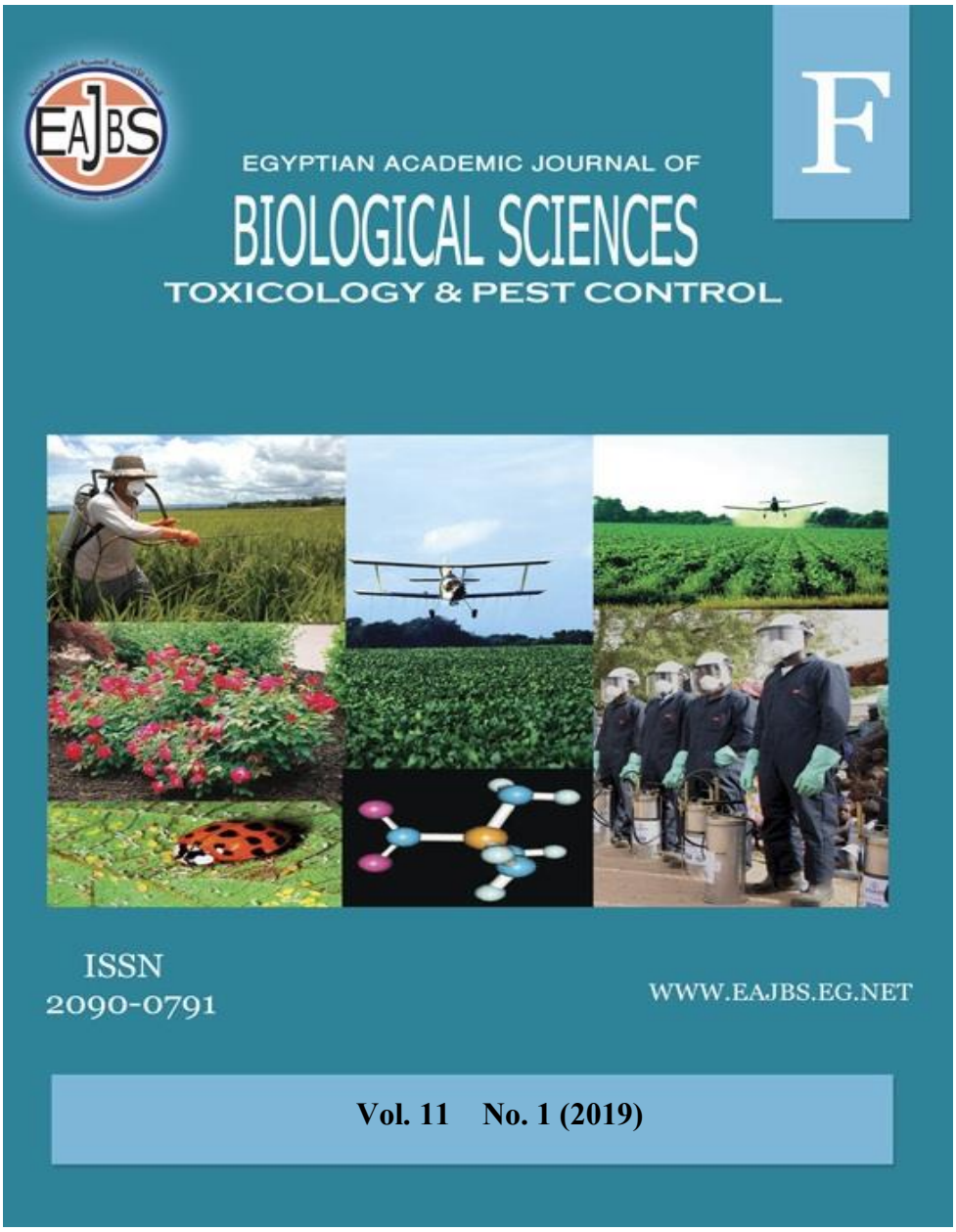

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Antioxidant Activity and Biological Studies of Two Medicinal Plant Extracts on *Spodoptera littoralis* (Boisd)

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ABSTRACT

Control management of *Spodoptera littoralis* (Boisd) infestation has been achieved by using chemical insecticides. However, the environment-friendly methods without unwanted side effects of these chemicals are becoming very important in modern pest management strategies. Natural products including plant ethanolic extracts and essential oils are some of the alternative approaches in pest control. The essential oils were extracted and their chemical composition was identified using a GC/MS spectrometer. Therefore, the present study was designed to evaluate the antioxidant activity of various extracts of sage and thyme plants by 2, 2-diphenyl-1-picrylhydrazil (DPPH) method. The obtained results revealed that concentrations at 25, 50, 100 and 200µg/ml of essential oils and ethanolic extract from both plants were more efficient in scavenging free radicals after 30 min when compared with butyl hydroxyl toluene(BHT) as synthetic standard. The concentrations 100 and 200µg/mL from ethanolic and essential oils were having higher activity than other extracts and standard .Furthermore, Sage and thyme extracts were investigated for their toxicity against cotton leafworm, *S. littoralis* in the laboratory, the percentages of cumulative mortality of larvae as well as the latent effects of the 4th instar of *S. littoralis* four concentrations (25, 50, 100 and 200 µg/mL) were applied. It was found that ethanolic extract of two plants was more efficient than essential oils on different stages of *S. littoralis*. The results showed that there were highly significant differences between all treatments and control some biological aspects.

INTRODUCTION

Cotton leafworm, *Spodoptera littoralis* (Boisd.), is a highly polyphagous pest with numerous hosts causing economically important loses. In Egypt, the Cotton leafworm, *Spodoptera littoralis* (Boisd), is considered one of the major pests attacking more than 112 host plants. Unfortunately, the rate of infestation may reach up to 119 048 egg-mass/ha, causing great damage to leaves, buds, flowers, and bolls (Temerak, 2002; El-Sheikh, 2012; El-Geddawy, *et al*. 2014; Ahmed, *et al*. 2015a, b).

