

Biological Activity of Essential Oil of Sage Plant Leaves *Salvia officinalis* L. against the Black Cutworm *Agrotis ipsilon* (Hubn.)

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Abstract: The black cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae) is one of the most destructive insect pest attacking different field crops. Biological activity of essential oil of Sage plant leaves was evaluated against 2nd instar larvae of *A. ipsilon*. Bioassay test showed that 4% Sage plant oil (*Salvia officinalis*) induced 96%, 63.6% starvation and antifeeding activity respectively, 75% of larval mortality recorded after 8 days of treatment, then all larvae died at the 10th day. The mortality percentage, starvation and antifeedant activity were positive concentration dependent. The oil disrupts the hormonal balance in the larvae when ingested with treated diet contained sub lethal concentration ($LC_{50} = 1.806\%$) which could be calculated after 8 days from larval treatment, producing malformed pupae which failed to give rise to adults, or developed into intermediate larval-pupal forms. The adult abnormalities failed to expand their wings and to emerge from the puparium. At the sub lethal concentration, the essential oil gave a relatively high percent of reduction (67.4% in egg deposition and 69.4% in egg hatchability and caused above 31.2% sterility among the resulting females. The survived larvae completed their life cycle with retarded growth. This was accompanied with reduction in larval weight, pupal weight, adult emergence and fecundity, increasing percentage of deformities and prolongation of the larval period. Pupation percentage decreased with 58% and in moths emergence with 24%. From this point of view, and so could be recommending usage of the mentioned plant essential oil for controlling the black cutworm on their hosts by spraying it as emulsion or as a toxic baits alternatives to chemical pesticides and decreasing the insect resistant buildup as a biological control method throughout the integrated pest control program. Present results showed that the essential oil from Sage plant leaves is potentially useful for management *A.ipsilon* insects population.

Keywords: Biological activity, Sage oil, *Salvia officinalis*, Black cutworm, *Agrotis ipsilon*

1. Introduction

The black cutworm, *A. ipsilon* is one of the most destructive insect pest attacking different field crops, such as cotton, soybean, corn, potatoes and tomatoes not only in Egypt but also in several countries of the throughout the year. Great losses occurred in yield due to *A. ipsilon* infestation especially at seedling stage (Ladhari et.al, 2013). The black cutworm control is currently based on heavy use of many insecticides, which damage the environment and/or pose a threat to public health via food residues, ground water or accidental exposure. The problems caused by pesticides and their residues have amplified the need for effective and biodegradable pesticides with great selectivity (Hazaa & Alam EL-Din, 2011). Alternative strategies have included the investigation for new type of insecticides, and re-evaluation and use of 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 have well-developed defense mechanism against herbivores and are excellent sources of new toxic substances for pests (Pickett et.al. 2006). Among various classes of natural substances that introduced as natural biopesticides are essential oils from aromatic plants (Isman & Grieneisen, 2014; Prakash et al., 2014). In recent year, more attention was paid to essential oils as pest control agents. In 1997, researchers described 866 different plant species that produce chemicals useful against and listed their 256 biologically active chemical

component (Prakash and Rao 1997) There are numerous researches on the pesticidal activity of essential oils from Lamiaceae family (Rajendran & Sriranjini, 2008; Isman et al., 2011; Ebadollahi & 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 humans (Upadhyay 2013). 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) (Lambrano et al., 2014). Sage plant known as garden true and Dalmatian Sage. Sage (*Salvia officinalis* L., Lamiaceae) has a wide range of biological activities such as anti-oxidative, anti-bacterial, hypoglycemic, anti-inflammatory, fungistatic, virustatic, astringent, eupeptic properties and anti-hydrotic effects (Eidi & Eidi, 2009).. Tayoub et al (2012) recorded that *S. officinalis* contained monoterpenes and sesquiterpenes by 57.3% and 41.7% respectively, also they found that *S.officinalis* oil has a fumigant toxic effect on larvae of khapra beetle *Trogoderma granarium* and showed that the essential oil is potentially useful for management of *T. granarium* insect population in stored products. The main goal of the present investigation was to evaluate the toxic activities of Sage plant *Salvia officinalis* leaves oil on different stages of the black cutworm *A. ipsilon* under controlled laboratory conditions for possible use as a safe biological method and alternative to chemical pesticides within the means of integrated pest control program.

