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Effects of *Ocimum sanctum* extract against biochemical aspects of *Spodoptera littoralis* (Lepidoptera: Noctuidae) larvae

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Abstract:

The methylene chloride leaves extract of *Ocimum sanctum* (L.) was studied against the 2nd and 4th instar larvae of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) strain. The LC50 value of the *O. sanctum* extract was lower against the 2nd instar larvae than the 4th instar and the time effect on the toxicity of the extract, where the LC50 after the 48 hrs was lower than after 24 hrs. against the two ages of the larvae. The biochemical aspects of *S. littoralis* larvae were detected using the LC50 of *O. sanctum* extract against the 2nd and 4th instar larvae. The *O. sanctum* extract exhibited variations of activities for each enzyme. It increased the activity of AST (Aspartate Transaminase) and beta esterases, on the contrary they decreased the activity of ALT (Alanine Transaminase), alpha esterases, Acetylcholinesterase (AchE), alkaline phosphatases, total protein and total lipids in both 2nd and 4th instar larvae, while *O. sanctum* extract decreased the activity of acid phosphatase only in the 2nd instar larvae and elevated it in the 4th instar larvae. Conversely, the carbohydrates increased in the 2nd and decreased in the 4th instar larvae. The results of the research show that the overall effects of *O. sanctum* extract on some biochemical components in *S. littoralis* larvae can facilitate the development of selective natural product as insecticides that can be employed in integrated pest management strategies.

Introduction

Ocimum sanctum (L.) (OS) is an herb belonging to the family Lamiaceae, known for its medicinal value in various traditional medicines in India and other Asian nations, particularly Ayurveda and Unani type of medicine (Satyavati,1987). The important bioactive constituents of *O. sanctum* are ursolic acid,-a triterpenoid and rosmarinic acid a phenylpropanoid. It contains volatile oil comprising mainly of eugenol and β-caryophyllene with minor terpenes like bornyl acetate, β-elemene, methyl eugenol, neral, β-pinene etc (Rastogi and Mehrsotra, 1998). Gupta *et al.* (2007) isolated three new compounds, ocimumoside A, (2) Ocimumoside B, and ocimarin, from an extract of the leaves of holy basil (*Ocimum sanctum*), together

with other known substances. apigenin, apigenin-7-O-beta- d-glucopyranoside, apigenin-7-O-beta- d-glucuronic acid, apigenin-7-O-beta- d-glucuronic acid 6"-methyl ester, luteolin-7- O-beta- d-glucuronic acid 6"-methyl ester, luteolin-7-O-beta-dglucopyranoside, luteolin-5-O-beta-d-glucopyranoside, 4-allyl-1-O-beta-dglucopyronosyl-2-ydroxybenzene and two known cerebrosides. Singh *et al.* (1991) found that the ethanol extract of OS leaves prevent the reduction in adrenergic neurotransmitters in brain of rats. Sukari *et al*. (1992) recorded a high toxicity of *O. sanctum* against *Tilapia mossambica*. While Chitwood (2002) mentioned the leaf extracts of lantana (*Lantana camara*), *Citrus* oil, tulsi (*Ocimum basilicum, O. sanctum*) and vetiver (*Vetiverazi zanoides*) are useful in controlling leaf miners in potato, beans, brinjal, tomato and chilies.

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is considered as the major pest in a wide range of cultivation including cotton, corn, soybeans, peanuts, and vegetables. This pest is not only widely spread in Egypt but also in other Middle East countries in addition to temperate zones in Asia and Africa (Salama *et al*., 1990). Over the last few decades, the intensive use of broad-spectrum insecticides against the Egyptian cotton leafworm, *S. littoralis* has led to the development of resistance to many registered pesticides making their control even more difficult (Miles and Lysandrou, 2002). The extensive use of these synthetic pesticides has given rise to problems such as residuals toxicity (pollution), pesticide resistance and harmful effects on beneficial insects, such as natural enemies, honey bees and beneficial birds. For the above mentioned reasons, the general trends in last three decade were the substitution of synthetic pesticides by natural products (Aydin and Gurkan, 2006).The aim of this research work is to study effects of *O. sanctum* extract against biochemical aspects of the Egyptian cotton leafworm, *S. littoralis* larvae.

