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Susceptibility of Field and Laboratory Strains of Cotton Leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) to Spinosad Pesticide under Laboratory Conditions

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Abstract

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The susceptibility of field and laboratory strains against all instars larvae of *S. littoralis* to spinosad pesticide after a 24- and 48-h exposure and under laboratory conditions was investigated. As a result against 1st instar larvae, the LC_{50} values after 24 h were 12 and 0.275 µg/ml for laboratory and field strain, respectively. In addition, the resistance ratio (RR) of 1st instar was 43.64-fold. In this interim, the 48 h LC_{50} values were 8.7 and 0.18 µg/ml for laboratory and field strain, respectively and the RR was 48.33-fold, which revealed the field strain was more susceptible to spinosad than the laboratory strain. Distinctly similar trend was shown for later instar larvae stages. For instance, in 6th instar larvae, the LC_{50} values after a 24-h exposure to spinosad were 1100 and 105 µg/ml for the laboratory and field strain, respectively, and the RR value was 10.48-fold. Furthermore, after a 48-h exposure, the LC_{50} values for laboratory and field strains were 500 and 42 µg/ml, respectively, with RR value being 11.90-fold. On the other hand, according to relative tolerance values, the 6th instar larvae were the most tolerant instar of all the instars tested. The susceptibility of 6th, 5th, and 4th instar larvae was comparable and significantly lower than that of 3rd, 2nd, and 1st instar larvae. However, the 1st instar was the least tolerant. The results implied that spinosad may play a potential role in the control of *S. littoralis* and, therefore, it is considered a promising tool in integrated pest management program to control Cotton leafworm which is becoming resistant to conventional pesticides in Egypt.

Keyword: spinosad; *Spodoptera littoralis*; cotton; insecticide resistance; integrated pest management (IPM)

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).

The control of Cotton leafworm is complicated due to its high resistance to most of the currently used pesticides classes. Their widely indiscriminate use moreover results in set up into environmental contamination, threat to wildlife populations, and serious public health concerns over food safety (Funderburk *et al*. 1993; Ahmed 2014; El-Geddawy *et al*. 2014). Recently, the global occurrence of Cotton leafworm and its growing resistance problem have presented an area of great needs for more effective and acceptable control methods such as alternative safe pesticide with the advantage of its respect to the environment.

On the other hand, integrated pest management (IPM) strives to find the right tactics or combina-

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tion of certain tactics to secure the main crop and to minimise the economic crisis. These control tactics include chemical, biological, genetic, culture, and physical controls (Pedigo 1996; Mesbah *et al*. 2007). In this trend, biopesticides have attracted attention and interest among those concerned to develop an environmentally friendly and safe tool towards the Integrated Crop Management (ICM). Moreover, biopesticides offer a unique opportunity in developing countries to explore and develop their own natural biopesticide resources in the field of crop protection. Such endeavours will assist in conserving foreign cash reserves, improve safety to applicators and consumers, and protect the environment (El-Geddawy *et al*. 2014). Hence, spinosad is considered a promising biopesticide in controlling many pests regardless of its potent toxicity and low toxicological effects on the environmental components (Nannan *et al*. 2000; Hendrix *et al*. 2001; Arora 2003; Pineda *et al*. 2007).

In this study, we aimed to assess the susceptibility of field and laboratory strains against all instars larvae of *S. littoralis* to spinosad pesticide after a 24- and 48-h exposure and under laboratory conditions.

MATERIAL AND METHODS

Laboratory strain. The laboratory strain of Cotton leafworm, *S. littoralis,* has been reared in the laboratory of the Plant Protection Department Research, Faculty of Agriculture, Assiut University, Egypt for more than 25 years (without any exposure to chemicals). Insects were reared under controlled conditions in the incubator at 26 ± 2°C and 65 ± 10% relative humidity with 8 light : 16 h darkness photoperiod. Larval jars were supplied with fresh Castor leaves, *Ricinus communis* L., as a source of food which was provided daily. The adults were kept separately and mated on the third day of emergence in clean jars (250 g), fed on 10% honey solution, and fresh green leaves of Tafla, *Nerium oleander* (L.) were provided for egg laying.

