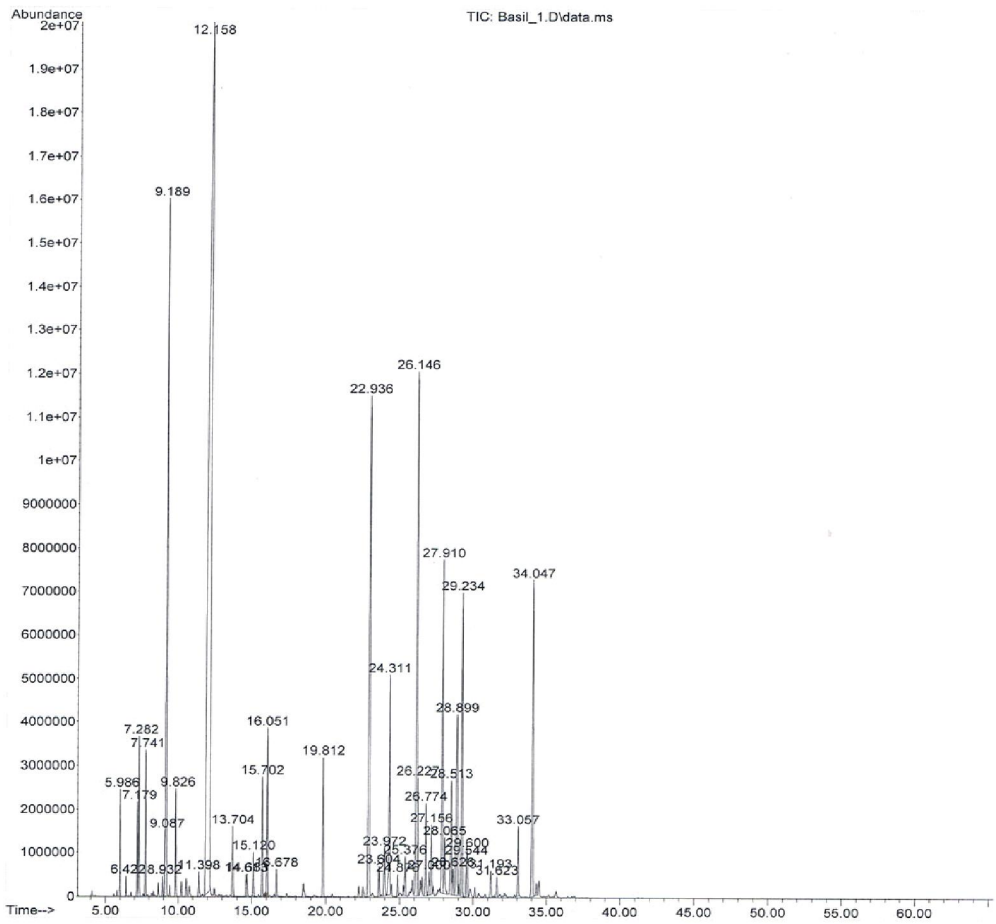


Fig. 1: High Performance Liquid Chromatography (HPLC) of the volatile oil of *Ocimum basilicum L.*

Table 1: The volatile compounds identified from the volatile oil of *Ocimum basilium* by using HPLC.

No.	Compound name	Retention time in min.(Rt.)	Peak area %
1	α-Pinene	5.98	0.75
2	Camphene	6.42	0.45
3	Sabinene	7.17	0.71
4	β- Pinene	7.28	1.33
5	β- Myrcene	7.73	1.16
6	O-Cymene	8.92	0.17
7	dl-Limonene	9.08	0.54
8	1.8-Cineole	9.18	8.4
9	β- Ocimene	8.82	0.92
10	α- Terpinolene	11.39	0.29
11	Linalool	12.15	33.9
12	(+)-2-Bornanone	13.7	0.86
13	Borenol	14.61	0.21
14	3-Cyclohexene-1-methanol	14.68	0.24
15	Terpinen-4-ol	15.12	0.44
16	β- Fenchyl alcohol or α-Terpineol	15.7	1.25
17	Chavicol	16.05	1.71
18	Caprylyl acetate	16.67	0.26
19	Bornyl acetate	19.81	1.57
20	Eugenol	22.93	8.31
21	α-Copaene	33.60	0.34
22	(-)-β- Bourbonene	23.97	0.66
23	β- Elemene	24.31	2.79
24	Methyl Eugenol	24.87	0.22
25	Trans- Caryophyllene	25.37	0.47
26	2,6-Dimethyl-6-(4-methyl-3-pentenyl)-bicyclo[3.1.1]hept-2-ene	26.14	8.04
27	α-Guaiene	26.22	1.11
28	α-Humulene	26.77	1.09
29	Cis-β-Farnesene	26.99	0.34
30	Germacene D	27.15	0.86
31	β-Cubebene	27.90	4.33
32	Trans-β-Farnesene	28.06	0.79
33	Bicyclo Germacene	28.51	1.54
34	α-Gurjuene	28.62	0.82
35	α-Bulnesene	28.89	3.31
36	α-Amorphene	29.23	3.95
37	Cis-Calamenene	29.54	0.41
38	δ-Cadinene	29.59	0.57
39	(+)-Aromadendrene	31.19	0.37
40	(+)-Spathulenol	31.62	0.25
41	α-Cubebene or Cadina-1,4-diene	33.05	0.84
42	α-7-H-Eudesma-3,5-diene	34.04	4.24