Materials and Methods

1. Plant materials:

The plant was collected from Khulias, Jeddah, Kingdom of Saudi Arabia, in February 2017.

2. Preparation of the extract:

A weight of 50 g fresh leaves of the plant was grounded and then macerated in 200 ml of methylene chloride solution and left 7 days, then filtered through Whatman No. 40 filter paper. The solvents were removed under reduced pressure using a rotary evaporator to obtain 1.61 g extract for *O. sanctum*.

3. Test insect:

The 2nd and 4th instar larvae of *S.littoralis* strain, used in this study, was obtained from the Faculty of Agriculture, Cairo University and was reared in the laboratory of the Pest Physiology Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt, for several generations away from any insecticidal contamination, under constant laboratory conditions as described by El-Defrawi *et al*. (1964).

4. Toxicity assay:

The concentrations 20, 10, 5 % (extract / diet) from *O. sanctum extract* were prepared. Ten larvae for each 2nd and 4th instar of *S. littoralis* were transferred individually to the surface of each treated diet kept in glass jars, four replicates for each concentration. Ten larvae were allowed to feed on untreated diet as a control treatment for each the 2nd and the 4th larvae. The glass jars kept under the previous controlled conditions and inspected daily. Mortality percentages were recorded after 24 and 48 hrs. the obtained data subjected to Ldp line analysis (**Bakr, 2007**) and the toxicity then estimated. In this paper, try to study the biochemical changes. So, samples for analysis been taken 48hrs. post treatment with LC50 of tested *O. sanctum* extract and before the onset of the mortality.

5. Preparation of insects for biochemical studies:

The preparation of samples involved the use of the 2nd and 4th instar larvae of *S. littoralis* after 48hrs. of all treatments at LC50 level and control. The larvae were homogenized in distilled water (50 mg /1 ml) using a Teflon homogenizer surrounded with jacket of crushed ice for three minutes. Homogenates were centrifuged at 8000 r.p.m. for 15 min. at 2℃ in a refrigerated centrifuge. The deposits were discarded and the supernatants, which is referred as enzyme extract, were used directly for the biochemical analysis (Amin, 1998).

6. Biochemical measurements:

- Transaminases; (ALT) alanine aminotransferase activity (GPT) and (AST) aspartate aminotransferase activities (GOT) were determined according to Reitman and Frankle (1957)
- Phosphatases were demonstrated according to Powell and Smith (1954)
- α- and β-esterases were detected according to Van Asperen (1962)
- AchE (acetyl cholinesterase) activity was measured according to Simpson *et al.* (1964)
- Total lipids Total lipids were estimated by the method of Knight *et al.* (1972)
- Total carbohydrates were determined according to Dubois *et al.* (1956)
- Total proteins were determined according to Bradford (1976)

7. Statistical analysis:

Significant differences were calculated by ANOVA and Duncan's multiple range tests. Differences among treatments were determined by Tukey's multiple range test ($P < 0.05$) CoStat - Statistics Software (CoStat, 2007).