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2. Materials and Methods

1. Insect culture: A standard culture of *A.ipsilon* has been maintained in the laboratory castor bean leaves under the laboratory condition of $28 \pm 2^\circ \text{C}$ and $55 \pm 5\% \text{RH}$. (EL-Kady et al 1997).
2. 2-Plant essential oil. Was obtained from Esotric essential oils. Republic of South Africa. Po.Box. 14007, Hatfield, 0028 Gauteng, South Africa. www.essentialoils.co.za. (fax (South Africa t27.12.3610061).
The company extracted the oil from the dried leaves by steam distillation.
3. Antifeedant tests: The leaf disc method described by Kubo & Nakanishi (1977) was followed to evaluate the antifeedant activity of different concentrations of essential oil of *S. officinalis*. The antifeedant effect was measured on 2nd instar larvae of *A.ipsilon* as starvation percentage according to Moustafa (1969).
4. Toxicity of the oil : The 2nd instar larvae were left to feed for 24 h. on leaf discs of Castor bean plant leaves treated with six different concentrations of essential oil (4, 3, 2, 1, 0.5 and 0.255%), (1ml. oil / 100ml. water) 0.5% Tween 80% was added as emulsifier. The dipping technique was followed. Untreated leaf discs were used for comparison. Ten replicates were used, 10 larvae each. The larvae left to fed for two days on the treated leaf discs, then the remained alive larvae after feeding were kept separately each in a small clean cups provided with fresh untreated castor bean leaves to prevent cannibalism habits and covered with muslin. Daily records were taken for larval mortality in order to obtain the LC₅₀ value.
5. Biological and biotic potential tests: The 2nd instar larvae were left to feed for 24 h. on leaf discs of Castor bean plant leaves treated with the concentration of LC₅₀ of the oil that was determined from the previous toxicity test, hundred replicates were used, one larva each. Percentage of pupation, pupal mortality, pupal malformation and pupal weight (two days after pupation). Pairs of moths (♀+♂) were kept each in jar (1 L.) with a strip of black paper for egg oviposition. Deposited eggs were counted and incubated at $28 \pm 2^\circ \text{C}$ and percentage of egg hatchability was recorded. The over-all latent effect on reproduction was expressed as percent sterility and calculating according to Topozada et al (1966).

$$a \times b$$

$$\% \text{ Sterility} = 100 - \frac{a \times b}{A \times B} \times 100$$

$$A \times B$$

a= No. of deposited eggs in treatment, b= % hatchability in treatment

B= No of deposited eggs in check, B= % hatchability in check.

Data were subjected to statistical analysis The average percent mortality of the tested larvae was calculated and corrected using Abbott's formula (Abbott, 1925). The corrected percentages of mortalities were statistically

computed according to the method of Finney (1971). Computed percentage of mortality was plotted versus the corresponding concentrations on logarithmic probability paper to obtain the corresponding Log-concentration probit lines. The lethal concentrations 50 and 95% were determined for established regression lines. The reduction percentages were calculated according (Abbott, 1925) with the equation.

$$\% \text{ Reduction} = \frac{C - T}{C} \times 100$$

Where C= mean number in the control, T = mean number in treated.

3. Results and Discussion

1. Antifeedant activity of *S.officinalis* oil on *A.ipsilon* larvae.

Data in Table (1) indicated that Sage oil caused 63.59% antifeedant activity when used at the highest concentration (4%) against the 2nd instar larvae of the black cutworm. Oil concentration played an important role in determining starvation effects as well as antifeedant activity of the tested oil. The higher the concentration the higher was the starvation percentage and vice versa. Sharaby et al (1994) mentioned that essential oil of *Dodonea viscosa* plant leaves caused antifeedant and starvation effects on *S. littoralis* larvae. ; **Sabbour & Abd El- Aziz (2002)** reported that *Eucalyptus* oil was effective as an antifeeding deterrent against the 3rd larval instar of *A. ipsilon* and *S. littoralis*; Ebadollahi (2013) recorded that essential oils from Apiaceae family have a lethal ovicidal, larvicidal, pupicidal and adulticidal and sublethal antifeedant, repellent, oviposition deterrent, growth inhibitory and progeny production against different insects. Taghizaeh Saroukola et al (2014) discussed the antifeedant activity and toxicity of some plant essential oils to Colorado potato beetle *Lipinotarsa decemlineata*, they showed that all essential oils tested *Saturija*, *Khuzistanica Thymus daenensis*, *Ocimum basilicum*, *Myrtus communis* *Mentha spicata*, and *Eugenia caryophyllus*, were effective on feeding deterrence on larvae and adults. Our results agreed with that mentioned by Shukla et al (2012) that the essential oils from flowers and leaves of crofton weed *Eupatorium adenophorum* and aerial part of Indian wormwood, *Artemisia nilagirica* showed significant antifeedant activity at 96h against the red palm weevil adults at 1000ppm. Recently Labrano et al (2014) determined the biological effects of essential oils isolated from *Cymbopogon nardus*, *C.flexuosus* and *C. martini* against two Lepidoptera larvae, the all tested oils showed antifeedant activity and dermal contact lethality against *Acharya fusca* and *Euprosterina elaeasa* (Lepidoptera: Eumecodidae) at various concentrations. All oils exhibited strong antifeedant and toxicity activity toward the insects.