These results agree with (Abou El-Ela, 2014) recorded basil oil was highly affect on last larval instars of *Acheroia gresilla*, LC_{50} and LC_{90} levels were 9.88 and 32.32 with basil oil, while eugenol recorded 4.34 and 21.98 at LC_{50} and LC_{90} levels. (Perumalsamy *et al*., 2014) recorded that, basil oil, *O. basilicum* had toxic effect on adult American house dust mites*, Dermatophagoides farinae Hughes* LC_{50} for 24h, methyl eugenol (5.78 µg / cm2) exhibited toxicity greater than benzyl benzoate (LC_{50} 8.41 µg / cm2) and N, N-diethyl-3-methylbenzamide (37.67 µg/cm2). Basil oil applied as 3 and 4 % sprays provided 97 and 100 % mortality against the mites, respectively.

Table 2: Toxic effect of basil oil on some cotton pests.

Treatment	Pests		LC_{50}	LC_{90}	Slope	Toxicity index		Relative potency	
			After 24 hours from treatment			After 24 hours from treatment			
Basil oil	Spiny bollworm larvae		41.34	178.76	2.01	LC_{50}	LC_{90}	LC_{50}	LC_{90}
						1.12	1.58	1.00	1.00
	Cotton leafworm larvae	1st	14.13	93.25	1.56	3.27	3.03	2.92	1.92
		2nd	17.91	93.38	1.81	2.58	3.02	2.31	1.91
		4th	19.26	81.74	2.04	2.40	3.45	2.15	2.19
	Red spider mite		0.46	2.82	1.63	100	100	89.36	63.36

Attia *et al.,* (2013) recorded that, high mortality rate when applied on *T. urticae* female which treated with *Citrus aurantium* and *Mentha pulegium* oil with LC_{50} at 4.09l / L and 5.39 µl / L and LC_{90} at 5.67 µl / L and 8.07 µl / L .(Hegab and Abd-El Atty 2013) they found the effect of the plant extracts, *Azadirachta indica*, *Citrullus colocynthis* and *Thymus vulgaris* formulations against different stages of spiny bollworm, *Earias*

insulana (Boisd.) at two concentrations (5 and 10 %) had caused the mortality of larval stage were (48.00 & 38.67 %), (40.00 & 45.33 %) and (38.67 &42.66 %), respectively. (Elumalai *et al.,*2010): Essential oil of Ginger, lime, and lavender (LC_{50}=15.3, 17.4, 19, LC_{95}=21.0, 20.3 and 53.1 ppm) showed highest mortality while the essential oil of calamus, basil, carnmint and rosemary (LC_{50}=66.7, 59.8, 53.1, 49.6, LC_{90}= 109.7, 125.3, 94.7 and 81.5 ppm) showed lowest larval mortality.

Latent effect of basil oil (20 %) on some biological aspects of the spiny bollworm:

Larval stage:

Data in Table (3) showed that, the concentration of basil oil (20 %) significantly prolonged larval duration; the mean period of larval duration was 15.83 days compared with 14.5 days for control, insignificant reduction in larval weight compared with control, the larval weight was 0.0761 g in the same concentration test as compared with 0.0819 g in control and highly significant increased in larval mortality percentage 63.89 % as compared with 12.5 % in untreated check

Pupal stage:

Data in Table (3) proved that, the tested concentration of basil oil (20 %) conc. caused insignificant reduction in pupal weight compared with control. The pupal weight was 0.0655 g for the tested concentration as compared with 0.0661 g for control treatment, pupal duration of the spiny bollworm was highly significant shortened pupal duration as influenced by concentration of basil oil (20 %) which recorded 10.50 days as compared with 11.00 days for control and highly significant effects of basil oil on pupation percentage as compared with control. The pupation percentages were 87.50 and 36.11 % for control and basil oil, respectively.

Table 3: Effect of basil oil on immature stages of the spiny bollworm.

