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Opportunity for Common Sage, *Salvia officinalis* (L) Essential Oil as a Natural Biocide against Cotton Leafworm, *Spodoptera littoralis* (Boisd.)

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Abstract Within the framework of diligent and continuous research for alternative tools and botanical products to control *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). *Salvia officinalis* (L.) (Lamiales: Lamiaceae) essential oil was assayed as an ovicidal and larvicidal. Chemical analysis of *S. officinalis* oil using GC-MS analysis showed monoterpenoids 1.8-Cineole (61.80%) and Camphor (17.18%) followed by monoterpenes Camphene (5.64%), α-Pinene (5.18%) and 2-β-Pinene (2.86%). Generally, the results of toxicity tests indicated that 1st instar larvae are more susceptible than 3rd instar one, LC_{50} and LC_{90} values were (1.963, 5.993 ppm) and (4.555, 26.073 ppm), respectively. On the other hand, treating three days old eggs possessed LC_{50} and LC_{90} 21.152, 396.892 ppm respectively. Biological results revealed that the oil elongated the larval duration, reduced pupal weight and increased pupal mortality than control. Meanwhile, the tested oil caused a reduction in AchE and increased LDH enzymes as compared to control.

Keywords *Spodoptera littoralis*, *Salvia officinalis*, ovicide, larvicide, enzymes

Introduction

Female adult of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) lays between 2300 to 2700 eggs [1]. Resulting larvae are very destructive attack the foliage of various economic crops such as cotton, clover, vegetables and fruits. Such polyphagous specimen causes enormous economic losses each year [2]. This pest built up resistance to several traditional insecticides which are commonly used in fields and bring about such serious problems as contamination of the environment and lethal effects in non-targed organisms. It also resulting in residues accumulation in different environmental components [3] and human health [4]. Therefore, efforts are being to find alternative methods to control pests. Plants may provide a potential option to currently use in insect control because it constitutes a rich source of bioactive chemicals. Plant essential oils have been receiving global attention and considered as potential alternatives to traditional insecticides (green pesticides) that can reduce the pest population and increase crop production have posted as alternative methods to synthetic insecticides.

Generally, essential oils, abundant in aromatic plant families as Lamiaceae are easily obtained and contain many bioactive compounds characterizing as insecticidal activities [5]. Leaves of Common Sage, *Salvia officinalis* L., and its essential oil possess antispasmodic, antiseptic, carminative, astringent and antihidrotic properties [6] and have antibacterial [7] and antifungal properties [8].

Common Sage, *Saliva officinalis* (Lamiaceae) derived its name from the Latin "Salvere" that means "to heal", regarded for its healing qualities. Common sage is well known in the Middle East in traditional medicine by possessing antimicrobial, antiflammatory, antiscabies and antisyphilitic properties [9, 10]. Many researchers

highlighted the action of its essential oil [EO] against different insects as: antifeedant, larvicidal, growth inhibitory, oviposition, deterrent and fertility reducer [11, 12].

In light of these information, the present investigation was designed to identify the chemical constituents of *S. officinalis* essential oil and to test their insecticidal action against *S. littoralis*.

Materials and Methods

1. Plant materials and isolation of its essential oil

The essential oil was extracted from the leaves of Common stage, *Saliva officinalis* of the family Lamiaceae. About 250 gm of *S. officinalis* leaves were bought from the local market, Sharquia Governorate, Egypt, (30°34′00″N and 31°30′00″E). The essential oil was extracted using a Clevenger-tube apparatus (Marcus and Lichtenstein 1979), where the Common stage leaves were subjected to hydro-distillation for 24 hours. The *S. officinalis* oil was separated, dried over anhydrous sodium sulfate to remove water after extraction and stored in dark glass bottles at 4 °C in a refrigerator until used. The isolated oil is a pale yellow liquid with a distinguished odor and taste of *S. officinalis.*

2. GC-MS analysis of the essential oil

The essential oil was analyzed on Gas Chromatography Mass Spectrometry (GC-Ms) HP 6890 Series A (Agilent) at National Research Center, Giza, Egypt. The constituents of oil were identified using computer matching and comparing the fragmentation patterns of their masses with those listed by [13].

3. Rearing techniques of *Spodoptera littoralis* larvae

A laboratory strain of cotton leafworm, *S. littoralis* were reared in Plant Protection Research Institute, Zagazig, Egypt, under constant conditions of 27±1 °C and 65±5 % R.H. % according to [14].

