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The Role of Mint Oil in Enhancing Spinosad Toxicity against the Cotton Leaf worm, *Spodoptera littoralis* in Relation to some Enzymes Activity

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ABSTRACT

This work aimed to study the effect of adding mint oil to Spinosad insecticide on the activity of some enzymes related to the toxicity of the compound against the cotton leaf worm, *Spodoptera littoralis*. The results showed that adding 0.3% mint oil to Spinosad solution increased its toxicity against *S. littoralis* 2nd instar larvae, where its LC₅₀ value was reduced from 20.482 ppm to 8.068 ppm after adding mint oil. The biochemical studies in the treated larvae with Spinosad combined with 0.3% mint oil showed a significant decrease in the activity of the enzymes; AChE, β-esterase, and phenoloxidase, while acid and alkaline phosphatases activity significantly increased; compared to treatment with Spinosad only. This data confirms the effect of mint oil on the activity of these enzymes towards Spinosad, which may explain the increased toxicity of Spinosad when mixed with mint oil against *S. littoralis*.

INTRODUCTION

The cotton leaf worm, *Spodoptera littoralis* (Boisd.) is considered one of the major destructive pests in Egypt. The larval stage was known as a leaf eater accepting almost all herbaceous plants (Abdel-Wahab, 2002). The wide use of different chemical insecticides for controlling *S. littoralis* caused the development of pesticide resistance (Ishaaya and Klein, 1990). Therefore, there is always a need for an alternative method for controlling the pest to minimize chemical insecticide's application and avoid the problem of evolved resistance in insect's field populations. Spinosad belongs to a new class of polyketide-macrolide insecticides. It has a novel mode of action, acting primarily at the nicotinic acetylcholine receptor in the nerve synapses. It might have some effects on the gamma-aminobutyric acid receptor and other nervous system components (Thompson *et al.*, 2000). Spinosad was considered an alternative reagent to classic insecticides, acted primarily as a stomach poison (Sparks *et al.*, 1998). It had been reported that pests have low susceptibility to Spinosad in the laboratory (Wang *et al.*, 2006). The addition of vegetable oils could increase either the uptake of the toxicant by the insect or reduce its evaporation dissipation or both. Park and Lee (2007) cleared that low insecticide toxicity in pests may be due to biochemical mechanisms including target site insensitivity to pesticides and increased detoxification rate by enhancing the production of metabolic enzymes. Alteration in the detoxification enzymes

might help in overcoming the insecticidal low toxicity that regulated by enzymes, where susceptibility variations were underlined mainly through three important tolerance mechanisms; decreased penetration, enhanced detoxification, and target-site insensitivity (Gunning *et al.*, 1996). Therefore, the present study aimed to investigate the effect of mixing mint oil with Spinosad on the activity of some enzymes in *S. littoralis* 2nd instar larvae.

MATERIALS AND METHODS

Tested Insect:

The 2nd instar larvae of the cotton leaf worm, *Spodoptera littoralis* (Boisduval), (Lepidoptera:Noctuidae) was obtained from a laboratory strain maintained in the Cotton Pest Research Department, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza. The culture was reared using the technique suggested by El-Defrawi *et al.* (1964).

Biopesticide:

Common name: Spinosad. Trade name: **Tracer** 24% SC. It is a natural bio-product formulation of a mixture of two natural metabolites, Spinosyn A (C41H65NO10) and D (C42H67NO10). It was produced by Dow Agro Sciences Co., UK.

Additive:

Vegetable mint oil. It contains menthol, menthone, carvenon, lemonin, and kadene. It was used at concentration 0.3% and it produced by El-captain Co. (CAP Pharm).

Surfactant agent:

Emulsifier (Sisi-6) was used at a concentration 0.3%. It provided by Central Agricultural Pesticides Laboratory (CAPL), Dokki, Giza.