Conc.%	Larval duration (days)	Larval weight (g)	Laval mortality %	Pupal weight (g)	Pupal duration (days)	Pupation %
20	15.83b	0.0761	63.89a	0.0655	10.50ab	36.11c
Control water	14.5c	0.0819	12.50b	0.0661	11.00a	87.50b
Control alcohol	17.25a	0.0816	6.67c	0.068	9.51b	93.33a
F. Test	**	NS	**	NS	*	**
$LSD_{0.05}$	0.67	0.011	5.83	0.0059	1.49	5.83

NS: Non-significant *: Significant **: Highly significant.

Adult emergence:

Data in Table (4) showed that, highly significant effects on adult emergence percentage resulted from treated larvae of the spiny bollworm with 20 % of basil oil concentration as compared with control. Adult emergence percentages recorded 68.75 % as compared with 88.32 % in control.

Male and female longevity:

Data in Table (4) showed that, insignificant influence in the male longevity of the spiny bollworm moths resulted from treated newly hatched larvae with basil oil (20 %) concentration as compared with control.

Sex ratio:

Statistical analysis of variance of the data in Table (4) show that, the sex ratio of male and female of the spiny bollworm moths resulted from treated newly hatched larvae with basil oil was highly significant effect as compared with control.

Number of deposited eggs/ female:

Data given in Table (4) showed that, the number of deposited eggs of female resulted from treated newly hatched larvae with basil oil (20 %) tested concentration was insignificant effect as compared with control.

Hatchability of eggs:

Present data in Table (4) proved that, the tested concentration of basil oil (20%) was decreased significantly, the mean hatchability percentages of eggs was 67.31% as compared with 96.00% eggs for control, respectively.

Pre-oviposition period:

The tested concentration of basil oil (20 %) was highly significant shortened Pre oviposition period than control Table (4).

Oviposition and Post Oviposition period:

Statistical analysis of the present data in Table (4) indicated that,, the oviposition and Post Oviposition period of female resulted from treated newly hatched larvae of spiny bollworm with basil oil (20 %) concentration was insignificant as compared with control.

Table 4: Biological activities of treated spiny bollworm moths with sub lethal conc. of basil oil.

Concentration %	Adult emergence %	Oviposition period / female / day			Adult longevity in days		Sex ratio%	Fecundity	Hatchability%
		Pre-Pre-oviposition period	Ovi-position Period	Post-oviposition period	Male	Female	Female		
20	68.75 b	1.33c	14.33	0.67	15.00	16.33	71.50a	224.50	67.31b
Control water	88.32 a	3.00a	14.00	0.33	13.67	17.33	51.78b	249.67	96.00a
Control alcohol	89.67 a	2.00b	12.00	0.67	13.67	15.00	51.33b	261.67	93.33a
F. test	**	**	NS	NS	NS	NS	*	NS	**
$LSD_{0.05}$	10.67	0.67	3.33	1.15	1.88	16.33	13.99	82.66	11.99

*NS: Non-significant *: Significant **: Highly significant.*

These results agree with(Aziza Sharabya *et al.,* 2009) stated Eugenol and peppermint oils, each at the 0.01% conc., caused a significant depression in the fecundity of *Phthorimaea operculella* moth and decreased the percentage of egg hatchability. Eugenol oil was much more effective than peppermint oil at 1 %. The results indicated that, dried powders of, *Ocimum basilicum* played a highly significant role in reducing egg deposition. The majority of the plant extracts at 1% conc. could protect potato tubers at different intervals. (Abou-El-Ela 2014) recorded basil oil and eugenol was highly affect on last larval instar of *Acheroia gresilla* and on mortalities of larval and pupal stages, pupation period showed 18.98 and 15.77 days as compared 17.45 days for control. Adult longevity for males observed (27.55 days) for basil oil, eugenol (24.54 days) as compared to (26.4 day) for control, for female was observed (33.4 days) for basil oil, eugenol (36.65 days) compared to (32 days) for control. Eggs laid/female was affected reaching (133.00 egg / female) for basil oil; eugenol was 66.4 egg /female. While recorded for the control being 339.00 egg/female. Basil oil recorded (94.8 %) eugenol (91.8 %) hatchability percentages, compared to control (96.6 %). (Eliopoulos *et al.,* 2015) toxicity data indicated that, larvae and pupae of *Ephestia kuehniella* and *Plodia interpunctella* were the most tolerant stages in all cases. Larval and pupal mortality were 21.00 and 38.00 % for basil oil, respectively.

Conclusions

HPLC analysis of the basil oil showed the presence of linalool and eugenol which exhibited toxic effect against different cotton pests and latent effect against newly hatched larvae of the spiny bollworm. Basil oil prolonged the larval duration and shortened pupal duration & pre-oviposition period and decrease the number of the deposited eggs / female and the rate of hatchability percentages.

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