4. Ovicidal action of *S. officinalis* oil

Three days-old egg masses of *S. littoralis* were used in this study. Five concentrations of *S. officinalis* oil were prepared using ethyl alcohol (95%) as a solvent (0.625, 1.25, 2.5, 5.00 and 10.00%) (v/v). Five egg masses were used for each tested concentration of *S. officinalis* oil. Control egg masses were dipped in ethyl alcohol (95%) only (dipped for 10 seconds). The treated egg-masses were left to dry in the air, and then transferred to Petri dishes, (Five egg-masses/ dish). Daily inspection for all treatments was performed until the untreated eggs hatched. The mortality percentages of egg-masses were recorded as average mortality percentages of each tested concentration using Abbott's formula [15].

5. Larvicidal action of *S. officinalis*

To study the larvicidal action of *S. officinalis* oil against both newly hatched larvae (1st instar larvae) and 3rd instar larvae of *S. littoralis*, the same precedent tested concentrations of the essential oil and control were prepared. Leaf discs (three cm diameter) of the fresh castor bean leaves were bunched with a cork borer and dipped in the tested concentrations for 10 seconds then left to dry, (leaf dip technique).

Larvae were transferred to the treated and non treated leaves, each tested concentration and control were represented by five replicates (20 larvae/ replicate). The larvae were allowed to feed on treated disks for 48 hours then on untreated ones. Mortality was recorded after 72 hours of treatment. Mortality percentages were corrected according to [15] to estimate the LC values.

6. Latent effects of *S. officinalis* essential oil on the 4th instar larvae of *S. littoralis*

Three replicates were used for each precedent used concentrations of essential oil and control (20 larvae/ replicate). The tested larvae were starved for 3-4 hours [16], then transferred into treated and non-treated disks (control). The disks were changed after 48 hours with fresh leaves. Larvae were checked daily until pupation under laboratory

conditions. Larval duration, prepupal mortality and pupal weight have represented the parameters of long-term bioactivity of *S. officinalis* essential oil.

7. Biochemical assay

7.1. Preparation of samples

The preparation of samples involved the use of 3rd instar larvae of *S. littoralis* after 72 hours of treatment with LC_{50} of *S. officinalis* oil and control. The healthy larvae were picked up and placed in clean jars, then starved for 4 hr. Five milligrams of treatment and control were homogenized in distilled water using a chilled glass Teflon tissue homogenizer (ST-2 Mechanic-Preczyina, Poland) surrounded by a jacket of crushed ice for three minutes. The homogenates were centrifuged at 8000 r.p.m for 15 minutes at 5°C in a refrigerated microcentrifuge to remove haemocytes.

The supernatants were transferred to clean tubes and stored in the freezer at -20°C until used. Three replicated were used for each biochemical assay for measuring the absorbance of colored substances or metabolic compounds, double beam ultraviolet/ visible spectrophotometer (Spectronic 1201, Milton Roy Co., USA).

7.2. Total protein assessment

Total protein concentration was estimated according to [17] using bovine serum albumin as a standard. Protein reagent was set by dissolving 100 mg of coomassie Brilliant blue G-250 (SIGMA) in 50 ml 95% ethanol. 100 ml of phosphoric acid 85% (w/v) were added to their solution. The resulting solution was diluted to obtain a final volume of 1 liter.

7.3. Determination of enzyme activities

7.3.1. Acetyl choline esterase (EC 3.1.1.7)

AChE activity was measured according to the method described by [18] using acetylcholine bromide (AchBr) as a substrate. The reaction mixture contained 200 µl enzyme solution, 0.5 ml of 0.1µ phosphate buffer (pH 7.0) and 0. 5 ml of 3 mM (AchBr).

7.3.2. Lactate dehydrogenase (LDH) (EC 1.1.1.27)

Lactate dehydrogenase (LDH) catalyzes the conversion of pyruvate to lactate. The rate of reduce in NADH is directly proportional to LDH activity that is determined photometrically at 340 nm according to [19] method. LDH catalyzed the reduction of pyruvate by NADH. The rate of reduction in the concentration of NADH, considered photometrically proportional to the catalytic concentration of LDH present in the sample.

8. Statistical analysis

Using the computed percentage of mortalities versus corresponding concentrations, Probit analysis was adopted according to [20]. This yields the toxicity indices (LC_{50} and LC_{90}) as well as the related parameters (slope, and chi-square, χ^2) for established toxicity regression lines. The toxicity index calculated according to [21].

