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Toxicological Evaluation of Selected Biopesticides and One Essential Oil in comparison with Indoxacarb pesticide on Cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) under laboratory Conditions

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ABSTRACT

The Cotton leafworm, *Spodoptera littoralis* (Boisd.) is considered one of the highly destructive insect pests in Egypt. In this study, we evaluate selected biopesticides [Emamectin benzoate (5% SG), Azadirachtin (0.03% EC), *Beauveria bassiana* ($1*10^8$ conidial /ml^3), and Azadirachtin (0.03% EC)] and one essential oil [Black pepper extract] in comparison with one chemical pesticide [Indoxacarb (15% SC)] on the second and fourth instar larvae of *Spodoptera littoralis*. However, Emamectin benzoate (5% SG) is the most potent compound among the tested compounds followed by Azadirachtin (0.03% EC), Black pepper extract, *Beauveria bassiana* ($1*10^8$ conidial /ml^3), Indoxacarb (15% SC), and Spinetoram (12% SC) compounds. Further, the toxicity increased dramatically after 48 h and continues increased after 72 h compared to 24 h data especially for Emamectin benzoate (5% SG) and Indoxacarb (15% SC) on second and fourth instar larvae. Moreover, according to the toxicity index, Emamectin benzoate (5% SG) is the most toxic compound (100%) followed by Azadirachtin (0.03% EC), Black pepper extract, and *Beauveria bassiana* ($1*10^8$ conidial /ml^3) which observed after 24 h exposure for both second and fourth instar larvae, in contrast, these results have been slightly changed after 48 and 72 h exposure. Importantly, the tolerance ratio (TR) ranged from 1.32 to 11.00 based on the 24, 48, 72 h exposure results. Accordingly, utilization of these biocontrol agents for controlling the Cotton leafworm provides a promising alternative to conventional insecticides in Egypt.

Keywords: Emamectin benzoate; Black pepper extract; Biopesticides; *Spodoptera littoralis*, Biopesticides

INTRODUCTION

The Cotton leafworm, *Spodoptera littoralis* (Boisd.) considered one of serious insect pests in Egypt in that it can attack many important field crops, vegetables, and fruits all year round [1,2,3,4]. However, the frequently use of conventional pesticides such as organophosphates, carbamates, and pyrethroids in controlling *Spodoptera littoralis* has been led to develop resistance and cross resistance to the pesticides that related to these groups regardless to the heavy and unwise uses [5,6,7,8,9]. Thus, the results of using heavy application of pesticides have resulted in residues of chemical pesticides in the environmental aspects [10,11]. From this point of view, it is very important to constrict the use of chemical pesticides that considered one of the environmental impacts and anticipate pesticide alternatives. One of the significant alternatives is biopesticides. Biopesticides share by more than 1% of the total world pesticide market in spit of their advantages, for instance, their safety to non-target organisms and mammalian systems, their environmental fit and the relative ease of registering a biopesticide in USA which may hopefully be followed eventually by the European Union (EU) [12]. Recently, biopesticides have been gaining increased attention and interesting in many countries. However, organic farms are being established in Egypt [13]. Thus, in this study, we

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evaluated the toxicity of selected biopesticides [Emamectin benzoate (5% SG), Azadirachtin (0.03% EC), *Beauveria bassiana* ($1*10^8$ conidial /ml^3), and Azadirachtin (0.03% EC)] and one essential oil [Black pepper extract] in comparison with one chemical pesticide [Indoxacarb (15% SC)] on the second and fourth instar larvae of *Spodoptera littoralis* under laboratory conditions.

Materials and Methods

Strain:

A laboratory susceptible strain of the Cotton leafworm, *Spodoptera littoralis* (Boisd.) reared in the laboratory of Plant Protection Department Research building, Faculty of Agriculture, Assiut University, for more than 25 years (without any exposure to chemicals). Insects were reared under controlled conditions in an rearing room at 26 ± 2°C and 65 ± 10% RH with 8:16 L:D photoperiod. Larval jars were supplied daily with fresh Castor leaves, *Ricinus communis* L., as a source of food which was provided daily. The adult were kept separately and mated on the third day of emergence in clean jars (250 g) adults were fed on 10% sugar solution, fresh green leaves of Tafla, *Nerium oleander* (L.) were provided for egg laying.