Alternative strategies have included the investigation for a new type of insecticides and re-evaluation traditional pest control agents. The adverse special effects of synthetic

pesticides have enlarged the requisite for effective and bio-degradable pesticides. Because of the power of plant-insect interactions, the plant has a well-developed defense mechanism against herbivores and are excellent sources of new toxic substances for pests (Pickett, *et.al*. 2006). During recent years, intensive research has been carried out to control agricultural pests by using natural insecticides of plant origin to decrease hazards in the environment. oil extracts or isolated active compounds have been shown to act as potent acute or chronic insecticides (Abdel Aziz, *et al*. 2007; Colomaa, *et al*. 2006) Also the physiological and biochemical effect of some plant oil extracts on various insects were studied by Emara, *et al*. 2002. There are numerous researches on the efficiency of essential oils from Lamiaceae family (Rajendran and Sriranjini, 2008; Isman, et *al*., 2011; Ebadollahi and Ashouri, 2011). The advantage of using plant essential oils is that they are easily available and they have been used extensively for medicinal purposes, implying that they have low or no toxicity to human (Upadhyay 2013). Among various classes of natural substances that introduced as natural biopesticides are essential oils from aromatic plants (Isman and Grieneisen, 2014and Prakash, *et al*., 2014).

The deleterious effects of plant products on insects can be manifested in several manners including toxicity, mortality, antifeedant growth inhibitor, suppression of reproductive behavior and reduction of fecundity and fertility, growth inhibition, perturbation of reproductive behavior (reduction of fecundity and fertility) (Hernández-lambraño, *et al*., 2014).

Sage (Lamiaceae family) is a perennial low shrub native of the Mediterranean region and its family reported to include more than 900 species (Pierozan, *et al*. 2009; Ilkiu-Vidal, *et al*. 2010) Its essential oil is added to meat, sausage, poultry stuffing, fish, soups, canned foods and other food products. Sage essential oil protected liver pates from oxidation processes and could be used as an alternative option to synthetic antioxidants such BHT and was used in dry fermented buffalo sausage too (Estévez, *et al*. 2007; Salem & Ibrahim, 2010)

Thyme (Lamiaceae family) is herbaceous plant of the platoon species, grows in mountainous areas, used as a beverage instead of or with tea, added to some food to give it an acceptable flavor, the plant is used in folk medicine frequently where it is prescribed to treat mouth infections, stomach, intestine and airways, coughing and gastroenteritis and expel intestinal worms, as well as to strengthen the heart (Mohamed, *et al*2013). Extracts from Thyme have been used in traditional medicine for the treatment of several respiratory diseases like asthma and bronchitis and for the treatment of other pathologies thanks to several properties such as antiseptic, antispasmodic, antitussive antimicrobial, antifungal, antioxidative, and antiviral (Ocana and Reglero 2012).

Control methods, which are used to limit the losses caused by S .littoralis, consist of treatments based on synthetic insecticides that are harmful as well for farmers, consumers and the environment. To seek for alternative ways to limit the use of these insecticides, essential oils and ethanol extract from the leaves of sage and thyme were tested in the laboratory. This work was designed to evaluate the biological and antioxidant activity effect of two medicinal plants extracts sage and Thyme treated on 4th larval instar of *Spodoptera littoralis*

MATERIALS AND METHODS

Chemicals and Reagents:

Pure ethanol, ether methanol were purchased from E. Merch Co. (Germany), and distilled before use.

Plants:

The selected plant for the study were garden Sage (*Salvia officinalis*) and thyme (*Thymus vulgaris*), (Lamiaceae),the plant leaves were collected from agricultural research center during may Month 2017, water rinsed then spread for drying in shade at room temperature about 2 – 3 weeks for dryness, then the leaves were ground by high-speed grinder.

Essential Oil Extraction:

A100 grams of plant leaves were separately subjected to hydro-distillation for over 3hours using a modified Clevenger apparatus according to Santana, *et al*. (2013). The resulted oil was dehydrated over anhydrous sodium sulfate and stored at -20 ºC until use.

GC / MS / MS analysis of essential oil

The analysis was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with a polar Agilent HP-5ms (5%-phenyl methyl polysiloxane) capillary column (30 m × 0.25 mm i. d. and 0.25 µm film thickness). The carrier gas was helium with the linear velocity of 1ml/min. The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature (Santana, *et al*., 2013).

Ethanolic Extraction:

A sample 50gof dried and ground leaves of each plant were macerated three times (48 hrs each) with300 mL ethyl alcohol70% at room temperature 25 ºC. The macerated samples were filtered and the ethanol was evaporated using rotary evaporator at 40ºC

Identification of Phenolic Compounds by HPL:

Separation and determination of phenolic compounds in ethanolic extracts were performed according to Belajova and Suhaj (2004) by HPLC (hp 1050, the used column was sprain SG X C-6 phenyl and the U.V. detector at 285 nm). The solvent system used was a gradient of A (CH_3COOH 2.5%), B (CH_3COOH 8%) and C (acetonitrile). The best separation was obtained with the following gradient: at 0min, 5% B; at 20 min, 10% B; at 50 min, 30% B; at 55 min, 50% B; at 60min, 100% B; at 100 min, 50% B and 50% C; at 110 min, 100% C until 120 min. The solvent flow rate was 1ml/min. and separation was performed at 35 °C. The volume injected was 10 ml. Peak identification was performed by comparing the relative retention time of each peak with those of known compounds.