Results and Discussion

Table (1) Showed that the LC50 of the *O. sanctum* extract to the 2nd instar larvae of *S. littoralis* is 47.62% after 24 hrs. and 0.99% after 48 hrs., where in Table (2) found the LC50 of the *O. sanctum* extract to the 4th instar larvae of S. *littoralis* is 140.06% after 24 hrs. and 27.02% after 48 hrs. The results showed, also, that the LC50 value of the *O. sanctum* extract was lower against the 2nd instar larvae of *S. littoralis* than against the 4th instar larvae and the time effect on the toxicity of the extract where the lC50 after the 48 hrs was lower than after 24hrs against the two ages of the larvae of *S. littoralis*. Anees (2008) found that, the LC50 values of *O. sanctum* leaf extract against the larvae of *Ae. aegypti* was 425.94, and against the larvae of *Cx. quinquefasciatus* was 592.60 ppm. Average of 60% mortality was recorded for *Periplenta americana* due to the toxicity of leaf extract of *O. sanctum* (Hazarikaa and Boruah, 2014). Yousef et al. (2016) mentioned that the LC50 values for *C. procera* extract was (0.14g /100 g diet) and recorded (0.0032 g/100 g diet) for *O. sanctum* against the cotton pink bollworm, *Pectinophora gossypiella (*Saunds.*)* they elucidate the high toxic effect of *O. sanctum* may be due to the presence of high amount of lead in the extract.

In Tables (3 and 4) *O. sanctum* extract caused decreased to the total protein non-significantly in the 2nd and significantly in the 4th instar larvae of *S. littoralis* compared with the control, the effect of *O. sanctum* is comparable to the effect of the novel insecticide pyridalyl was obtained by Dahi *et al.* (2011), they observed a conspicuous depletion in total protein content in both 4th and 6th treated larval instar with LC50 of the novel insecticide pyridalyl. Similar results were obtained by (Sokar, 1995) and (Assar *et al.*, 2016) for the total protein of the same species treated with teflubenzuron and hexaflumuron. Wilkinson (1976) stated that protein help to synthesize microsomal detoxifying enzymes which assist in the detoxificaition of toxicants that enter into the insect body. Proteins are the most important components of biochemical of insect that bind the

Table (1): Mortality percentage of the 2nd instar larvae of *Spodoptera littoralis* treated with *Ocimum sanctum* extract.

Conc.	Corrected Mortality % ± SD	
	After 24 hrs.	After 48 hrs.
20%	38.46±0.00 b	92.30±2.5 a
10%	41.02±2.5 ab	74.35±2.8 b
5%	48.71±4.08 a	79.48±4.0 b
control	0±0 c	0±0 c
Lc50	47.6284	0.9945
Slope	0.3447+/-0.2141	0.9609+/-0.2397
F value	62	176.44
LSD	0.8320	0.9434
P	.0000***	.0000***

Means in the same column with the same letter(s) are not significantly different. (P<0.05)
*SD = standard deviation LSD : least significant difference. ***: highly significant*

Table (2): Mortality percentage of the 4th instar larvae of *Spodoptera littoralis* treated with *Ocimum sanctum* extract.

Conc.	Corrected Mortality %± SD	
	After 24 hrs	After 48 hrs
20%	20±4.08 a	42.50± 2.50 a
10%	7.5±2.50 b	22.50±4.78 b
5%	7.5±4.78 b	12.50±2.88 b
control	0.00±0.00 b	0.00±0.00 c
Lc50	140.0685	27.0286
Slope	1.0747+/-0.6386	1.6206+/-0.5363
F value	6	33.333
LSD	1.04302	0.9434
P	.0097**	.0000***

Means in the same column with the same letter(s) are not significantly different. (P<0.05)
****: highly significant SD = standard deviation LSD: least significant difference*

foreign compounds. In general, the problem of protein synthesis is intimately related to metabolism of nucleic acids.

Total carbohydrates show a non-significantly increase 12.26% higher in the 2nd instar larvae of *S. littoralis* was treated with LC50 of *O. sanctum* extract than in the control. These observations agreed with Assar *et al.* (2016), indicated that all tested insecticides (emamectin, spinetoram, hexaflumuron and teflubenzuron) led to increase in total carbohydrates compared with control. In contrast, total carbohydrates in the 4th instar larvae was non-significantly decreased (-3.69%) than in the control. That agree with Osman and Abou-Zeid (2015), they noticed decrease of total carbohydrate when they used the plant extract of *Capsicum annuum L*, and Organophosphorous insecticide Profenofos (selecron) and the mixture of them for controlling 4th instar larvae of cotton leaf worm under the semi field circumstances. The disturbance in carbohydrate content can be understood in the light of the ability of the organism to modify the synthesis of certain metabolite and disrupt the functionality of the organism (Rodriguez-Ortega *et al.*, 2003). The obtained results in this work recorded non-significantly decrease in total lipids values in the 2nd instar larvae and significantly in the 4th

instar larvae, which were treated with LC50 of *O. sanctum* extract.