Compounds:

The following compounds, Emamectin benzoate (5% SG), Azadirachtin (0.03% EC), *Beauveria bassiana* ($1*10^8$ conidial /ml^3), Indoxacarb (15% SC), and Spinetoram (12% SC) have been purchased from pesticide store in Assiut and Cairo cities.

Black pepper extract:

The concerned spice Black pepper (*Piper nigrum* L.) was purchased from the local market. The Black pepper extract prepared according to method adopted by Freedman et al. (1979) [14] and Abo-Elghar (1985) [15] with some modification. The seeds were grounded in an electrical mil and sieved through 0.5 mm sieve. Petroleum ether (40-60) solvent was used for extraction process.

A sample of 50 g powder was soaked in 350 ml of petroleum ether for 72 h in bottle with tight stoppers, and then the bottle was shaken for 30 min by shaker and then filtered. The extracted powder was soaked again two times in additional volumes of petroleum ether for 24 h, and filtered by using filter paper. The filterate portions were mixed and the solvent was evaporated until dryness. The crude oil obtained was weight.

Bioassay test:

Two sets of three replicates each contain 10 newly molted 2nd and 5 newly molted 4th instar larvae for each concentration of each tested compounds were used. The compound concentrations were dissolved in distilled water and for Black pepper extract in acetone. Treatment of larvae was conducted by the leaf dipping technique. Fresh and clean castor leaves, *Ricinus communis* L., were immersed for 10 second in the prepared concentrations of the tested compounds. The treated leaves were then left to dry at room temperature before being offered to the newly molted 2nd and 4th instar of *Spodoptera littoralis* larvae. Larvae were left to feed on treated leaves for 24, 48, 72 h exposure. The same numbers of larvae was used for control experiments in which larvae were offered fresh clean castor leaves dipped in distilled water for biopesticide compounds and for Black pepper extract in acetone. The percentages of mortality in larvae were recorded at 24, 48, and 72 h. The corrected mortality percentage was calculated according to Abott's formula [16].

The mortality data were pooled and analyzed (the LC_{50}, LC_{90}, and their 95% confidence limits values) using Proban probit analysis program version 1.1 [17]. Figures were done by using GraphPad Prism 6.01 software (San Diego, CA, USA).

Results and Discussions

The toxicity of selected biopesticides and Indoxacarb on second instar larvae of *Spodoptera littoralis* after 24, 48, and 72 h was shown in Table 1. However, the LC_{50} for the most toxic compound, Emamectin benzoate (5% SG), was 0.02 ppm and the LC_{50} of lowest toxic compound, Spinetoram (12% SC), was 9.36 ppm whereas the moderate toxic compounds were Azadirachtin (0.03% EC), Black pepper extract, *Beauveria bassiana* ($1*10^8$ conidial /ml^3), and Indoxacarb (15% SC), and the values of LC_{50} were 0.39, 0.74, 1.02, and 1.47 ppm respectively. After 48 h, the LC_{50} values dramatically decreased and the LC_{50} values for Emamectin benzoate (5% SG) and Indoxacarb (15% SC) were 0.005 and 0.25 ppm respectively. By 72 h, the LC_{50} values continued to decrease to be 0.001 and 0.04 ppm respectively, and the most interested finding was that the LC_{50} value of *Beauveria bassiana* ($1*10^8$ conidial /ml^3) was decreased significantly to be 0.05 ppm after 72 h exposure . On the other side, the same trend was particularly notable with slightly changed in the results of the toxicity of selected biopesticides and Indoxacarb on fourth instar larvae of *Spodoptera littoralis* after 24, 48, and 72 h (Table 2).