2, 2 Diphenyl-1-picrylhydrazyl (DPPH) Radical Assay:

Scavenging effect of 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical was measured by the method of Brand-Williams, *et al*.(1995). Where, 0.1 ml of 1 mM methanol solution of DPPH was incubated with various concentrations of each plant extract (25, 50, 100 and 200 µg/ml). After 15 and 30 min incubation periods at room temperature, the absorbance of the resulting solution was recorded at 517 nm using a spectrophotometer (Jen way 6305 UV/Vis Spectrophotometer). In addition to measuring the color of tea extract without DPPH. DPPH radical scavenging activity was expressed as the inhibition percentage and was calculated as:

Absorbance of control - absorbance of sample/absorbance of control × 100. Standard butylated hydroxyl toluene (BHT) was used for comparison.

Rearing of the Cotton Leaf Worm, *S. littoralis:*

The culture of the cotton leafworm was reared under laboratory conditions (27 ± 2°C and 60±5% RH), the egg masses were allowed to hatch in clean jars provided with castor bean leaves, *Ricinus communis,* the larvae continued their development till pupation. The pupae were collected in separated jars until adult emergence. Moths were fed on 10% sugar solution. The colony was reared for several generations before using the 4^{th} instar larvae in the bioassay experiments Bakr, *et al.*, (2010).

Biological Studies:

Laboratory experiments were conducted to determine the efficiency of the concerned plant extracts, against 4th instar of *S.littoralis* larvae reared at 27 ± 2 °C and 55 – 65% R.H. All experiments were carried out using dipping method, in which fresh castor leaves were dipped for 20 seconds in the different tested extracts. Then they were left in shad to dry. Two hundred and seventy larvae were divided into three replicates each of 10 larvae where they were kept in plastic cups. Larval mortality was daily recorded until pupation. Mortality percentage was determined according to (Meglla, 1984) as following:

% Mortality of larvae = No. of dead larvae/ Total No. of larvae× 100

Larval malformation % = number of malformed larvae / number of tested larvae × 100

All malformed pupae were counted. The pupal malformation percent was calculated by using the following equation:

Pupation %= No. of pupae / No. of tested larvae × 100

Pupal malformation % = No. of malformed pupae / Total No. of pupae × 100

A percentage of adult emergences were counted by using the following equation:

Emergence of adults% = No. of emerged adults/ Total No. of pupae ×100

Adults malformation was estimated by any change in color, size, shape or failure to grown. All malformed Adults were counted and removed immediately.

Adults malformation % = No. of malformed Adults / Total No. of adults × 100

Some adults lay an egg to complete the life cycle, then counted egg hatchability%

Egg hatchability % = No. of hatched egg / Total No. of egg × 100

Statistical Analysis:

Data were subjected to an analysis of variance, and the means were compared using the "Least Significant Difference (LSD)" test at 0.01 levels, as recommended by Snedecor and Cochran (1982).

RESULTS AND DISCUSSION

Essential Oil Composition:

The hydro-distillation of Sage, *(Salvia officinalis*) and thyme *(Thymus vulgaris*), were produced a colorless essential oil. The yield of salvia essential oil was 1.7 % on a dry basis. This is in agreement with others values found in literature, which reported yields of 1.1 to 2.8% (Couladis, *et al*. 2002; Velièkov, *et al*. 2003; Lima *et al*. 2004; Klaus *et al.* 2009) and the dried thymus contains up to 1.1% essential oil This is in accordance with Dauqan and Abdullah (2017), which reported yields of 2.5%, Porte and Gopoy (2008) found that 1.1 %.The identified compounds along with their relative percentages are given in Table (1). The data revealed that the major components of Sage oil were α-terpinolene (20.24%), α-pinene (16.80%), β-pinene (10.14%), .terpnene (9.82%) and borneol (8.79%) as the main components. Other important constituents of this oil were terpinen-4-O l (5.33%), γ-terpinene (4. 68%) tricyclene (3.41%), cyperene (3.20%), thymol (3.04%), limonene (2.12%), β-caryophyllene (1.12%). This is in accordance with Porte, *et al*., (2013) found that the major constituents of the oil were α-thujone (40.90 %), camphor (26.12 %), α-pinene (5.85 %) and β-thujone (5.62 %). However, a total of 19 compounds were identified from Thyme essential oils as the major compound reached to linalool (25.50%), mycrene (15,67%), thymol (13.76 %) , 1,8-Cineol (11.94 %), limonene(8.10%) other important constituents of this oil were α-terpineol (6.31%), Other notable components included cis-sabinene (0.16%) , β-caryophyllene oxide(0.14%)and terpinolene(0.13%).This results with agreement with

Eqbaland Abdullah (2017) the main components are thymol, carvacrol, p-cymene, γ-terpinene, linalool, β-myrcene, terpinen-4-ol, and some compounds occur partly as glycosides, Also, Prasanth *et al*. (2014) showed that the essential oil from T. vulgaris a high content of oxygenated monoterpenes(56.53%) and low contents of monoterpene hydrocarbons (28.69%), sesquiterpene hydrocarbons (5.04%) and oxygenated sesquiterpenes (1.84%). The predominant compound among the essential oil components was thymol (51.34%) while the amount of all other components of the oil was less than 19%.

Table 1: The relative percentage of essential oil compositions of Thyme and Sage plants (identified by GC / MS / MS).