The same results were obtained by Assar *et al.* (2016) were stated the reduction in total lipids with hexaflumuron and teflubenzuron as IGR, s against 4th instar larvae of *S. littoralis*. Similar reduction in total lipids was recorded by (El-Sheikh *et al.*, 2013). Different results were obtained by (Abdel-Mageed *et al.*, 2018), in they work recorded significantly increase in total lipids values 6.63, 5.74, 6.09 for flufenoxuron, chlorfluazuron, triflumuron respectively, while it was 4.33 for control. And elucidate the exceptional cases of increasing lipid content in *S. littoralis* treated with Chitin synthesis inhibitors (CSIs) may indicate its pronounced interference with not only the synthesis of lipids but also their mobilization as promoted to convert into other metabolites or fatty acids.

Table (3): Effect of LC.50 of *Ocimum sanctum* extract on some biomolecules of treated 2nd instar *Spodoptera littoralis*.

Biomolecules	Mean of the enzyme activity ± SD		Change%	F value	LSD	P
	Treatment	Control				
Total carbohydrate *(mg/g.b.wt)*	16.2±0.43 a	14.43±0.66 a	12.26	4.8937	2.21729	0.0914 ns
Total lipids *(mg/g.b.wt)*	6.4±0.26 a	6.96±0.24 a	-8.0	2.5130	0.9924	0.01881 ns
Total protein *(mg/g.b.wt)*	33.53±1.35 a	38.1±1.47 a	-11.99	5.2078	5.5559	0.0846 ns

Means in the same row with the same letter(s) are not significantly (ns.) different. (P<0.05), SD = standard deviation LSD: least significant difference.

Table (4): Effect of LC.50 of *Ocimum sanctum* extract on some biomolecules of treated 4th instar *Spodoptera littoralis*.

Biomolecules	**Mean of the enzyme activity ± SD**		**Change%**	**F value**	**LSD**	**P**
	Treatment	**Control**				
Total carbohydrate *(mg/g.b.wt)*	**10.43±0.12 a**	**10. 83±0.27 a**	**-3.69**	**1.8**	**0.8277**	**0.2508 ns**
Total lipids *(mg/g.b.wt)*	**3.8±0.115 b**	**6.10±0.118 a**	**-37.70**	**194.0191**	**0.45911**	**0.0002 *****
Total protein*(mg/g.b.wt)*	**43±1.60 b**	**57.76±1.47 a**	**-25.55**	**45.7670**	**6.06031**	**0.0025 ****

*Means in the same row with the same letter(s) are not significantly (ns.) different. (P<0.05), ***: highly significant SD = standard deviation LSD: least significant difference.*

In Tables (5 and 6) *O. sanctum* extract caused (ALT) alanine aminotransferase activity (GPT) decreased significantly (-38.38) in the 2nd instar *S. littoralis* larvae than in the control, where significant elevation to (AST) aspartate aminotransferase activities (GOT) (71.34) in comparing to the control. Also caused significant increased to AST (138.20) and significant decreased to ALT (-45.08) of the 4th instar *S. littoralis* larvae than in control. Declined level of (ALT) in *S. littoralis* larvae by *O. sanctum* extract, in the present study, in agreement with decreased activity in *S. Littoralis* by several insect growth regulators (IGRs) and CSIs, for example, hexaflumuron (Sokar, 1995), teflubenzuron, flufenoxuron and pyriproxyfen (El-Kordy *et al.*, 1995), and flufenoxuron, Chlorfluazuron and Triflumuron (Abdel-Mageed *et al.*, 2018). Azmi *et al.* (1998) dissected the inhibited activity of (GPT) in haemolymph of *S. littoralis* larvae by CSIs can be understood since pyruvate is the precursors of Krebs cycle compounds, related to the mitochondrial oxidation phenomenon and ATP products. Anyhow, diverse effects of the tested CSIs on GPT activity in larvae could be due to their effects on the synthesis or functional levels of this enzyme directly or indirectly by varying the cell cytomorphology (Nath,