Table 1: Percentage starvation and antifeedant activity of *A. ipsilon* 2nd instar larvae affected by *S.officinalis* essential oil

% Oil concentration	Larval weight mg. after feeding 24 h.	% Starvation	% Leaf disc eating	% Antifeeding activity
4	0.556	69.02	29.07	63.59
3	1.263	29.47	52.43	34.32
2	1.519	15.16	60.90	23.71
1	1.590	11.19	67.71	15.18
0.5	1.686	5.82	74.83	6.26
0.25	1.690	5.59	75.12	5.90
Check	1.790	-----	79.83	--
Starved larva	0.002	---	-----	----

2. Toxicity of the oil.

Table (2) shows that the mortality percentage decreased as the oil concentration of *S. officinalis* decreased. High percentage of larval mortality was (75%) recorded at 4% concentration., after 8 days from feeding, the larvae still at the 2nd instar then all died after 14 days. At 3% concentration the larvae reached the 4th instar, then they stopped feeding, degenerated and died within 3 days. The lower concentration 2% caused 56% kill. Data indicated the satisfactory insecticidal activity of the oil. LC50 could be calculated after 8 days from feeding on the treated leaf discs of Castor bean and determined with 1.806 % as

illustrated in figure (1). Our result consistent with the view of Varma & Dubey (2001) stated that Mint *M. arensis* has toxic effect on larvae of *Sitophilus oryzaea*, *Tribolium castanum*. **Pavela (2005)** tested 34 essential oils for insecticidal activity against larvae of *Spodoptera littoralis* and concluded that they were toxic or highly toxic.. In similar findings **Tripathy & Singh (2005)** in India reported larval mortality in *Helicoverpa armigera* as induced by some vegetable oils (mustard oil, sesame oil, linseed oil, castor oil, cottonseed oil and groundnut oil). Also Our results in agreement with reported by Sharaby et al, (2012) that the essential oils such as Garlic *Allium sativum*, Mint *Mintha piperita* and Eucalyptus *Eucalyptus globulus* are known for their pest control properties for Controlling the Grasshopper *Heteracris littoralis*.

Table 2: Toxicity of *S.officinalis* essential oil on 2nd instar larvae of *A.ipsilon* after 8 days from feeding on treated castor bean leaves for 8 days

Oil concentration ml. oil/100 ml. water.	4%	3%	2%	1%	0.5%	0.25%
% of larval mortality	75	62	56	35	15	2