The toxicity of all compounds on second instar larvae was increased after 48 and 72 h (Fig. 1). For example, Emamectin benzoate (5% SG) increased in toxicity from 4.00-fold after 48 h to 20.00-fold after 72 h compared to the toxicity after 24 h, however, *Beauveria bassiana* ($1*10^8$ conidial /ml^3) compound

increased in toxicity from 2.13-fold after 48 h to 20.40-fold after 72 h. Furthermore, Indoxacarb (15% SC) increased in toxicity from 5.88-fold after 48 h to 36.75-fold after 72 h (Fig. 1). Furthermore, this trend was shown strongly on fourth instar larvae which increased after 48 and 72 h (Fig. 2), for instance, the toxicity of Emamectin benzoate (5% SG) increased from 15.00-fold after 48 h to 50.00-fold after 72 h compared to the toxicity data after 24 h whereas, for Indoxacarb (15% SC), the toxicity increased from 7.83-fold after 48 h to 28.20-fold after 72 h compared to the toxicity data after 24 h. Similar trend was slightly observed on the toxicity of all other compounds.

Table 1: Toxicity of select biopesticides and one essential oil in comparison with Indoxacarb on 2nd instar larvae of *Spodoptera littoralis* after 24, 48, and 72 h exposure.

Compounds	After 24 h			After 48 h			After 72 h		
	LC_{50} (95%CL)[a]	LC_{90} (95%CL)[a]	Slope (SE)	LC_{50} (95%CL)[a]	LC_{90} (95%CL)[a]	Slope (SE)	LC_{50} (95%CL)[a]	LC_{90} (95%CL)[a]	Slope (SE)
Emamectin benzoate (5% SG)	0.02 (0.009-0.10)	25.99 (23.07-62.28)	0.43 (0.08)	0.005 (0.002-0.01)	4.07 (0.66-8.84)	0.44 (0.08)	0.001 (0.0006-0.003)	0.47 (0.10-7.66)	0.47 (0.09)
Azadirachtin (0.03% EC)	0.39 (0.29-0.66)	1.58 (0.86-6.41)	2.13 (0.44)	0.14 (0.11-0.18)	0.44 (0.32-0.78)	2.59 (0.43)	0.10 (0.08-0.13)	0.34 (0.25-0.58)	2.59 (0.45)
Black pepper extract	0.74 (0.45-1.05)	4.68 (2.68-18.91)	1.60 (0.37)	0.51 (0.29-0.70)	2.35 (1.58-5.44)	1.93 (0.41)	0.38 (0.21-0.51)	1.27 (0.94-2.25)	2.46 (0.53)
Beauveria bassiana ($1*10^8$ conidial /ml^3)	1.02 (0.81-3.57)	1.20 (0.22-9.41)	1.19 (0.42)	0.48 (0.26-2.45)	1.05 (0.89-3.11)	0.06 (0.15)	0.05 (0.03-0.24)	0.51 (0.45-4.97)	0.07 (0.27)
Indoxacarb (15% SC)	1.47 (0.46-8.90)	603.53 (561.59-781.01)	0.48 (0.09)	0.25 (0.08-1.04)	194.65 (120.85-233.61)	0.44 (0.08)	0.04 (0.01-0.14)	29.91 (24.69-65.60)	0.45 (0.08)
Spinetoram (12% SC)	9.36 (8.31-11.57)	86.67 (56.43-97.07)	1.23 (0.50)	4.28 (1.70-7.67)	74.86 (38.90-123.67)	0.95 (0.21)	2.42 (0.89-4.33)	37.40 (19.08-67.50)	1.07 (0.22)

Table 2: Toxicity of select biopesticides and one essential oil in comparison with Indoxacarb on 4th instar larvae of *Spodoptera littoralis* after 24, 48, and 72 h exposure.