2000), or the neurosecretory hormonal pattern (Abdel-Mageed *et al.*, 2018).

On the other hand, increased the activity of AST in *S. littoralis* larvae by *O. sanctum* extract, in agreement with Sokar (1995), El-Kordy *et al.* (1995) and Abdel-Mageed *et al.* (2018) treated *S. littoralis* with several IGRs or insecticides, e.g. hexaflumuron, flufenoxuron, pyriproxyfen or teflubenzuron, Chlorfluazuron and Triflumuron.

Table (5): Effect of LC 50 of *Ocimum sanctum* extract on enzymes activity of 2nd instar larvae of *Spodoptera littoralis*.

Enzymes	Mean of the enzyme activity ± SD		Change%	F value	LSD	P
	Treatment	control				
AST *(U/g.b.wt)*	**8.91±0.14 a**	**5.20± 0.10 b**	**71.34**	**444.64**	**0.48**	**.0000 *****
ALT *(U/g.b.wt)*	**2.36±0.14 b**	**3.83±0.17 a**	**-38.38**	**41.1914**	**0.634478**	**.0030 ****
Alkaline phosphatase *(mU/g.b.wt)*	**2988.33±57.32 b**	**6054±101.23 a**	**-50.64**	**694.348**	**323.0169**	**0.0000 *****
Acid phosphatase *(mU/g.b.wt)*	**329±8.02 b**	**433.33±10.13 a**	**-24.07**	**65.1389**	**35.89151**	**0.0013 ****
∞-esterase *(ugα-naphthol/min/g.b.wt)*	**704.66±9.70 a**	**746±25.73 a**	**-5.54**	**0.14739**	**57.85561**	**0.7206 ns**
β-esterase *(ugβ-naphthol/min/g.b.wt*	**1425±19.31 a**	**1206.66±12.57 b**	**18.15**	**89.7541**	**63.9855**	**.0007 *****
AchE *(ugAchBr/min/g.b.wt)*	**193.33±8.81 b**	**533.66± 14.49 a**	**-63.77**	**402.331**	**47.10874**	**0.0000 *****

Means in the same row with the same letter(s) are not significantly (ns.) different. (P<0.05),
****: highly significant*
SD = standard deviation
LSD: least significant difference.

Table (6): Effect of LC50 concentrations of *Ocimum sanctum* extract on enzymes activity of 4th instar larvae of *Spodoptera littoralis*.

Enzymes	Mean of the enzyme activity ± SD		Change%	F value	LSD	P
	Treatment	control				
AST *(U/g.b.wt)*	**10.4±0.152 a**	**4.366±0.185 b**	**138.20**	**630.01923**	**0.667374**	**.0000 *****
ALT *(U/g.b.wt)*	**1.72±0.06 b**	**3.19±0.15 a**	**-45.08**	**76.5673**	**0.46642**	**0.0009 *****
Alkaline phosphatase *(mU/g.b.wt)*	**470.33±10.17 b**	**1063.33±31.79 a**	**-55.76**	**315.506**	**92.6915**	**0.0001 *****
Acid phosphatase *(mU/g.b.wt)*	**232.33±7.310 a**	**217±6.92 a**	**7.06**	**2.3176**	**27.9642**	**0.2026 ns**
∞-esterase *(ugα-naphthol/min/g.b.wt)*	**339±7.37 b**	**425.33±7.31 a**	**-20.29**	**69.1556**	**28.8239**	**0.0011 *****
β-esterase *(ugβ-naphthol/min/g.b.wt*	**1098.33±7.83 a**	**1032.33±17.07 b**	**6.39**	**12.343**	**52.1564**	**0.0246 ***
AchE *(ugAchBr/min/g.b.wt)*	**744±8.32 b**	**824±12.12 a**	**-9.70**	**29.58397**	**40.83671**	**0.0055 ****