Bioassay:

To investigate the interaction between Spinosad and the additive mint oil, a series of seven aqueous concentrations (3, 6, 12, 24, 48, 72 and 144 ppm) of Spinosad were prepared as Spinosad only or in combination with 0.3% mint oil+0.3% emulsifier (Abd El-Hafez *et al.*, 2013). The leaf-dipping technique using fresh castor bean leaves was used according to the method of Shepard (1958). The percentages mortality after 72 h of treatment of *S. littoralis* 2nd instar larvae were corrected using Abbott's formula, Abbott (1925). The LC₅₀ values of Spinosad (only and in combination with mint oil) were determined after 72 h of treatment according to the method described by Finney (1971) through LDP line software computerized program. The synergistic ratio (SR) based on the LC₅₀ values was calculated according to the method of Sun and Johnson (1960).

Biochemical Studies:

Sample Preparation for Biochemical Analysis:

Two groups of *S. littoralis* 2nd instar larvae were treated with the median lethal concentration (LC₅₀) value of Spinosad (only or in combination with 0.3% mint oil). The 1st group was treated with Spinosad only while the 2nd group was treated with Spinosad in combination with 0.3% mint oil. The survived healthy treated larvae in the two groups were separated after 120 hrs. of treatment. These healthy treated larvae were starved for 4 hours, then kept frozen (-5°C) until larval homogenation. The larvae were weighted then homogenated in distilled water with the fixed ratio (0.5 gm.b.wt to 10 ml d. water). The samples were centrifuged at 8000 rpm for 15 min undercooling (4°C) to remove the remnant of tissues. The supernatant fluid was divided into small aliquots (0.5 ml) to be used in the enzymes assay.

Determination of Acetylcholinesterase (AChE) Activity:

AChE activity was measured according to the method described by Simpson *et al.* (1964), using acetylcholine bromide (AchBr) as a substrate.

Determination of A- and B-Esterases Activity:

α - and β -esterases activity was determined according to the method described by Van Asperen (1962) using α -naphthyl acetate or β -naphthyl acetate as substrates, respectively.

Determination of Acid and Alkaline Phosphatases Activity:

Acid and alkaline phosphatases were measured according to the method described by Powell and Smith (1954).

Determination of Phenoloxidase Activity:

Phenoloxidase activity was determined according to the method of Ishaaya (1971).

Statistical Analysis:

The data were subjected to analysis of variance using (ANOVA) in SAS program, (SAS Institute, 1998). Mean separation was conducted using the least significant difference (LSD) in the same program at significant level $P \leq 0.05$. Activity ratio = enzymatic activity in treated larvae/ enzymatic activity in control.

RESULTS AND DISCUSSION

Synergistic Action of Adding 0.3% Mint Oil on Spinosad Toxicity against *S. littoralis* 2nd Instar Larvae:

Table (1) showed the values of LC₅₀ after 72 h of treatment of *S. littoralis* 2nd instar larvae. Data revealed that the LC₅₀ value of Spinosad (LC₅₀= 20.482 ppm) is reduced sharply when combined with 0.3% mint oil to reach 8.068 ppm. This synergistic action of mint oil addition could be attributed to oil properties that might increase toxicity and penetration of the insecticide. Adding vegetable oils might increase the uptake of the toxicant by the insect or reduced its evaporation dissipation or both (Abdel-Hafez and Abdel-Aziz, 2010). Degradation of Spinosad in the environment occurs mainly by photo and microbial degradation (Thompson and Hutchins, 1999). Vegetable and mineral oils could increase the adhesion, wetting and spreading properties of pesticides on the surface of the targets, decreasing pesticide loss and improving pest control (Abhilash and Patil, 2006). In some insect species, oils inhibit respiration and this, in turn may synergize the toxicity of insecticides that act on the nervous system. The present result is agreed with Abdel-Hafez and Abdel-Aziz (2010) they reported that emulsified oils of tagetes and sesame enhanced the toxicity and persistence of Spinosad against *S. littoralis* 2nd instar larvae.

Table (1): Synergistic effect of 0.3% mint oil combination on Spinosad toxicity against the 2nd instar larvae of *S. littoralis* after 72 h of treatment.