The biological results were subjected to one-way analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range test ($p < 0.05$) [22]. Data were subjected to statistical analyses using the software package Costat® Statistical Software [23] a product of Cohort Software, Monterey, California, USA.

The biochemical data are presented as mean + SE and the statistical significance was analyzed using Student's 't' test, $P<0.05$ was considered significant [22].

Results

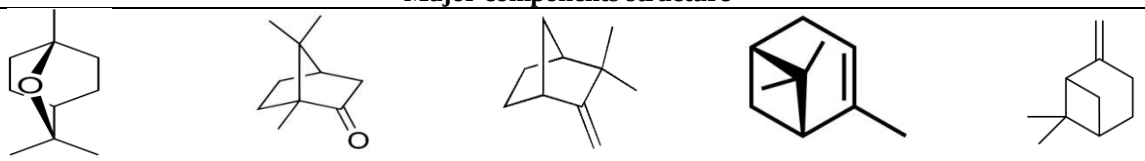
1. Chemical characterization of *S. officinalis* essential oil

The chemical composition of the leaves of *S. officinalis* was shown in Table (1) and Fig. (1). Twenty eight compounds were identified representing 99.92% of the total essential oil using GC-Ms. The main componenets were oxygenated monoterpenes or monoterpenoids 1.8-Cineole (61.80%) and Camphor (17.18%) followed by monoterpenes Camphene (5.64%), α-Pinene (5.18%) and 2-β-Pinene (2.86%).

Table 1: Chemical composition of essential oil of *S. officinalis* leaves

No.	Chemical compounds	Molecular formula	Molecular weight	Retintion time	Area %
1	Cis-Salvene	C_9H_{16}	124	6.69	0.04
2	Tricyclene	$C_{10}H_{16}$	136	7.25	0.33
3	α-Pinene	$C_{10}H_{16}$	136	7.57	5.18
4	Camphene	$C_{10}H_{16}$	136	8.16	5.64
5	2-β-Pinene	$C_{10}H_{16}$	136	9.09	2.86
6	β-Myrcene	$C_{10}H_{16}$	136	9.44	0.31
7	Sabinene	$C_{10}H_{16}$	136	10.02	0.09
8	α-Phellandrene	$C_{10}H_{16}$	136	10.12	0.11
9	α-Caryophyllene	$C_{15}H_{24}$	204	10.48	0.34
10	L-Limonene	$C_{10}H_{16}$	136	10.95	1.00
11	1,8-Cineole	$C_{10}H_{18}O$	154	11.10	61.80
12	Cis-sabinene hydrate	$C_{10}H_{18}O$	154	11.58	0.03
13	trans-β-Ocimene	$C_{10}H_{16}$	136	12.09	0.13
14	Alloocimene	$C_{10}H_{16}$	136	13.15	0.26
15	α-Thujone	$C_{10}H_{16}O$	152	14.13	0.60
16	3-Thujanone	$C_{10}H_{16}O$	152	14.61	0.21
17	(-)-Camphor	$C_{10}H_{16}O$	152	15.88	17.18
18	Isopinocamphone	$C_{10}H_{16}O$	152	16.38	0.31
19	Borneol	$C_{10}H_{18}O$	154	16.85	1.22
20	Myrtenal	$C_{10}H_{16}O$	152	17.27	0.04
21	(+)-α-Terpineol	$C_{10}H_{18}O$	154	17.95	0.18
22	l-Bornyl acetate	$C_{12}H_{20}O_2$	196	21.09	0.71
23	α-terpenyl acetate	$C_{12}H_{20}O_2$	196	23.62	0.19
24	α-ylangene	$C_{15}H_{24}$	204	25.67	0.06
25	α-humulene	$C_{15}H_{24}$	204	26.21	0.66
26	Alloaromadendrene	$C_{15}H_{24}$	204	26.93	0.07
27	Viridiflorol	$C_{15}H_{26}O$	222	27.60	0.24
28	Ledene	$C_{15}H_{24}$	204	28.86	0.13
Total %		99.92%			

Major components structure



61.80% 1,8-Cineole | 17.18% Camphor | 5.64% Camphene | 5.18% α-Pinene | 2.86% 2-β-Pinene