Means in the same row with the same letter(s) are not significantly (ns.) different. (P<0.05),
****: highly significant SD = standard deviation LSD: least significant difference*

The obtained data show that significantly low activity of alkaline phosphatase (ALK-P) was noticed in 2nd and 4th instar larvae of *S. littoralis* was treated with *O. sanctum* extract (-50.64 and -55.76%, respectively) lower than in control. At the same respect, *O. sanctum* extract caused significant decrease in acid

phosphatase (AC-P) activity -24.07% in the 2nd instar larvae compared to the control. These results are in agreement with those obtained on *S. littoralis* by (El-Barky *et al.*, 2008 and El-Sheikh, 2012) using spinetoram with significant decrease in both acid and alkaline phosphatases. On the contrast, *O. sanctum* extract caused increased in (AC-P) activity 7. 06 % in the 4th instar larvae compared to control. Some increase in the activity of acid phosphatase in the same insect recorded by Sokar (1995) using hexaflumuron. Sridhara and Bhat (1963) stated that the increase or decrease of both phosphatase enzymes development is reflected in increase or decrease in acid-soluble phosphorus content.

O. sanctum extract caused decreased to the alpha esterase activity non-significantly, in the 2nd instar larvae of *S. littoralis* recorded -5.54% followed by 4th instar larvae which have -20.29% significantly lower than the control level. Assar *et al.* (2016) concluded that both alpha and beta esterases in *S. littoralis* was highly inhibited with hexaflumuron., On the other hand, *O. sanctum* extract increased significantly the activity of beta esterase enzymes in the 2nd and 4th instar larvae to 18.15 and 6.39%, respectively, higher than in the control. Bakr *et al.* (2013) noticed that IGR's may be cause different levels of significant changes in alpha and beta esterases on *S. littoralis*.

The most common resistance mechanisms in insects are modified levels or activities of esterase detoxification enzymes. These esterases comprise six families of proteins belonging to the a/ß hydrolase fold superfamily (Oakeshott *et al.,* 1993 and Cygler *et al.,* 1993). Numerous studies have demonstrated that esterases play an important role in participate to insecticide detoxifications in insect and other arthrsopod species (Mouches *et al.,* 1986).

Acetylcholinesterase (AChE) significantly decreased in the 2nd and 4th instar larvae of *S. littoralis, which were treated* with LC50 of *O. sanctum* extract recorded -63.77 and -9.70%, respectively, lower than in control. AChE is a key enzyme in the nervous system, terminating nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine. In insects, AChE is the only cholinesterase (Salgado, 1998). It is one of the most known defensive esterases as it is the major target for organophosphate and carbamate insecticides. This property led to the development of inhibitors of this enzyme as insecticides, they covalently bind to the active site. (Aldridge, 1950) and cause the death of the insect. (Fournier *et al.,* 1992).

The effect of *O. sanctum* extract on the enzymes; esterses , AChE, AST, ALT and phosphatases, may be useful in the management of insect populations where insecticide resistance has developed as a result of altered enzyme activities. It could be concluded that *O. sanctum* extract effects on the cotton leaf-worm are significant and could be added to its toxic effects, so it is suggested that *O. sanctum* is potentially potent substitution to chemical pesticides for control of *S. littoralis.*

Conflict of Interest

The present study was performed in absence of any conflict of interest.

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