Field strain. One field strain of all the eggs masses was collected from different localities in Assiut Governorate, Egypt and reared for one generation under the same laboratory conditions as the laboratory strain described above. However, this field strain was used for all experiments.

Pesticide. The formulation of spinosad used in the bioassay was Spintor® (24% SC, registration No. 1050)

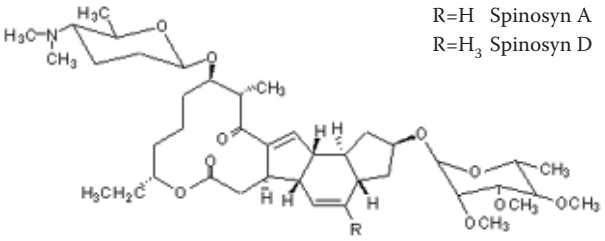

Figure 1. The structures of the spinosyn A and D

obtained from Dow AgroSciences Co., Cairo, Egypt. The product is a mixture of two active components, Spinosyn A and D produced by fermentation of the soil actinomycetes, *Sacharopolyspora spinosa* (Figure 1).

Bioassay test. Initially, a pilot test was conducted to choose the range of concentrations used for field or laboratory strain. Spinosad was dissolved in distilled water at different concentrations and leaves of castor bean (approximate radius (r) = 5 cm) were dipped in each concentration for 10 s and left to dry under laboratory conditions. Leaves were put on the bottom of plastic cans covered with a sieved lid. Then 10 larvae of 1st, 2nd or 3rd instar were added, and for 4th, 5th, and 6th instar 5 larvae were used. Four replicates were performed for each concentration and control (leaves dipped in distilled water only). The technique was performed for various instars of field and laboratory strains. The dead larvae were recorded 24 and 48 h after exposure and the percentage of mortality was estimated and corrected according to Abott's formula (Abott 1925).

Bioassay data were pooled and analysed (the LC_{50}, LC_{90}, and 95% confidence limit values) according to the methods described by Litchfield and Wilcoxon (1949) and Swaroop *et al*. (1966). However, the resistance ratio (RR) was calculated by dividing the LC_{50} value of the laboratory strain by the LC_{50} value of the field strain. Further, relative tolerance (RT) was calculated by dividing the LC_{50} value of the 6th instar larvae of the laboratory strain by the LC_{50} value of the 1st instar larvae of the laboratory strain.

RESULTS

The LC_{50} and LC_{90} values for all instars after a 24- and 48-h exposure are shown in Table 1, and RT of various *S. littoralis* larval instars to spinosad after a 24- and 48-h exposure on the basis of LC_{50} values is shown in Table 2.

Initially, the LC_{50} and LC_{90} values for 1st instar larvae after a 24-h exposure to spinosad were 0.275

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Table 1. Probit analysis parameters of various larval instars of field and laboratory strain of *S. littoralis* after being fed on spinosad treated leaves for 24 and 48 hours

Instar	Strain	Treated leaves for 24 h								Treated leaves for 48 h							
		LC_{50} (µg/ml)	confidence limits of LC_{50}		LC_{90} (µg/ml)	confidence limits of LC_{90}		slope	RR*	LC_{50} (µg/ml)	confidence limits of LC_{50}		LC_{90} (µg/ml)	confidence limits of LC_{90}		slope	RR*
			upper	lower		upper	lower				upper	lower		upper	lower		
1st	laboratory	12	15.617	9.221	58	75.48	44.57	3.33	43.64	8.7	11.27	6.72	33	42.75	25.47	2.78	48.33
	field	0.275	0.379	0.199	1.8	2.48	1.31	4.33		0.18	0.26	0.12	1.2	1.75	0.82	4.42	
2nd	laboratory	31.5	39.38	25.20	120	150.01	96.00	2.77	9.84	19.0	23.00	15.70	62.0	75.04	51.22	2.13	12.18
	field	3.2	4.68	2.19	44	64.37	30.08	6.98		1.56	2.42	1.13	6.6	9.66	4.51	3.42	
3rd	laboratory	180	263.24	123.08	1050	1535.10	718.19	3.94	20.93	57	79.04	41.11	180	249.61	129.80	2.50	23.75
	field	8.6	13.12	5.64	47	71.67	30.82	3.91		2.4	3.62	1.59	11.0	16.60	7.29	3.16	
4th	laboratory	380	526.79	274.11	1000	1386.30	721.34	2.11	15.20	230	318.95	165.88	7.40	1026.16	533.64	2.50	20.91
	field	25	45.67	13.68	540	986.58	295.57	10.82		11	19.75	6.13	125	224.45	69.61	6.62	
5th	laboratory	600	842.82	427.14	2350	3301.05	1672.96	3.00	13.33	350	510.40	240.01	1700	2478.60	1165.98	3.38	14.58
	field	45	76.71	26.39	540	920.70	316.72	6.86		24	41.63	13.84	250	433.57	144.15	5.92	
6th	laboratory	1100	1632.7	741.10	6600	9796.38	4446.54	4.16	10.48	500	790.18	316.38	3500	5531.40	2214.63	4.38	11.90
	field	105	163.60	67.39	840	1308.72	539.15	4.96		42	67.26	26.23	240	384.31	149.88	3.73	