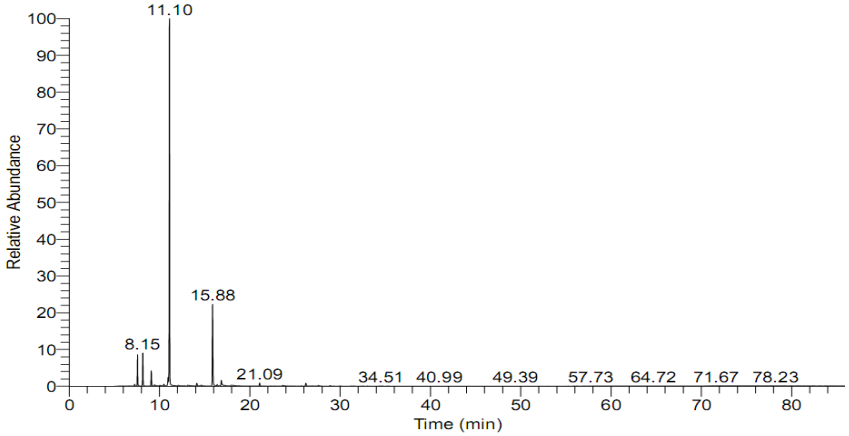


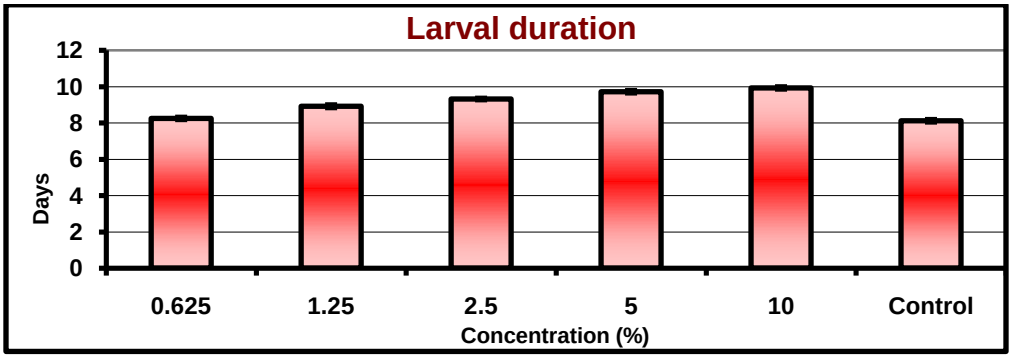
Figure 1: Gas chromatography profile of the essential oil of S. officinalis leaves

2. Susceptibility of certain stages of *S. littoralis* to the essential oil of *S. officinalis*

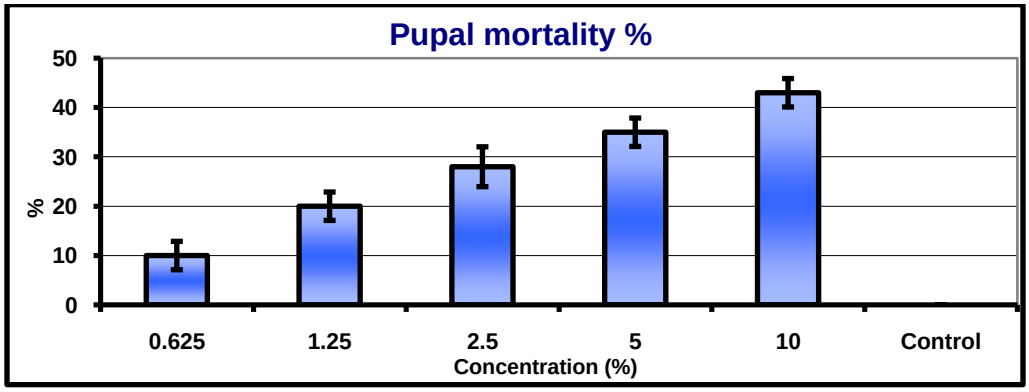
Essential oil (EO) of *S. officinalis* leaves possess toxicity effect against both eggs and larvae of *S. littoralis*. Based on LC values, EO revealed that LC_{50} and LC_{90} values of three days-old eggs, 1st and 3rd instar larvae were (21.152, 396.892 %), (1.963, 5.993 %) and (4.555, 26.073 %), respectively, Table (2).