Thyme			Sage		
Name of compound	RT	Area %	Name of compound	RT	Area %
α-camphene	5.201	0.45	Salvene	8.55	0.37
Sabinene	6.543	0.69	Tricyclene	8.91	3.41
α-pinene	7.854	1.07	α-pinene	9.05	16.80
B-pinene	8.960	2.03	α-thujene	9.23	1.36
Mycrene	9.383	15.67	Camphene	9.30	7.08
Limonene	9.892	8.10	B-pinene	9.56	10.14
α-terpinene	10.781	4.53	Mycrene	10.24	0.83
γ-terpinene	10.940	0.83	Limonene	10.73	2.12
p-cymene	11.561	2.62	γ-Terpinene	11.06	4.68
1,8-Cineol	12.043	11.94	Terpinene	11.31	9.82
α-terpineol	14.015	6.31	α-terpinolene	11.53	20.24
Linalool	14.293	25.50	α-terpineol	13.18	0.43
Thymol	15.927	13.76	Terpinen-4-ol	14.05	5.33
Carvacrol	16.714	2.47	Borneol	14.94	8.79
Terpinolene	18.023	0.13	Thymol	16.02	3.04
Terpinyl acetate	18.641	2.78	Cyperene	18.13	3.20
β-caryophyllene	21.633	0.81	p-Cimenene	19.26	1.06
β-caryophyllene oxide	25.345	0.14	β-caryophyllene	21.43	1.15
cis-Sabinene	26.219	0.16	Carveol	22.05	0.11

Phenolic Compounds:

HPLC analysis of the phenolic compound in the ethanol extract from leaves salvia and thymus (Table 2) showed the presence of 17 and 15 compounds in two plants. The major phenolic acids identified were previously detected in Salvia, p-coumaric acid (37.0%), chlorogenic acid (12.03%), and luteolin-7-O-glucoside (11.15%), with luteolin, rosmarinic acid and caffeic acid. However, in thymus extract p-coumaric acid was the predominant phenolic compound (30.95 %), followed by *p*-vanillin (21.74 %), gallic acid (9.07%) and caffeic acid (8.75%). These results were in agreement with the results Dincer *et al.*, (2013) and Roby*et al* (2013) showed that HPLC analysis of methanolic extract the presence of: rosmarenic acid, methyl rosmarenate, caffeic acid, cinnamic acid, chlorogenic acid and quinic acid as phenolic acids, besides some flavonoids such as ferulic acid, apigenin, luteolin and quercetin.Also, Lu and Foo (2000) showed that the flavonoid and phenolic glycosides from Salvia officinalis. a high content of lycosides cis-p-coumaric acid 4-O-(2'-O-beta-D-apiofuranosyl)-beta-D-glucopyranoside and trans-p-coumaric acid 4-O-(2'-O-beta-D-apiofuranosyl)-beta-D-glucopyranoside. Also, they isolated and identified 4-hydroxyacetophenone 4-O-(6'-O-beta-D-apiofuranosyl)-beta-D-glucopyranoside, luteolin 7-O-beta-D-glucoside, 7- and 3'-O-beta-D-glucuronide, 6-hydroxyluteolin 7-O-beta-D-glucoside and 7-O-glucuronide, and 6,8-di-C-beta-D-glucosylapigenin.

Table 2. Phenolic compounds of Thymus and Salvia of ethanolic extract identified by HPLC analysis

Thymus			Salvia		
Name of compound	RT	Area %	Name of compound	RT	Area %
Gallic acid	4.62	9.07	Pyrogallic acid	3.40	3.29
Hydroquinone	6.11	1.13	Gallic acid	3.87	0.56
Chlorogenic acid	7.32	8.40	Unknown	4.18	0.45
p-Hydroxy benzoic acid	8.14	1.22	Caffeic acid	7.31	0.52
Apigenin-o-pentoside	9.33	0.19	Caffeic acid glucoside	9.03	0.43
Caffeic acid	10.8	8.75	Rosmainic acid	11.04	9.49
Vanillin	11.3	21.74	Unknown	12.27	0.97
Rosmainic acid	11.6	2.35	Apigenindiglucoside	13.73	5.84
p-Coumaric acid	12.73	30.95	Quercitrin	16.53	6.41
m-Coumaric acid	17.92	7.24	Isoquercitin	17.21	0.46
Unknown	19.23	0.25	o-Coumaric acid	18.89	0.94
Ferulic acid	22.01	5.11	p-Coumaric acid	19.81	37.0
Luteolin-7-o-glucoside	25.5	1.24	Salicylic acid	21.22	0.41
Luteolin-diglucuronide	30.81	2.04	Chlorogenic acid	23.05	12.03
Unknown	32.53	0.31	Ferulic acid	25.42	0.70
			Luteolin	27.05	9.35
			Luteolin-7-o-glucoside	28.68	11.15

Antioxidant Activity:

The antioxidant activity of two plants of different extracts at different concentrations (25, 50, 100 and 200 ppm) was evaluated as free radical DPPH scavenging and their results are presented in Tables (3, 4). The results obtained show that the antioxidant activity of essential oil of Thymus and Salvia compared with ethanolic extract depends on which different compounds contain this oil. The results indicated that the DPPH scavenging activities (%) were increased significantly with increasing the concentration from 25 to 200 ppm essential oil (20.21, 36.39, 30.33 and 34.14% Inhibition after 15 min respectively) of thymus. While, 11.18, 12.15, 24.01 and 36.88 respectively of salvia.While, 34.21, 47.30, 65.04 and 76.30 % Inhibition after 30 min respectively of thymus. But, 29.01, 40.32, 44.05 and 62.43respectively, of salvia. The data illustrated in this table revealed that relative percentages inhibition of the DPPH scavenging activity of ethanolic extract,(14.75, 19.87, 27.21 and 30.21after 15 min respectively) of thymus. While, 3.18, 8.04, 18.01 and 25.84 respectively of sage. While, 20.75, 23.87, 36.28 and 41.21after 30 min respectively of thymus. But, 28.54, 27.48, 33.59 and 54.65respectively, of salvia. Therefore, both extract and essential oil can be regarded as possessing moderate and weak antioxidant activities, in comparison to the synthetic BHA and the ethanol extract of sage, according to Kaur and Kapoor (2002).These results with the agreement with Rasmy *et al.*, (2012) found that ethanolic extract were more efficient in scavenging free radicals. Because, it's contained the high level of total phenolic compounds in salvia. Also, Grigore, *et al*., (2010) and Miura, *et al*., (2002) showed that both sample of thymus a good antioxidant capacity, dose dependent, slightly higher for volatile oil fraction.