*Resistance ratio (RR) calculated by dividing the LC_{50} value of laboratory strain by the LC_{50} value of field strain

Table 2. Relative tolerance (RT) of various *S. littoralis* larval instars to spinosad after a 24-h and 48-h exposure on the basis of LC_{50} values

Instar	Strain	RT* after 24-h exposure					RT* after 48-h exposure				
		1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
6th	laboratory	91.70	34.92	6.11	2.89	1.83	57.47	26.32	8.77	2.17	1.43
	field	381.00	32.8	12.12	4.2	2.33	233.30	26.92	17.50	3.81	1.75
5th	laboratory	50.00	19.05	3.33	1.58		40.23	18.42	6.14	1.52	
	field	163.64	14.06	5.23	1.80		133.33	15.38	10.00	2.81	
4rh	laboratory	31.67	12.06	2.11			26.44	12.10	4.04		
	field	90.91	7.81	2.91			61.11	7.05	4.58		
3rd	laboratory	15.00	5.71				6.65	3.00			
	field	31.27	2.69				13.33	1.54			
2nd	laboratory	2.63					2.18				
	field	11.64					8.67				

*RT calculated, for example, by dividing the LC_{50} value of 6th instar larvae of laboratory strain by the LC_{50} value of 1st instar larvae of laboratory strain

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and 0.18 µg/ml for the field strain, and 12.0 and 58.0 µg/ml for the laboratory strain, respectively.

However, comparing the LC_{50} and LC_{90} values after a 24-h action of spinosad tested against 1st instar of both strains, it was concluded that the field strain is more susceptible (43.64-fold on the basis of LC_{50} value or 32.22-fold on the basis of LC_{90} value) than the laboratory strain. At the exposure period of 48 h, the LC_{50} value of the laboratory strain was 48.33-fold the LC_{50} value of the field strain whereas on the basis of LC_{50} value it was 27.5-fold.

Further, the 24-h LC_{50} and LC_{90} values of spinosad tested against 2nd instar larvae of field strain were 3.2 and 44.0 µg/ml, and for laboratory strain, the values were 31.50 and 120.0 µg/ml, thus the rate of resistance of laboratory strain was 9.84-fold on the basis of LC_{50} and 2.73-fold on the LC_{90} basis, as compared with field strain. Comparing the LC_{50} value of 2nd instar larvae with that of 1st instar larvae, the 2nd instar is more tolerant (more than 10-fold in the field strain and more than 2-fold in the laboratory strain).

In regards to the LC_{50} value of spinosad that was tested against field strain, the value was 8.6 µg/ml as compared with the LC_{50} value of laboratory strain (180.0 µg/ml) indicating that laboratory strain is 20.73-fold more resistant than field strain. The LC_{90} value for field strain was 47.0 µg/ml while for laboratory strain it was 1050.0 µg/ml. On the basis of LC_{90} value, laboratory strain is 22.3-fold more resistant as compared with field strain. The 24 h LC_{50} value of spinosad tested against 3rd instar larvae of field strain was more than two-fold than that for 2nd instar larvae, whereas in the laboratory strain LC_{50} value for 3rd instar larvae was more than fold the LC_{50} value of 2nd instar larvae.