Table 2: Susceptibility of different stages of *S. littoralis* the tested oil

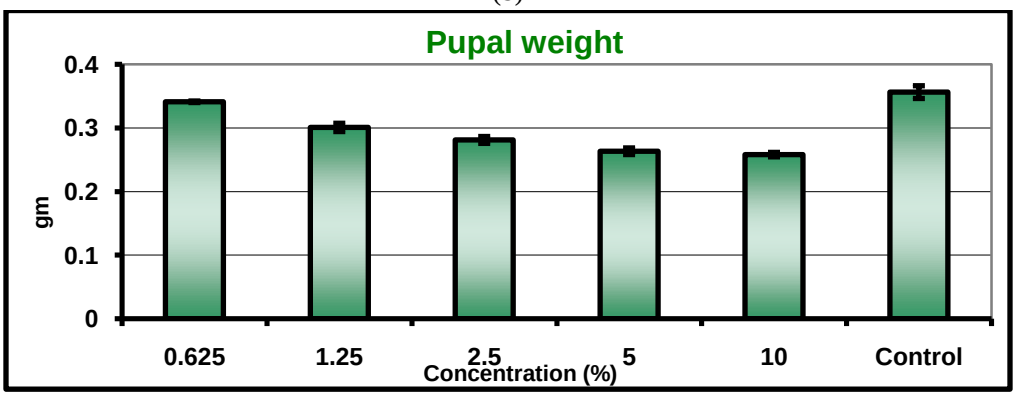
Stage	LC_{50} (Lower-Upper)	LC_{90} (Lower-Upper)	Chi square ($\chi 2$)	Slope±SE	Toxicity index
3 days-old eggs	21.152 (12.96-50.24)	396.892 (101.58-2194.99)	1.20	1.117± 0.175	9.28
1st instar larvae	1.963 (1.26-2.91)	5.993 (4.69-13.27)	11.19	2.644± 0.197	100.00
3rd instar larvae	4.555 (3.83-5.57	26.073 (17.99-44.08)	1.82	1.691± 0.161	43.09



(a)



(b)



(c)

Figure 2: Changes in the measured biological attributes

3. Bioactivity of *S. officinalis* essential oil

Essential oil induced a disruption in the growth of treated 4th instar larvae of *S. littoralis* compared to control including prolongation in the larval stage, reduction in the pupal weight, as well as prepupal mortality.

The lowest used concentration (0.625 %) led to the lowest significant larval duration of 8.25±0.060 days to the highest significant duration 9.93±0.061 day at 10.00 % (highest concentration). Control lasted 8.12±.0067 days, P=0.0001, Fig. (2a).

Generally, the lethal prepupal values were directly related with increasing tested oil concentration. The mortality percentages were recorded 10.00±2.88, 20.00±2.89, 28.00±4.04, 35.00±2.87 and 43.00±2.85% for 0.625, 1.25, 2.50, 5.00 and 10.00 %, respectively, whereas control did not give any pupal mortality, P=0.000, Fig. (2b).

The reduction in pupal weight becomes more evident by increasing the used oil concentrations that ranged between the lowest significant weight 0.3412±0.012 gm after treated with the lowest concentration (0.0625 %) to the maximum significant reduction 0.2581±0.003 gm at the highest concentration (10 %). Control pupae gave 0.3564±0.011 gm, P=0.0000, Fig. (2c).

4. Biochemical responses

The changes in AchE and LDH enzyme activities on 3rd instar larvae of *S. littoralis* a response of treatment with LC_{50} of oil as well as control (using ethyl alcohol only) after 72 hours of treatment were detected.

4.1. Acetylcholin esterase (AchE)

AchE activity showed a significant decline after treatment with the essential oil of *S. officinalis* (1.84±0.0416 µg Ach Br/ minutes/ mg protein) as compared to control (2.05± 0.055µg Ach Br/ minutes/ mg protein), P=0.0391, Table (3).

4.2. Lactate dehydrogenase (LDH)

The Common Sage essential oil caused a significant elevation in LDH activity (20.67±0.981 $Ux10^3$/ mg protein) than control (16.12±0.494 $Ux10^3$/mg protein), P=0.0144, (Table, 3)

Table 3: Changes in some biochemical parameters in *S. littoralis* larvae treated with common sage, *S. officinalis* essential oil

Treatments	Acetyl choline esterase (AchE) µg AchBr/ min/ mg protein	Lactate dehydrogenase (LDH) U X 10^3 mg protein
S. officinalis	1.84±0.04	26.67±0.98
Control	2.05± 0.05	16.12±0.49
P	0.0391	0.0144

Notes: Each datum represents the mean of three replicates.

-Data expressed as Mean ± Standard Error (SE). Significance different ($P < 0.05$), highly significant ($P < 0.01$).