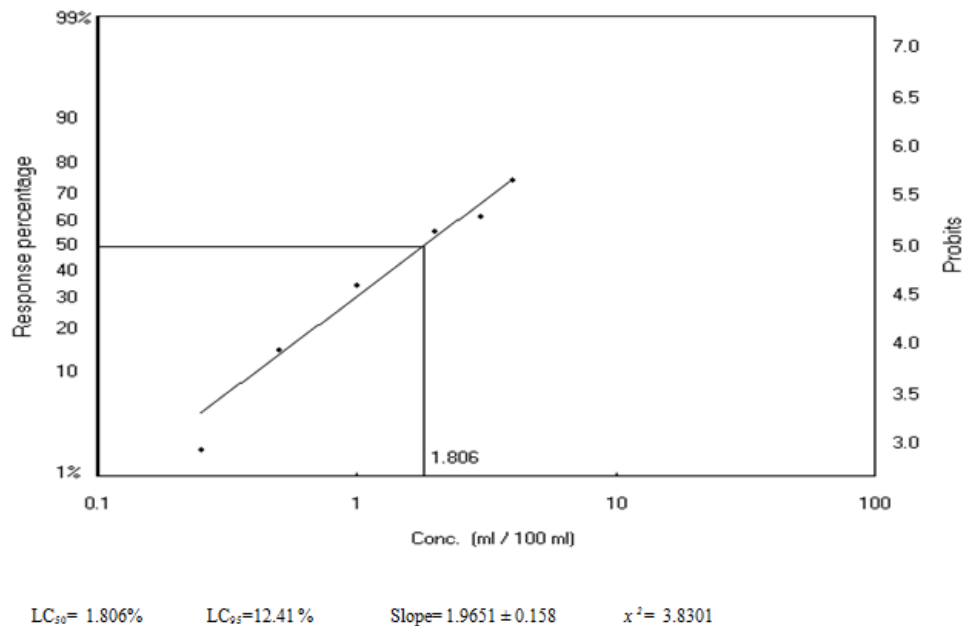


Figure 1: Toxicity regression line of *S.officinalis*.essential oil on *A.ipsilon* 2nd instar larvae

3. Biological aspects of *S. officinalis* essential oil on *A. ipsilon*.

Table (3) cleared that the essential oil of sage caused great an obvious retardation in larval development of survived larvae with 44.4%, the pupation percentage was decreased from 95-40%, the high reduction 57.9% in pupation and pupal morphogenesis (deformed incomplete pupae as

larval- pupal intermediates), , reduction in moths emergence with (23.5%). In table (4) The deposited eggs of females ensuring from larvae treated with the LC₅₀ concentration were 320 compared with 980 eggs/♀ for the control untreated check showing reduction percentage with 69.5%. The essential oil also gave relatively high percent of reduction (69.5%) in egg hatchability. The oil caused above (32 %) sterility among the treated females. Our

results indicate that essential oil proved to have insecticidal activity, has properties similar to juvenile hormone on the *A. ipsilon* larvae that caused retardation in larval and pupal development, such disruption in insect development and malformation through pupal and adults formation is in agreement with the findings of (EL-Nagar, 1979., Awad, 1980., Sharaby, 1987., Sharaby, 1988., Sharaby and Magd EL-Din, 1990, Sharaby et al. 2012 and Sharaby et al, 2014). From the above mentioned results, it could be concluded that essential oil of Sage plant leaves *S. officinalis* at a LC₅₀ concentration (1.806% ml/ml) showed satisfactory insecticidal properties against 2nd instar larvae of the black cutworm. The relatively high biological activity of the oil may be attributed to its main components of terpenes and sesquiterpenes (Camphene, &- pinene, B- pinene, myrcene, limonine, 1, 8-cineole, &- thujone, B-thujone, camphor, linalool, bornylacetate and Borneol., Tayoub et al (2012), this is in agreement with the findings of Sharaby et al 1987 and Sharaby et al, 2014).

Table 3: Biological aspects of Sage *S. officinalis* plant leaves on *A. ipsilon*

Treatment	Larval Duration In days ±SE	Larval Weight mg±SE	Pupal duration In days ±SE	Pupal weight mg. ±SE	% Pupation	% pupal deformation	% Moths emergence
LC ₅₀ Oil Conc. 1.806 %	29±15.3b	395±30.7b	13.5±0.19a	289.7±13.6a	40%	9%	75%
Check test	16.2±0.11a	478±7.6 a	14±0.43a	282.6±22.2a	95%	0	98%

Table 4: Biological aspects of Sage *S. officinalis* plant leaves on *A. ipsilon*

Treatments	% Moth deformation	No. of deposited eggs	% egg hatchability	% Sterility
LC ₅₀ Oil Conc. 1.806%	3%	320±0.09b	28.13%	31.19%
Check test	0	980±0.12a	91.84%	0

The toxic effect is almost certainly due to one or more of the component of the essential oil particularly monoterpenes that are found in *S. officinalis* oil several reports indicate that monoterpenoids cause insect mortality by inhibiting acetyl-cholinesterase enzyme activity (Houghton et al, 2006). Previous reports by (Enan, 2001), suggests that toxicity of constituents of an essential oil is related to the octopaminergic nervous system of insects. There is another suggestion that some monoterpenes may inhibit cytochrome P450-dependent mono oxygenases (De-Oliveira et al, 1997). The above citation suggests that target sites of mode of action of monoterpenes may be various and the essential oils act at multiple level in the insects, so the possibility of generating resistance is little probable (Gutierrez et al, 2009).

4. Conclusion

Essential oil of *S. officinalis* (Leaves) have toxicities against different stages of *A. ipsilon*. These findings demonstrated the potential of the essential oil for further development into a botanical pesticide in the control of the black cutworm *A. ipsilon* may be in the field as toxic bait traps or as spraying emulsion on plants through integrated pest management program, anyway our study needs more further field investigations.

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