Compounds	After 24 h			After 48 h			After 72 h		
	LC_{50} (95%CL)[a]	LC_{90} (95%CL)[a]	Slope (SE)	LC_{50} (95%CL)[a]	LC_{90} (95%CL)[a]	Slope (SE)	LC_{50} (95%CL)[a]	LC_{90} (95%CL)[a]	Slope (SE)
Emamectin benzoate (5% SG)	0.15 (0.05-0.68)	79.98 (68.55-95.89)	0.47 (0.08)	0.01 (0.005-0.04)	12.25 (1.66-27.27)	0.43 (0.08)	0.003 (0.001-0.01)	1.07 (0.25-3.88)	0.52 (0.08)
Azadirachtin (0.03% EC)	0.71 (0.46-1.28)	3.04 (1.26-4.66)	2.03 (0.54)	0.22 (0.17-0.31)	0.94 (0.57-1.59)	2.08 (0.39)	0.15 (0.11-0.19)	0.49 (0.35-0.91)	2.51 (0.42)
Black pepper extract	0.99 (0.70-1.36)	4.91 (2.93-5.17)	1.84 (0.38)	0.68 (0.44-0.92)	3.26 (2.09-8.39)	1.88 (0.39)	0.50 (0.32-0.66)	1.80 (1.30-3.38)	2.30 (0.45)
Beauveria bassiana ($1*10^8$ conidial /ml^3)	1.76 (1.56-6.68)	1.85 (1.66-4.09)	1.25 (0.49)	1.03 (0.46 -1.57)	1.23 (1.04-6.83)	0.93 (0.36)	0.55 (0.21-1.68)	0.81 (0.79-9.26)	1.31 (0.36)
Indoxacarb (15% SC)	2.82 (0.84-22.11)	477.17 (293.34-542.66)	0.48 (0.95)	0.36 (0.11-1.78)	383.44 (328.91-586.42)	0.42 (0.08)	0.10 (0.02-0.36)	104.75 (100.89-283.77)	0.42 (0.08)
Spinetoram (12% SC)	15.07 (11.64-21.73)	147.99 (132.09-174.51)	1.20 (0.45)	8.11 (6.34-10.91)	86.62 (64.19-98.53)	1.24 (0.47)	5.09 (3.23-7.16)	63.17 (47.97-86.76)	0.95 (0.38)

Moreover, the toxicity index of the most toxic compound after 24 h, Emamectin benzoate (5% SG), was 100% on both second and fourth instar larvae (Fig. 3). The toxicity index for Azadirachtin (0.03% EC), Black pepper extract, *Beauveria bassiana* ($1*10^8$ conidial /ml^3), Indoxacarb (15% SC), and Spinetoram (12% SC) after 24 h was (5.13%, 2.70%, 1.96%, 1.36%, and 0.21% respectively) on second instar, and (21.13%, 15.15%, 8.52%, 5.32%, and 1.00% respectively) on fourth instar, whereas, the lowest toxicity index was slightly changed dramatically after 48 and 72 h for most of the compounds. The tolerance ratio (TR) between fourth and second instar larvae was shown in Figure 4. For 24, 48, and 72 h exposure, the highest TR was met with *Beauveria bassiana* ($1*10^8$ conidial /ml^3) after 72 h (TR=11.00) and Emamectin benzoate (5% SG) after 24 h (TR=7.50), however, in general, The most interested finding was that the rest of the TR ranged from 1.32 to 3.00 which shown unmarked variation between second and fourth instar larvae in the response of most of the compounds.