Table 3: DPPH scavenging activity of essential oil and ethanolic extract of Thymus and Salvia

Concentration (ppm)	(%) Inhibition after 15 min				
		Thyme		Sage	
	Standard (BHT)	essential oil	ethanolic extract	essential oil	ethanolic extract
200	$55.18^{a} \pm 0.07$	$34.14^{b} \pm 0.23$	$30.21^{c} \pm 0.94$	$36.88^{b} \pm 0.47$	$25.84^{cd} \pm 0.14$
100	$39.32^{a} \pm 0.18$	$30.33^{a} \pm 0.31$	$27.28^{b} \pm 0.07$	$24.01^{b} \pm 1.01$	$18.01^{c} \pm 0.05$
50	$22.06^{b} \pm 0.23$	$36.39^{a} \pm 0.08$	$19.87^{b} \pm 0.14$	$12.15^{c} \pm 0.03$	$8.04^{d} \pm 0.03$
25	$12.01^{c} \pm 0.11$	$20.21^{a} \pm 1.01$	$14.75^{b} \pm 1.07$	$11.18^{c} \pm 0.27$	$3.18^{d} \pm 0.27$

Each value in the table was obtained by calculating average of three experiments a standard deviation , each different letters mean significant differences (p<0.05).

Table 4: DPPH scavenging activity of essential oil and ethanolic extract of Thymus and Salvia

Concentration (ppm)	(%) Inhibition after 30 min				
		Thymus		Salvia	
	Standard (BHT)	essential oil	ethanolic extract	essential oil	ethanolic extract
200	$72.33^{a} \pm 0.43$	$76.30^{a} \pm 0.38$	$41.21^{c} \pm 0.26$	$62.43^{b} \pm 0.11$	$54.65^{b} \pm 1.21$
100	$47.10^{b} \pm 0.28$	$65.04^{a} \pm 0.21$	$36.28^{c} \pm 0.07$	$44.05^{b} \pm 1.01$	$33.59^{c} \pm 0.01$
50	$37.15^{b} \pm 0.04$	$47.30^{a} \pm 1.25$	$23.87^{c} \pm 0.14$	$40.32^{a} \pm 0.47$	$27.48^{c} \pm 0.36$
25	$26.37^{b} \pm 0.06$	$34.21^{a} \pm 0.09$	$20.75^{c} \pm 1.07$	$29.01^{b} \pm 0.14$	$28.54^{b} \pm 1.28$

Each value in the table was obtained by calculating average of three experiments a standard deviation, each row different letters mean significant differences ($p<0.05$).

Effect of Plant Extracts on 4th instar Larvae of *S. littoralis:*

The experiments included the percentage of larval mortality, malformed larvae, pupation and malformed pupae. Also, percentages of adult emergence, malformed adult and egg hatchability.

Table 5: latent biological effect of *S. officinalis* (leaves) on developmental stages of *S. littoralis*

	Conc. (ppm)	Larval mortality (%)	Malformed Larvae (%)	Pupation (%)	Malformed Pupae (%)	Adult Emergence (%)	Malformed Adult (%)	Egg Hatchability (%)
ethanolic extract	200	78.6±4.5b	0±0.0c	20.8±0.0e	0±0.0c	0±0.0d	0±0.0c	13.3±2.4d
	100	85.3±0.1b	0±0.0c	14.7±4.3d	11.6±3.3b	3.1±0.1d	0±0.0c	7.7±4.3c
	50	27.1±0.1c	10.3±5.1b	62.6±2.7c	23.7±5.1a	38.9±0.2c	31.5±0.2a	8.6±4.3b
	25	6.8±0.3d	15.8±2.2a	77.4±5.2b	21.6±6.3a	55.8±0.6b	18.4±0.36b	12.5±6.3b
	Control	2.1±0.15d	0±0.0c	97.9±3.4a	0±0.0c	100±0.0a	0±0.0c	95.7±5.2a
	F. value	248.1	34.9	556.9	30.6	206.32	309.72	315.8
	LSD 5%	9.1	4.1	5.7	6.7	9.36	2.7	7.5
essential oil	200	32.3±0.17a	31.6±0.3c	36.1±0.35e	18.5±4.4d	17.6±2.4b	0±0.0b	0±0.0d
	100	25.5±0.32b	30.4±0.21c	44.1±0.3d	25.6±5.35c	18.5±4.5b	0±0.0b	0±0.0d
	50	0±0.0c	42.7±0.2b	57.3±0.5b	43.0±4.31a	14.3±4.3bc	5.1±0.35a	24.1±5.5b
	25	0±0.0c	50.3±0.3a	49.7±0.5c	37.5±2.5b	12.3±4.3c	4.5±0.4a	14.5±4.2c
	Control	0±0.0c	0±0.0d	100±0.0a	0±0.0e	98.7±2.2a	0±0.0b	97.5±3.4a
	F. value	640.8	896.259	690.9	232.6	599.1	308.739	336.7
	LSD 5%	2.1	2.1	3.1	3.6	4.9	0.5	7.3

Means within the same column that have the same letters are not significantly different ($P<0.001$)using Least Significant Differences LSD