-Treated larvae at level of LC_{50} of *S. officinalis* essential oil.

Discussion

In the present study, Egyptian samples of the essential oil (EO) of *S. officinalis* leaves contain 28 chemical compounds using GC-MS. The major constituents descried as follows: 1.8 cineole (61.80%), camphor (17.18%), comphene (5.64%), α-pinene (5.18%) and 2-β-pinene (2.86%). The EO of *S. officinalis* leaves from the Jordan samples showed that 1, 8- cineole (39.50-50.30%) camphor (8.80-2.5%, α-thujone (1.2-3.7%) and β-thujone (0.1-3.1%) were the basic components [24]. Whereas, in Tunisia, the major constituents were β-Thujone (20.1%), 1.8 cineole (15.91%), camphor (14.79%) and viridiflorol (9.06%) [12].

Generally, the composition of *S. officinalis* EO is highly influenced by organ, environmental and genetic factors, climate conditions and the extraction methods. These changes in the chemical profile of EO related to changing in the number of chemical compounds and stereochemical types of molecules extracted [25].

The essential oil of *S. officinalis* leaves possess toxicity as well as biological and biochemical activities against *S. littoralis*. These properties displayed by the tested oil against *S. littoralis* seems to be related to its major constituents detected in the essential oil principally terpenes such as 1.8 cineole, camphor, camphene, α-pinene and β-pinene.

The most occurrence terpenes in EO are monoterpenes ($C_{10}H_{16}$) which recorded (14.91%). However, monoterpenoids ($C_{10}H_{16}O$ and $C_{10}H_{18}O$) were represented by (81.57%) of essential oil. [26] found that terpenoids are the major source of insecticidal substances, which perform protection against insects in the plants, demonstrating a good insecticidal performance in the experimental methods. Previous reports regarding the insecticidal activity of both 1.8 cineole and camphor against different pests [27, 28].

According to LC_{50} and LC_{90} data showed that the insecticidal activity of this oil against both 1st and 3rd instar larvae of *S. littoralis* more potent than its ovicidal activity, that's maybe attributed with its mode of action. Therefore, *S. officinalis* leaves maybe act as stomach poison than contact action. Generally, terpenes had different toxicity on eggs and larvae with different effects on growth and development. [29] reported that the insecticidal activity of *S. officinalis* might be due to the presence of terpenes and sesquiterpenes. The active components are 1.8 cinieole, camphor, camphene, α-pinene, β-pinene, myrcene, limonene and borneol that have a high toxic effect.

All the tested concentrations of *S. officinalis* had biological activities against 4th instar larvae of *S. littoralis* represented as significant larval duration, the mortality of prepupal period and pupal weight as compared to control. Generally, in all cases, increased biological effects were directly associated with increased oil concentrations. That's could be due to the toxic components of this essential oil. In general, the essential oils are also known to reduce the growth of insects and act as antifeedants and moulting inhibitors [30].

Several EO_S and their constituents have properties similar to juvenile hormone and act as insect growth regulators, disrupting growth [31]. Additionally, monoterpenoids are lipophilic that have fast penetration properties into insects that consequently interfere with biochemical and physiological functions [32].

The current results observed a significant decline in AchE enzyme activity after treated with *S. officinalis* and significant elevation in LDH enzyme activity as compared to control. AchE is a key enzyme in detecting the neurotoxicity by hydrolyzing the neurotransmitter acetylcholine in cholinergic synapses of the nervous system and is the target site of several neurotoxic insecticides. [33] that leads to overstimulation of the nervous system and subsequently cellular death.

Many reports indicated that monoterpenoids were lethal to the insects through inhibition the activity of AChE enzyme [34], 1.8 cineole was the inhibitoriest effect on AChE activity [35].

Furthermore, LDH enzyme used as an indicator for cellular damage and cytotoxicity of toxic agents [36, 37], thus the elevation in the LDH usually found in the tissue break down and in cellular death.

Conclusion

Continuous efforts are being made to combat the wide speared polyphagous *Spodoptera littoralis*, one of the most important pests in both Egypt and the world to find out modern, non-traditional and environmentally friendly agents as alternatives to conventional pesticides. This study could contribute to assessing the possibility of using *Salvia officinalis* as a potential insecticide against *S. littoralis*. Its extracted essential oil act as a source of biologically active compounds which may prove to be an efficient insecticide incorporated in IPM programmes.

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