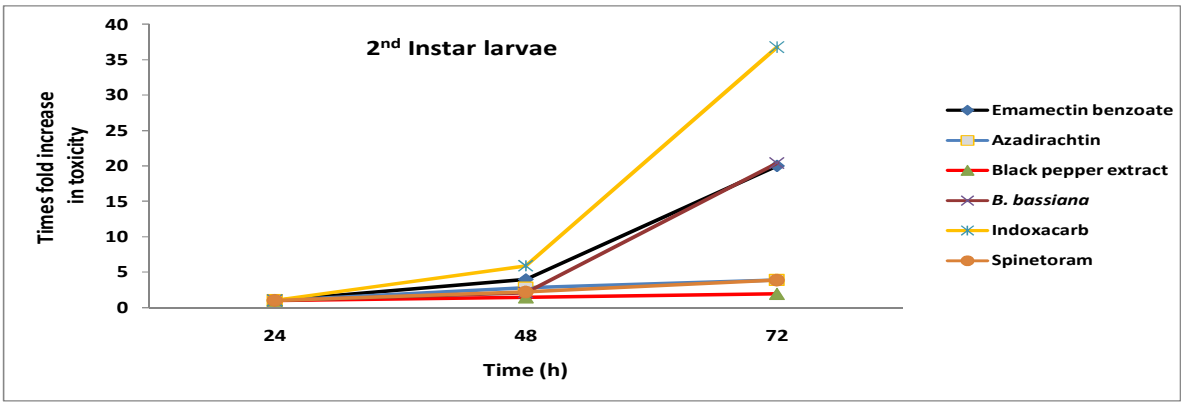

Fig. 1: Times fold increase in toxicity of selected biopesticides and one essential oil in comparison with Indoxacarb on 2nd larvae of *Spodoptera littoralis* after 24, 48, and 72 h exposure.

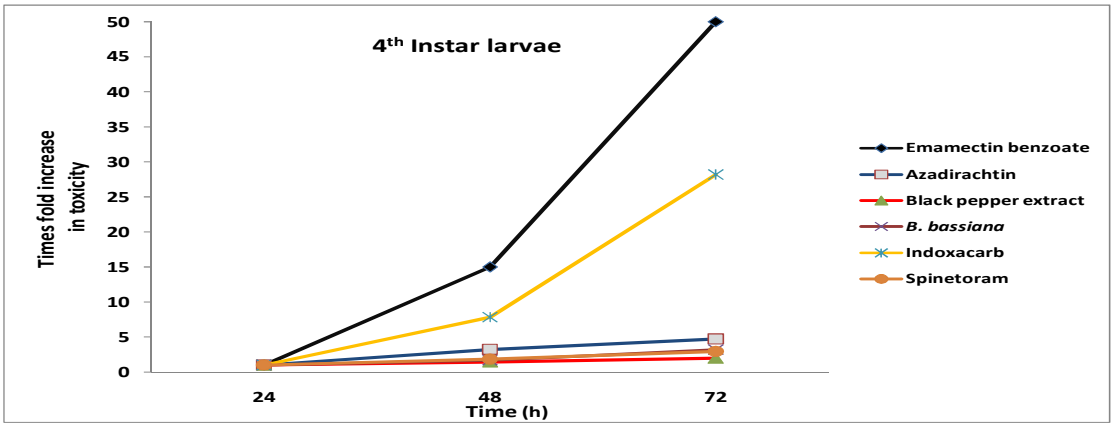

Fig. 2: Times fold increase in toxicity of selected biopesticides and one essential oil in comparison with Indoxacarb on 4th larvae of *Spodoptera littoralis* after 24, 48, and 72 h exposure.