Treatments	Time after exposure (hrs.)	LC ₅₀ (ppm)	95%(FL)		Slope	*SR
			Lower	Upper		
Spinosad	72	20.482	15.827	26.338	0.898 ± 0.092	-----
Spinosad+Mint oil	72	8.068	5.470	10.915	1.909 ± 0.134	2.539

*SR= Synergistic ratio

Biochemical Studies:

The effect of the LC₅₀ value of Spinosad (only or in combination with 0.3% mint oil) was evaluated on some biochemical parameters of *S. littoralis* 2nd instar larval body homogenate after 120 h of treatment.

1.Acetylcholinesterase (AChE) Activity in the Treated and Untreated Larvae of *S. littoralis*:

Data represented in Table (2) recorded an inhibition in AChE activity in the treated 2nd

instar larvae with Spinosad only ($108.667 \mu\text{g}$ acetylcholine bromide released/min./gm.b.wt) or combined with mint oil ($53.333 \mu\text{g}$ acetylcholine bromide released/min./gm.b.wt) compared to the untreated larvae ($120.000 \mu\text{g}$ acetylcholine bromide released/min./gm.b.wt). In this respect, more significant reduction in AChE activity was induced in treated larvae with Spinosad when combined with 0.3% mint oil. AChE terminates the nerve impulses in cholinergic synapses of the nervous system by hydrolyzing the neurotransmitter acetylcholine (Salgado *et al.*, 1998). Spinosad as a bio-control agent acted through excitation of the pest nervous system, which in turn induce alteration in the function of nicotinic and GABA – gated ion channels which leads to involuntary muscle contractions and tremors (Thompson *et al.*, 1995). The results agree with those of Abd El- Mageed and El-Gohary (2006) who found that Spinosad induced a reduction in the AChE activity by -39.29% lower than the control in the laboratory strain of *S. littoralis*. They concluded that the change of Spinosad response could be associated with the reduction in AChE activity. In this field of the study, Megahed *et al.* (2013) reported that Spinosad induced a reduction in AChE activity in the treated 4th instar larvae of *S. littoralis*. However, Price (1988) stated that the sensitivity of AChE did not necessarily mean that pests would be susceptible to the particular chemical when it was used as an insecticide, as the toxicity of a particular compound depended on many factors including cuticle penetration and metabolic processes.

Table (2): Acetylcholinesterase (AChE) activity in *S. littoralis* 2nd instar larval body homogenate after 120 h of treatment with spinosad (only or mixed with 0.3% mint oil).

Treatments	AChE activity (μg acetyl choline bromide released/min./gm.b.wt)	
	Mean \pm SE	Activity ratio
Control	$120.000^{\text{a}} \pm 0.577$	
Spinosad	$108.667^{\text{b}} \pm 0.333$	0.906
Spinosad+ mint oil	$53.333^{\text{c}} \pm 0.882$	0.444
LSD	2.209	-----

Means followed by different letters are significantly different, ($P < 0.05$).

2. α - and B-Esterases Activity in the Treated and Untreated Larvae of *S. littoralis*:

The data represented in Table (3) recorded a reduction in α - and β -esterases activity in the treated 2nd instar larvae of *S. littoralis*, compared with the untreated larvae. When Spinosad combined with 0.3% mint oil, a significant reduction in β -esterase activity was observed, while α -esterase activity had a non-significant reduction, compared to treatment with Spinosad only. General esterases are a large and diverse group of hydrolases that hydrolyse numerous substrates including esters and certain non-ester compounds. Numerous studies have demonstrated that esterases play an important role in conferring or contributing to insecticide detoxifications in insect and other arthropod species (Mouches *et al.*, 1986). The present results are in agreement with Hassan and Abdel-Hafez (2009), they reported that Spinosad reduced the activity of esterases in the haemolymph of the 2nd larval instar of *S. littoralis* compared to the untreated larvae. Whereas, Abd El-Mageed and El-gohary (2006) found that α -esterase activity decreased while β -esterase activity significantly increased after treating resistant strain of *S. littoralis* 4th instar larvae with Spinosad.