Effect of Age Leaves Extracts against *S. littoralis:*

Data in (Table 5) indicated the biological studies of ethanolic extract of sage leaves against the 4th larvae of *S.littoralis* difference were difference significant between larval mortality%, malformed larvae%, pupation% and malformed pupae%. Also, percentages of adult emergence, malformed adult%, egg hatchability% compared with control, there was also are markable increase in the concentrations as it was 100ppm to become a percentage of larval mortality 85.3% comparing with control larvae. However, low concentration (25ppm) gave the highest percentage of malformation of larvae 15.8%. These results showed that pupation percentage was 20.8 , 14.7%, 62.6% and 77.4% at concentrations, 200, 100, 50 and 25ppm, respectively, compared with 97.9% control, while malformation of pupae was 0, 11.6% 23.7% and 21.6 at the same concentrations, comparing to zero of control. Data indicated that the percentage of adult emergence recorded 0, 3.1%, 38.9% and 55.8% for 200, 100, 50 and 25 ppm, respectively, compared with 100% of control. While the concentrations, 50 and 25ppm gave percentage of malformed adult 31.5% and 18.4%, respect. In addition, egg hatchability recorded 8.6% and 12.5% at previous same concentrations.

Data presented in (Table 5) show that the efficacy of essential oil of *S.officinalis* leaves against the 4th instar larvae of *S.littoralis*, there was a significant difference between percentages of larvae mortality which was treated with different concentrations of essential oil compared with control. The increasing of the concentrations,200 ppm and

100 ppm recorded (32.3% and 25.5%) as larval mortality comparing to 2.1% of control larvae, while the concentrations, 50 ppm and 25ppm caused insignificant difference compared with the control of larval mortality. The essential oil was more effective on malformation of larvae (Fig 1) and caused 31.6, 30.4, 42.7 and 50.3% at concentrations, 200, 100, 50 and 25ppm, respectively.(low concentration gave highest percentage of malformed larvae).

The results showed that pupation percentages of *S.littoralis* treated with essential oil of *S.officinalis* were significantly different among the concentrations, where pupation percentages of *S.littoralis* recorded 36.1%, 44.1%, 57.3% and 49.7% at concentrations,200, 100, 50 and 25ppm .respectively ,compared with control 100%, while malformation percentage of pupae was 18.5%, 25.6%, 43% and 37.5% at the same concentrations. The data showed that adult emergence percentage of *S.littoralis* was 17.6, 18.5, 14.3 and 12.3% at previous concentrations, compared with control 98.7%. But malformation percentage of adults was 5.1% and 4.5% at concentrations, 50 and 25 ppm. Also egg hatchability (%) recorded 24.1 and 14.5% at concentrations, 50 and 25 ppm. In conclusion, it was found that ethanolic extract of *S.officinalis* was more effective than essential oil on different stages of *S.littoralis*.

T. vulgaris* (leaves) Extracts against *S. littoralis:

Effect of *T. vulgaris* (leaves) extracts on some biological aspects of *S. Littoralis***,** the results in (Table 6) indicated the toxicological activity of ethanolic extract of *T. vulgaris* leaves against the larvae of *S.littoralis*.The results clarified that it is significant between percentage of larvae mortality, malformation of larvae%, pupation%, malformation of pupae%, the emergence of adults % malformation of Adults% and egg hatchability(%) compared with control (Fig.2). Percentage of larval mortality was96.3%, 72.2%, 44.7% and 16.4% at concentrations, 200, 100, 50 and 25 ppm, respectively, comparing to 2.1% of control, where malformation of larvae % recorded 0, 11.6%, 31.1% and 43.6% at the same concentrations. These results showed that the lowest pupation percentage recorded by 3.7% at conc. 200ppm, comparing to 97.9% of control, while malformation of pupae recorded 0, 8.4% 12.7% and 17.5 at conc. 200, 100, 50 and 25ppm, respectively. Data indicated that percentage of adult emergence was 0, 7.8%, 11.5% and 22.5% at concentrations, 200, 100, 50 and 25ppm, respectively, comparing to 100% of control, where percentage of malformed adult recorded 0, 3.1%, 6.7% and 18.9%at the same concentrations. Egg hatchability (%) was 12.4% and 5.7% at conc., 100 and 50 ppm, respectively.

Table 6: latent biological effect of *T. vulgaris* (leaves) on developmental stages of *S. littoralis*

	Conc. (ppm)	Larval mortality (%)	Malformed Larvae (%)	Pupation (%)	Malformed Pupae (%)	Adult Emergence (%)	Malformed Adult (%)	Egg Hatchability (%)
Ethanolic extract	200	96.3±3.3a	0±0.0d	3.7±2.1e	0±0.0d	0±0.0d	0±0.0d	0±0.0d
	100	72.2±2.2b	11.6±3.3c	16.2±5.2d	8.4±3.1c	7.8±4.3c	3.1±2.1c	12.4±4.4b
	50	44.7±2.2c	31.1±4.8b	24.2±3.4c	12.7±4.7b	11.5±5.4c	6.7±2.2b	5.7±3.5c
	25	16.4±3.4d	43.6±3.2a	40.0±4.1b	17.5±3.2a	22.5±3.3b	18.9±3.4a	0±0.0d
	Control	2.1±1.1e	0±0.0d	97.9±3.8a	0±0.0d	100±0.0a	0±0.0d	95.7±5.2a
	F. value	645	147.4	273	52	820.5	94.1	1032.1
	LSD 5%	1.57	5.22	7.2	3.5	4.6	2.5	4.2
essential oil	200	31.6±0.2a	30.9±5.7c	37.5±7.2d	0±0.0c	15.7±5.3cd	14.7±0.1a	0±0.0d
	100	13.8±0.6c	47.2±4.2a	39.0±4.4cd	0±0.0c	12.9±5.2d	15.7±0.2b	0±0.0d
	50	15.3±0.3b	39.5±4.3b	45.2±5.6bc	27.1±0.1a	18.1±10.1c	0±0.0c	10.8±2.4c
	25	0±0.0d	48.3±7.5a	51.7±10.5b	19.1±0.3b	32.6±4.2b	21.4±0.3a	26.7±6.7b
	Control	0±0.0d	0±0.0d	100±0.0a	0±0.0c	98.7±3.3a	0±0.0c	97.5±3.5a
	F. value	421.8	188.9	148	243.4	577	306.9	640
	LSD 5%	2.1	4.7	6.9	2.7	4.7	2.1	5.2

Means within the same column that have the same letters are not significantly different ($P<0.001$) using Least Significant Differences LSD.