Furthermore, the 24-h LC_{50} and LC_{90} values of spinosad action on 4th instar larvae of laboratory strain were 380 and 1000 µg/ml and for field strain the values were 25 and 540 µg/ml. Laboratory strain showed 15.2-fold higher resistance than field strain on the basis of LC_{50} value, but it was only 1.85 µg/ml based on LC_{90} value. After a 48-h exposure, the LC_{50} value of spinosad against laboratory and field strain was 30 and 11 µg/ml, whereas the LC_{90} value was 1026.16 and 284.45 µg/ml, respectively. The laboratory strain showed 20.9- and 5.92-fold resistance than the field strain according to LC_{50} and LC_{90} values.

On the basis of a 24-h LC_{50} value of spinosad, 4th instar larvae of laboratory strain showed 31.67-, 21.06-, and 2.11-fold resistance as compared with 1st, 2nd, and 3rd instar larvae, respectively. For field strain the resistance was 90.91-, 7.81-, and 2.91-fold, respectively.

Interestingly, the slope values for laboratory and field strains after a 24- and 48-h exposure indicate that field strain is more homogeneous in response to spinosad than laboratory strain.

The 24-h LC_{50} value was 600 and 45 µg/ml for laboratory and field strains, whereas the LC_{90} value in respective was 2350 and 540 µg/ml. After a 48-h exposure, the LC_{50} values were 350 and 24 µg/ml and LC_{90} values were 1700 and 250 µg/ml. Based on the 24-h LC_{50} value of laboratory and field strain, it might be concluded that laboratory strain was 13.33-fold more resistant than field strain.

The 24-h LC_{50} value of spinosad tested against 5th instar larvae of laboratory strain was 50-, 19.05-, 3.33-, and 1.58-fold that of 1st, 2nd, 3rd, and 4th instar larvae, respectively. For field strain, the values were 163.64-, 14.06-, 5.23-, and 1.8-fold, respectively (Table 2). The 5th instar larvae were significantly more resistant than 1st and 2nd instars, and slightly more tolerant than 3rd and 4th instars.

The relatively high slope value for field strain in both the 24- and 48-h exposure revealed a more homogeneous response of field strain to spinosad than laboratory strain. The 24-h LC_{50} value of spinosad tested against 6th instar larvae was 1100 and 105 µg/ml for laboratory and field strain giving 10.48-fold RR. The 24-h LC_{90} values were 6600 and 840 µg/ml for laboratory and field strains with 7.86 RR. The 48-h LC_{50} values were 500 and 42 µg/ml for laboratory and field strains with resistance rate of 11.90, whereas the 48-h LC_{90} values were 3500 and 240 µg/ml for the two strains with a 14.58-fold RR. Comparing the slope value after the 24- and 48-h exposure, it seems that 6th instars of both strains exhibit similar response to spinosad.

The 24-h LC_{50} value of spinosad tested against 6th instar larvae of laboratory strain was 91.7-, 34.92-, 6.11-, 2.89-, and 1.83-fold the LC_{50} value of 1st, 2nd, 3rd, 4th, and 5th instar larvae, respectively. For field strain, RT was 381-, 32.8-, 12.21-, 4.2-, and 2.33-fold, respectively. The same trend was observed in 48-h exposure results (Table 2). The 6th instar larvae of both strains showed a higher significant tolerance to spinosad than 1st and 2nd instars, a moderate tolerance compared to 3rd instar. However, in response to spinosad 5th instar was comparable with 6th instar.