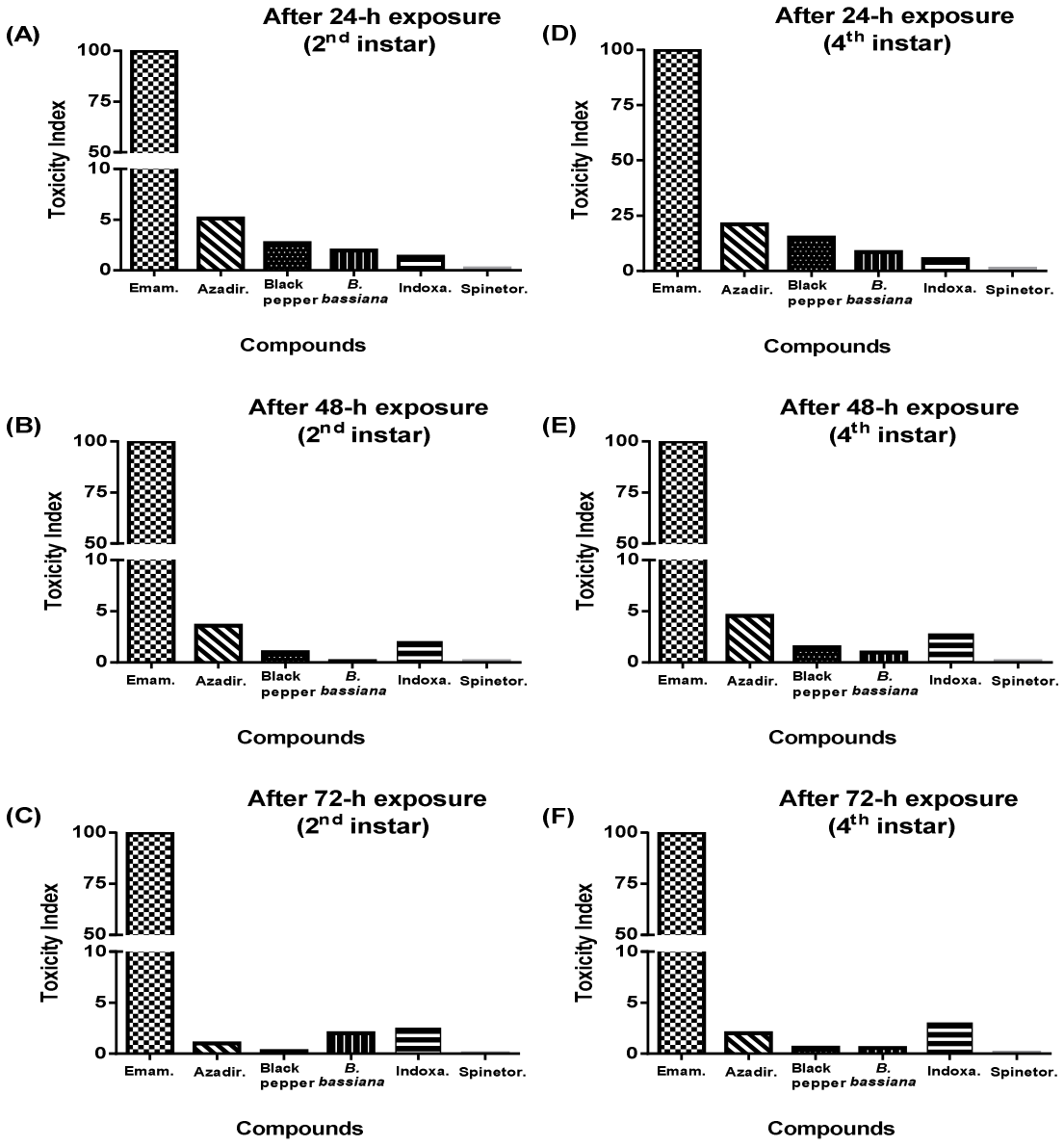

Fig. 3: Toxicity index of selected biopesticides and one essential oil in comparison with Indoxacarb on 2nd larvae of *Spodoptera littoralis* (A, B, and C) and on 4th larvae of *Spodoptera littoralis* (D, E, and F) after 24, 48, and 72 h exposure. Toxicity index = [(LC_{50} of the most toxic tested compound/LC_{50} of the tested compound) ×100].

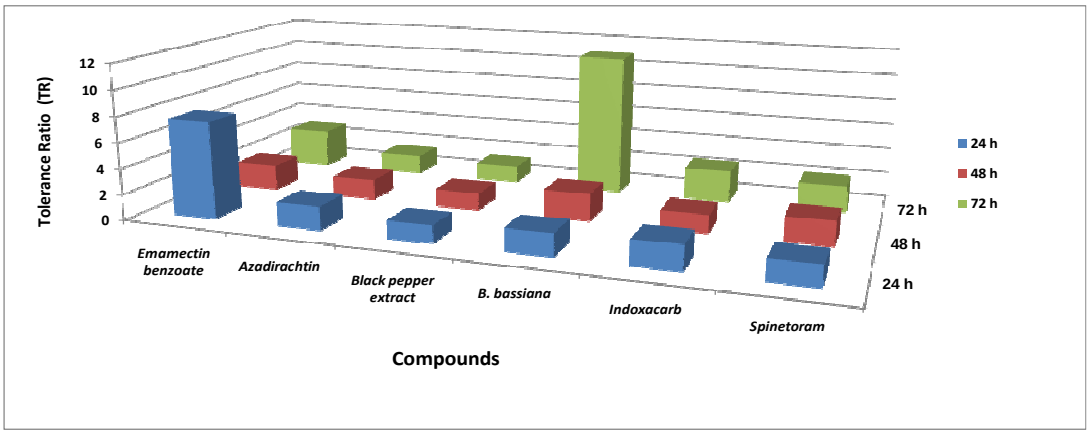

Fig. 4: Tolerance ratio of the 4th instar larvae of *Spodoptera littoralis* to biopesticides and one essential oil in comparison with Indoxacarb after 24, 48, and 72 h exposure on the basis of LC_{50} values. Tolerance ratio (TR) = LC_{50} for 4th instar larvae/LC_{50} for 2nd instar larvae).