Data presented in (Table 6) show the efficacy of essential oil of *T. vulgaris* leaves against the 4th instar larvae of *S.littoralis*, there was significant between percentage of larvae mortality, malformation of larvae%, pupation%, malformation of pupae%, emergence of adults% malformation of Adults% and egg hatchability (%) compared with control (Fig.3). The percentage of larval mortality recorded 31.6%, 13.8, 15.3 and 0%, at concentrations, 200, 100, 50 and 25ppm, respectively. The oil extracts were more effective on malformation of larvae, malformation of larvae percentage was 30.9, 47.2, 39.5 and 48.3% at the same concentrations, therefore low concentration gave the highest percentage of malformed larvae.

The results showed that pupation percentages of *S.littoralis* treated with essential oil of *T. vulgaris* were significant among the concentrations, where pupation percentages of *S.littoralis* was 37.5, 39.0, 45.2 and 51.7 at concentrations,200, 100, 50 and 25ppm, respect compared with control 100%, but malformation percentage of pupae recorded 27.1 and 19.1 only at the concentrations, 50 and 25ppm.

The data showed that adult emergence percentage of *S.littoralis* was 15.7, 12.9, 18.1 and 32.6at concentrations, 200, 100, 50 and 25ppm, respectively compared with control 98.7%. but malformation percentage of adults recorded 23.1, 15.7, 0 and 21.4% at the same concentrations, Also egg hatchability (%) was 10.8and 26.7%at concentrations, 50 and 25 ppm. In conclusion, it was found that ethanolic extract of *T. vulgaris* was more effective than essential oil different stages of *S.littoralis*.

The foregoing results indicate that the tested plant extracts have properties which cause feeding deterrence, larval mortality, retardation in the developmental stages, pupal and adult morphogenesis, reduction in fecundity and viability of *S. liioralis* and persistent on cotton plants and this may be correlated to the chemical constituents of these plants under studies.

These results are in agreement with those reported by Abdel Fattah *et al.,* (2009) revealed that the botanical volatile oils used, had morphogenic effects against *S. nudiseta* stages. These include larval-pupal intermediates, pupal-adult intermediates, deformed adults with crumpled wings and/ or deformed thorax and abdomen. Also some adults couldn't emerge and remained in their puparia. Also, Sahayaraj, *et al.,* (2010) proofed that plant extracts extended the larval, pupal and adult periods; reduced the pupal weight, pupal and adult emergence and caused larval–pupal deformities. In addition to there is a significant reduction in adult emergence of *S.littoralis* as compared with control as a result of using wild plant extracts by Shadia and Azza. (2007).

It is evident that these chemicals as constituents of the tested plants have properties which inhibit feeding and cause retardation in the larval development, pupal and adult morphogenesis of *S. littoralis*.

Therefore, it can be concluded that, *S.officinalis* and *T. vulgaris* extracts were effective in suppressing the population size of *S. littoralis* either directly through their acute toxic effects on the larvae and egg masses or indirectly through their delayed effects on the pupae and adults and minimizing the cotton infestation by the cotton leafworm *S. littoralis* at the vegetative growth stage.

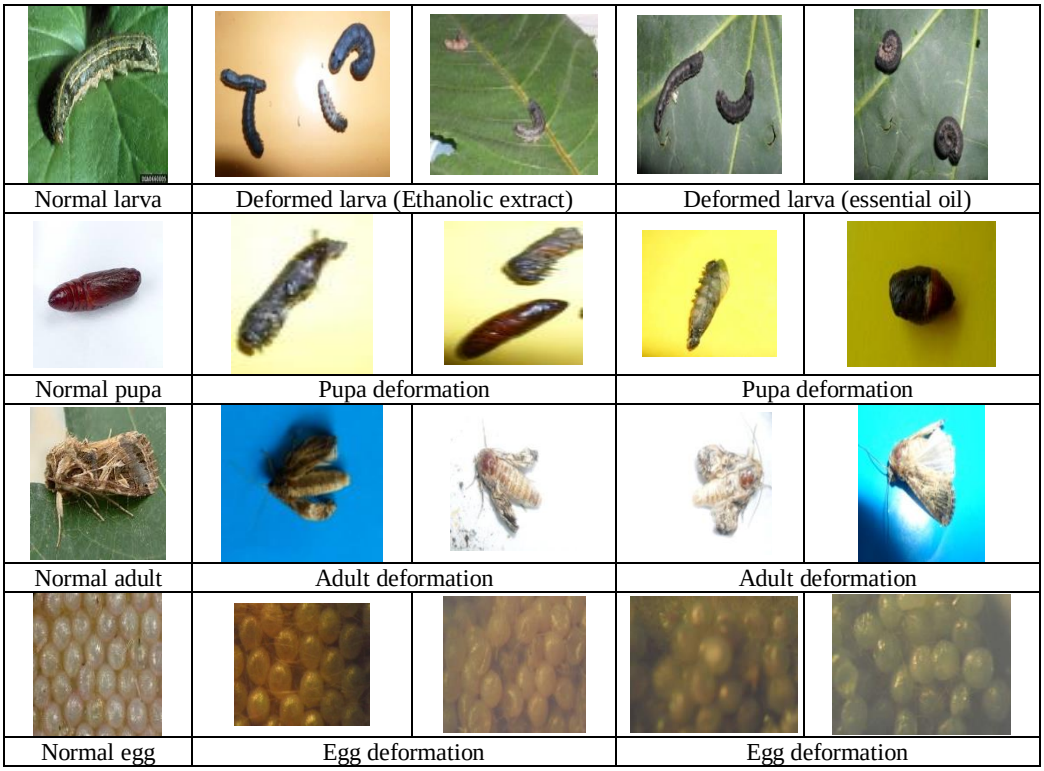

Fig. 1: Normal and abnormal of different stages of *S. littoralis* treated with *S.officinalis* extracts (Ethanolic and essential oil)