Table (3): α - and β -esterases activity in *S. littoralis* 2nd instar larval body homogenate after 120 h of treatment with Spinosad (only or combined with 0.3% mint oil).

Treatments	α -esterase ($\mu\text{g } \alpha\text{-naphthol/min./gm.b.wt}$)		β -esterase ($\mu\text{g } \beta\text{-naphthol/min./gm.b.wt}$)	
	Mean \pm SE	Activity ratio	Mean \pm SE	Activity ratio
Control	2491.000 ^a \pm 5.859		1266.000 ^a \pm 1.528	
Spinosad	2260.000 ^b \pm 10.408	0.907	928.000 ^b \pm 2.517	0.733
Spinosad+mint oil	2274.333 ^b \pm 5.364	0.913	824.333 ^c \pm 2.333	0.651
LSD	26.160	-----	7.505	-----

Means followed by different letters are significantly different, ($P < 0.05$).

3. Phosphatases (ACP and ALP) Activity in the Treated and Untreated Larvae of *S. littoralis*:

The obtained results in Table (4) show a significant reduction in acid and alkaline phosphatases (ACP and ALP) activity after treatment with Spinosad compared with the untreated larvae. This inhibition in the enzyme's activity was reduced when Spinosad combined with 0.3% mint oil. Data revealed significant differences in both enzymes' activity in treated larvae with either Spinosad only or in combination with 0.3% mint oil.

The reduction in ACP and ALP activities might be related to the action of treatment, which created alkaline media to the enzymes which led to enzyme inhibition (Farag, 2015). This inhibition may be due to the binding between the bio-agent and the phosphatase enzymes. In addition, this enzyme inhibition might be due to strong reduction of ecdysone which is followed by subsequent reduction in number of lysosomes and in turn decrease levels of ACP (Hassan, 2002). ACP and ALP are important biomarkers because they are involved in adaptive cellular response to the potential cytotoxicity and genotoxicity of pollutants (Leohner *et al.*, 2001). The obtained data are in accordance with those of Abd El-Mageed and El-gohary (2006), they reported that Spinosad induced high significant reduction in ALP activity compared to the untreated larvae of *S. littoralis*. The current results are also in harmony with those recorded by Assar *et al.* (2016) that using Spinetoram against *S. littoralis* larvae caused a significant reduction in both ACP and ALP activities compared to the untreated larvae. Yan *et al.* (2012a) explained that the treatment of *Lymantria dispar* L. larvae with Spinosad LC₅₀ value affected ALP activity, where it was increased first significantly and then reduced at different time intervals. On the other hand, Aziz *et al.* (2013) reported that any organ was directly exposed to toxicants or xenobiotics, enzymes activity might be increased or decreased due to the denaturation of their active sites.

4. Phenoloxidase Activity in the Treated and Untreated Larvae of *S. littoralis*:

Results represented in Table (5) showed that phenoloxidase activity increased in all the treated larvae than that in the untreated ones. This increase was significant in treatment with Spinosad only (10.533 O.D.units/min./gm.b.wt), while it was non-significant in its mixture with mint oil that recorded 6.633 O.D.units/min./gm.b.wt, compared to the activity in the untreated larvae (6.320 O.D.units/min./gm.b.wt). Therefore, these data could explain the enhanced toxicity of the tested Spinosad+mint oil mixture than that of Spinosad only. For that may be using mint oil interrupted the defense mechanism of the pest to the bio-agent. Insects defend themselves against pathogens through innate mechanisms; as increased phenoloxidase activity (Valadez-Lira *et al.*, 2012). The present results are in accordance

with those of Piri *et al.* (2014), they found that Spinosad induced a significant increase in phenoloxidase activity in *Glyphodes pyloalis* (Walker) larvae compared to the untreated larvae. Phenoloxidase is an important component of insect immune systems. Its activity had been shown to be correlated with resistance to some parasites or pathogens across species (Nigam *et al.*, 1997). Using phenoloxidase activity as a physiological parameter might also help in the determination of immune response activation against entomopathogenic microbial infections (Narayanan, 2004).