DISCUSSION

Generally, spinosad showed great effects against *S. littoralis* larvae. However, the effect was significant

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on field strain in comparison with laboratory strain. The LC_{50} value of spinosad effect on 1st instar after a 48-h exposure was 0.18 and 8.7 µg/ml for field and laboratory strain, respectively. Furthermore, the value increased in the successive instars to reach 500 and 42 µg/ml for 6th instar larvae of laboratory and field strains, respectively. For all instars, and at a 24- and 48-h exposure, the field strain was more susceptible than laboratory strain. Interestingly, the resistance rate was much higher in 1st, 3rd, and 4th instars. The reasons behind the high level of insensitivity of the laboratory strain to spinosad could be the lacking exposure of the laboratory strain to natural microorganisms or the lack of the exposure towards the severity of pesticides selective pressure in comparison to field strain which leads to a resistant trend against spinosad the active ingredient of which is a microorganism. Therefore, the laboratory strain may invalidate their ability to the microbial pesticides or biopesticides such as spinosad. Plus, the reasons could be xenobiotic metabolism changes or altered toxicokinetics and behaviour differences. Further, there are some correlations between the high level of resistance to spinosad and various kinds of insecticides, especially those having a similar mode of action (e.g. neonicotinoids, sulfoximine, and nereistoxin analogs) which lead to cross-resistance. However, in agreement with the present findings, Temerak (2002) found that spinosad was more active against field strain of *S. littoralis* larvae than laboratory strain. The LC_{50} values of spinosad against 1st–6th instar larvae of field strain were 0.54, 1.19, 1.866, 18.17, 40.5, and 61.02 µg/ml, however, for laboratory strain they were 4.84, 10.9, 66.86, 1559.63, 2690.39, and 4013.23 µg/ml, respectively. The LC_{50} values for the last three instars of laboratory strain (4th, 5th and 6th) were much higher than those recorded in the present study whereas; in field strain the reverse was noticed. This may be due to the variation in the strain and the environmental condition prevailing in the area. In contrast, Ayadin and Gürkan (2006) evaluated lethal dose bioassays of spinosad on 3rd instar larvae of *S. littoralis* using the leaf dip method. The LC_{50} values for field and susceptible strains were 43.691 and 10.037 µg/ml, respectively. The field strain was approximately 4.4-fold less sensitive than the susceptible strain. Saunders and Bret (1997) stated that spinosad undergoes photodegradation when exposed to sunlight and is rapidly metabolised when washed into soil. On the other hand, the variation in the result of spinosad toxicity may be due to the application techniques used. Redding and Nead (1998) found that using hollow cone nozzles (TX6) with 41.3.7 kPa (60 psi) for the application of tracer provided better coverage control as compared with the same type of nozzles at lower pressure (27.3.5 kPa or 40 psi) or different nozzles (TX15 and 8003 flat fan) at the same or lower pressures. The higher tolerance of laboratory strain than of field strain was confirmed by many authors. During their research on natural product, Abo-Elghar *et al.* (1994) found that laboratory strain of cotton leafworm was more tolerant to *Bacillus thuringiensis* and Abamectin (fermentation of the actinomycete, *Streptomyces avermitilitis*) than field strain. In spinosad bioassays, Mascarenhas *et al.* (1998) demonstrated that field strains of *Spodoptera exigua* had significantly lower LC_{50} values than reference strain. Moulton *et al.* (1999) found that field population of 2nd and 3rd instar of *Spodoptera exigua* was 3- to 70-fold less susceptible to spinosad than a reference laboratory population. Against, field collected strains of the soybean pest, *Pseudoplusia includens*. Mascarenhas and Boethel (1997) found in spinosad bioassay that field strain had lower LC_{50} than the susceptible USDA reference strain.

In conclusion, spinosad showed variable degree of toxicity against *S. littoralis*. According to the present investigation and the available literature, spinosad proved to be the most active biopesticide against cotton pests. Its efficacy was comparable to that of synthetic insecticides.

One essential key of the pest management strategy is the use of safe and alternative products to ensure that continual selectivity will not occur and that any possibility of pest resistance is avoided, or at least significantly delayed. The naturalyte insect control class represented by spinosad provides the potential option because the members of this class show no cross-resistance to other product classes including pyrethroids, carbamates, organophsophates, and even newer classes, such as fipronils, imidaclopid, and avermectins. Biochemical and molecular biological investigation should follow to better elucidate the mode of action of spinosad on *S. littoralis*. Thus spinosad has gained a great interest especially after the establishment of organic farms in Egypt.

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