The efficiency of different tested biopesticide compounds in the control of the 2nd and 4th instar larvae of the Cotton leafworm, *Spodoptera littoralis* (Boisd.) varied tremendously according to the instar of larvae and the structure of the tested biopesticides. Generally, the 2nd instar larvae were more sensitive to the six tested compounds than the 4th instar larvae of *Spodoptera littoralis*. However, Emamectin benzoate is considered the most potent biopesticide among all compounds tested.

Emamectin benzoate is isolated from fermentation of *Streptomyces avermitilis*, a naturally occurring soil actinomycete and it acts by stimulating the release of γ-aminobutyric acid [Gamma Amino Butyric Acid (GABA)], an inhibitory neurotransmitter, thus causing paralysis of the lepidoptera, which stop feeding within hours of ingestion [18]. Further, in agreed with our results, Ahmad et al. [19] stated that Emamectin benzoate, a derivative of Abamectin, is quite effective against a number of lepidopterous insect pests including *Spodoptera exigua* and *Spodoptera litura*. Moreover, in agreement with earlier findings, Saeed et al. [20] evaluated four different pesticides, Emamectin benzoate, Lufenuron, Chlorpyrifos, and Cypermethrin on 2nd instar larvae of *Spodoptera exigua* under laboratory conditions and they found that among all the tested pesticides, Emamectin benzoate gave the lowest LC_{50} value i.e., 0.005 mg/l (95% FL: 0.004-0.007 mg/l) followed by Lufenuron i.e., LC_{50} value 0.65 mg/l (95% FL: 0.38-0.93 mg/l). On another study, The LC_{50} value of Emamectin benzoate was 0.53 ug/ml against third instar larvae of *Spodoptera litura* in leaf disc ingestion method compared with another pest, *Heliothis virescens* (F.) (0.040 ug/ml) [21], *Spodoptera exigua* (Hubner) (0.007 ug/ml), and *Spodoptera litura* (0.025 ug/ml) in leaf dip method [22] which shown highly toxic biopesticide among the biopesticides that tested and which is in agreed with our results. In the same tendency, El-Naggar, 2013 [23] examined three biopesticides, (Spinosad, Spinetoram and Emamectin benzoate) and two insect growth regulator; (Flufenoxuron and Triflumuron) on some biological and physiological aspects using a laboratory strain of *Spodoptera littoralis* (Boisd.) 4th larval instar and he found that based on the LC_{50} values, Emamectin benzoate was the most toxic insecticides followed by flufenoxuron while spinosad was the least toxic one. Also, Megahed et al. (2013) [24] evaluated three biopesticides, Emamectin benzoate "Proclaim", Abamectin "Romacten" and Spinosad "Tracer") on the 4th larval instar of the Cotton leafworm, *Spodoptera littoralis* by leaf dipping technique, however, Emamectin benzoate shown the highest toxic biopesticide after 24 h (LC_{50}=0.17 ppm). The toxicological efficacy of Emamectin benzoate was reported by many authors on other pests of different crops such as Corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) [25]. Importantly, Emamectin benzoate shown lack of resistance against different insect pests worldwide which stressed its incorporation into integrated resistance management programs (IRM) and integrated pest management (IPM) [26].

In conclusion, Emamectin benzoate is considered the most toxic biopesticide among the compounds tested on 2nd and 4th instar larvae of Cotton leafworm, *Spodoptera littoralis*, however, field studies can enhance the effectiveness of these and other pesticides for long term and effective management of *Spodoptera littoralis*.

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