Table (4): Alkaline and acid phosphatases activity in *S. littoralis* 2nd instar larval body homogenate after 120 h of treatment with Spinosad (only or combined with 0.3% mint oil).

Treatments	Alkaline phosphatase (mU/gm.b.wt)		Acid phosphatase (mU/gm.b.wt)	
	Mean ± SE	Activity ratio	Mean ± SE	Activity ratio
Control	3278.333 ^a ± 7.265		625.667 ^a ± 4.978	
Spinosad	1886.667 ^c ± 10.138	0.575	551.667 ^c ± 6.009	0.882
Spinosad+mint oil	2428.000 ^b ± 4.163	0.741	594.000 ^b ± 8.327	0.949
LSD	26.270	-----	22.799	-----

U= unit of enzyme activity.

Means followed by different letters are significantly different, (P < 0.05).

Table (5): Phenoloxidase activity in *S. littoralis* 2nd instar larval body homogenate after 120 h of treatment with Spinosad (only or combined with 0.3% mint oil).

Treatments	Phenoloxidase (O.D.units/min./gm.b.wt)	
	Mean ± SE	Activity ratio
Control	6.320 ^b ± 0.117	
Spinosad	10.533 ^a ± 0.291	1.667
Spinosad + mint oil	6.633 ^b ± 0.145	1.050
LSD	0.690	-----

Means followed by different letters are significantly different, (P < 0.05).

In conclusion, the present laboratory investigation suggest that adding mint oil could enhance Spinosad performance and it is considered a useful addition for controlling *S. littoralis* larvae affecting its enzymes activity, enhancing its susceptibility to the tested bio-agent and reducing the cost of its control via reducing the insecticidal rates used, but further laboratory, semi-field and open field experiments are still needed to confirm the results.

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ARABIC SUMMARY

دور زيت النعناع في تحسين سمية سبينوساد ضد دودة ورق القطن سبودوبترا ليتوراليس وعلاقته بنشاط بعض الإنزيمات.

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- 1- معهد بحوث وقاية النباتات - مركز البحوث الزراعية.
- 2- قسم علم الحشرات - كلية العلوم - جامعة عين شمس.

استهدف هذا البحث دراسة تأثير اضافة زيت النعناع الي سبينوساد علي نشاط بعض الانزيمات المتعلقة بسمية المركب ضد دودة ورق القطن سبودوبترا ليتوراليس. أثبتت النتائج المتحصل عليها أن اضافة زيت النعناع بتركيز 0,3% الي محلول المبيد الحيوي سبينوساد أدى الي زيادة سمية المركب ضد دودة ورق القطن سبودوبترا ليتوراليس حيث انخفضت نسبة التركيز نصف المميت مقارنة بالمبيد منفردا من 20,482 الي 8,068 جزء في المليون عند خلط المبيد بزيت النعناع. كما أوضحت الدراسات البيوكيميائية في اليرقات المعاملة بمخلوط سبينوساد مع 0,3% من زيت النعناع انخفاض معنوي في نشاط إنزيمات الأستيل كولين إسترايز، بينما إسترايز و الفينول أوكسيديز، بينما زاد نشاط إنزيمات الفوسفاتيز القاعدي و الفوسفاتيز الحامضي مقارنة بنشاط هذه الإنزيمات في حالة المعاملة بسبينوساد منفردا، مما يؤكّد أهمية دور زيت النعناع في التأثير علي نشاط هذه الإنزيمات تجاه سبينوساد، وقد يُعد تفسيرا لزيادة سمية المبيد الحيوي سبينوساد عند خلطه بزيت النعناع ضد دودة ورق القطن سبودوبترا ليتوراليس.