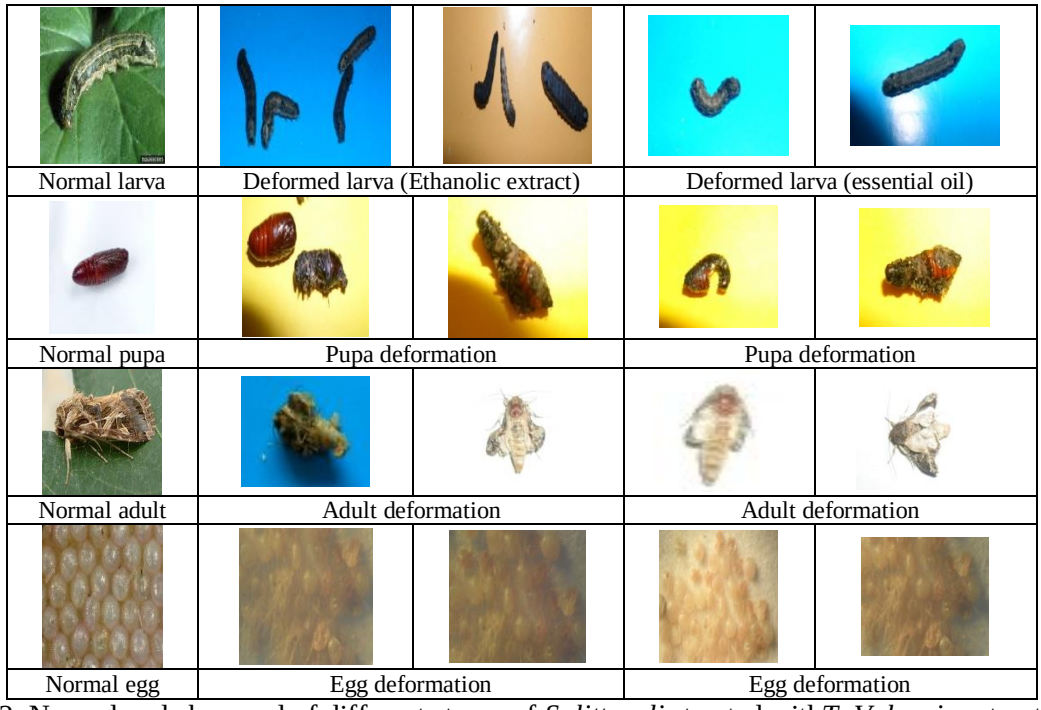

Fig. 2: Normal and abnormal of different stages of *S. littoralis* treated with*T. Vulgaris* extracts (Ethanolic and essential oil)

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ARABIC SUMMARY

دراسات علي النشاط المضاد للاكسده و الاقتدار الحيوي لاثنين من المستخلصات النباتيه الطبيه علي دوده ورق القطن

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تتحقق الاداره المتكامله لمكافحه الاصابه بدوده ورق القطن باستعمال المبيدات الكيمياويه و ذلك بطرق صديقه للبيئه بدون التاثيرات الجانبيه الغير مرغوبه لكي تصبح هذه المركبات الكيماويه ذات اهميه في استراتيجيه و خطه المكافحه المتكامله للافات. المركبات الطبيعية بما في ذلك المستخلصات النباتية والزيوت العطريه هي بعض الطرق البديلة في مكافحة الآفات. تم استخلاص الزيوت العطرية وتم التعرف على تركيبتها الكيميائية باستخدام مقياس الطيف GC/MS ولذلك ، تم تصميم هذه الدراسة لتقييم نشاط مضادات الأكسدة من مستخلصات مختلفة من نباتات المريمية والزعتر بواسطة طريقة (DPPH) بواسطة 2 ، 2-ثنائي فينيل 1-بيكريل هيدرازيل. وقد أظهرت النتائج التي تم الحصول عليها أن التركيزات عند 25 ، 50 ، 100 و 200 ميكروجرام / مل من الزيوت العطريه والمستخلص الايثانولي من كلا النباتين كانت أكثر كفاءة في ازاله الشقوق الحرة بعد 30 دقيقة مقارنة مع تولوين هيدروكسيل (BHT) ككنترول. كانت التركيزات 100 و 200 ميكروجرام / مل من الإيثانول والزيوت العطريه لها نشاط أعلى من المستخلصات الأخرى . وعلاوة على ذلك ، تم دراسة مستخلصات المريميه والزعتر لسميتها ضد دودة ورق القطن في المعمل، و كانت النسب المئوية للموت التراكمى لليرقات وكذلك التاثير المتاخر للعمر الرابع من تركيزات 25 ، 50 ، 100 و 200 ميكروجرام / مل . وقد وجد أن المستخلص الإيثانولي لنباتين كانا أكثر تأثيرا من مستخلص الزيت في المراحل المختلفة لدودة ورق القطن. وأظهرت النتائج أن هناك فروق معنويه عاليه بين جميع المعاملات والتحكم في بعض الجوانب البيولوجية.