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# Toxicological Evaluation of certain Pesticides on Potato Tuber Moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) under Laboratory Conditions

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**ABSTRACT:** Potato Tuber Moth, *Phthorimaea operculella* (Zeller), is one of the most dangerous pests infesting the potato crop in Egypt. Herein, five selected pesticides (Indoxacarb, sulfoxaflor, emamectin benzoate, thiamethoxam + chlorantraniliprole, and *Bacillus thuringiensis*) were evaluated on 1<sup>st</sup> instar larvae of *P. operculella* under laboratory conditions. As results, indoxacarb was the most potent pesticide through selected pesticides with LC<sub>50</sub> value of 55.08 µg/ml after 24-h of exposure. After 72-h of treatment, emamectin benzoate was the most toxic pesticide among selected pesticides with LC<sub>50</sub> value of 22.96 µg/ml. Further investigations are required to set these selected pesticides within integrated pest management (IPM) programs to control *P. operculella* in Egypt.

**Keywords:** Potatoes, *Phthorimaea operculella*, Sulfoxaflor, Emamectin benzoate, Chemical control, Integrated Pest Management (IPM)

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is considered a pivotal part of vegetable production and a significant crop in Egypt, widely cultivated worldwide. Millions of people in many countries depend on potatoes for food, so potatoes can assignment in pursuing food security and eradication of malnutrition (IYP, 2008; Barrell *et al.*, 2013). During this period, the potato tuber moth, *Phthorimaea operculella* (Zeller, 1873) (Lepidoptera: Gelechiidae) (PTM), is recognized as a significant insect pest (Kroschel *et al.*, 2013; CABI, 2018) that affects a wide range of host plants, particularly solanaceous crops. PTM has become a growing agricultural concern in tropical regions and other areas due to its ability to develop resistance to pesticides (Amiri and Bakhsh, 2019). Additionally, its high reproductive capacity, adaptability to diverse climatic conditions (Vaneva-Gancheva and Dimitrov, 2013; Kroschel *et al.*, 2016), and habit of spending most of its life inside tubers make its control challenging. Farmers have attempted to combat this pest by treating their crops with Bt, azadirachtin, methamidophos, acetamiprid, methomyl, and imidacloprid, as these compounds have demonstrated effectiveness in pest management (Kroschel & Koch, 1996; Rondon, 2010; Vaneva-Gancheva & Dimitrov, 2013). Importantly, chlorantraniliprole could be a confident alternative for the control of *T. solanivora* populations resistant to neurotoxicity pesticides belonging to the class's organophosphates, pyrethroids, and carbamates (Bacca *et al.*, 2021).

*Bacillus thuringiensis* sp. *kurstaki* has proven effective in controlling the potato tuber moth (Gomaa and El-Nenaey, 2006). Emamectin benzoate, a highly efficient semisynthetic antibiotic pesticide, exhibits dual action as both a contact and stomach poison for pests. It is derived from the fermented product of avermectin B1 (Kary et al., 2018). Chemical control measures are most effective during the early stages of *P. operculella*, before the larvae penetrate the tuber and the effectiveness of control diminishes significantly (Valderrama et al., 2007). Sulfoxaflor and flupyradifurone have been found to have minimal harmful effects on biological control agents. Therefore, they are suitable for chemical control with low toxicity to natural enemies and can be incorporated into Integrated Pest Management (IPM) programs (Wanumen et al., 2016; Nawaz et al., 2018; Barrania et al., 2019).

Acetamiprid and indoxacarb realized high mortality percent of > 90% on newly larvae of *P. operculella*, while imidacloprid demonstrates the lowest larvicidal effect under laboratory conditions (Vaneva and Dimitrov 2013), while in Bt was record 20-60%, where they bind to specific receptors in pest species of Lepidoptera and Coleoptera as possible alternatives to chemicals (Gill et al., 1992). Chlorantraniliprole was effective on *T. solanivora* little larvae before penetration tuber, so contact with the pesticide topically or through penetration maintains its effectiveness for a period that may reach a week after treatment (Bacca et al., 2021). Bacterial formulations of Bt are widely used in organic farming, display noticeable toxicity on more species of the order Lepidoptera, and are registered for controlling many insect pests on agricultural crops (Otvos et al., 2005). At the laboratory this study focused on the toxicity evaluation of five selected pesticides (indoxacarb, sulfoxaflor, emamectin benzoate, thiamethoxam + chlorantraniliprole, and Bt) with different modes of action on 1<sup>st</sup> instar larvae of *P. operculella*.

## MATERIALS AND METHODS

### Experimental area

A laboratory experiment was conducted at the Plant Protection Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.

### Rearing of *P. operculella*

A laboratory colony of *P. operculella* was established using a stock obtained from The Biocides Unit of the Plant Protection Research Institute in Giza Governorate, Egypt. The colony was initiated by infesting potato tubers obtained from potato fields in June 2019 in Giza Governorate, Egypt.

The infested tubers, containing different unripe stages, were confined in cages (30 × 30 × 50 cm) with fine layer of clean sand and small fresh potato tubers in each cage were used as larval food and the accomplishment of the development of PTM yeasty stages and increase the population density of each cage, a piece of cotton sodden with 20% sugar solution was used for moth feeding. Potato tubers were punctuated superficially to ease the penetration of the neonate larvae of PTM.

This process was achieved just before larval hatching so that the puncture wounds will not over before larval entry. Added new potato tubers every 10 days for egg precipitation and to keep a continual culture (Etzel, 1985; Mandour, 1997). These cages were kept under 26 ± 1 °C and 60 ± 5% R.H. of these conditions. However, hatching occurred after 3 - 4 days. After 12-15 days, the full grown larvae of PTM dropped from tubers into the sand layer for pupation (Khandge et al., 1979; Mandour, 1997).

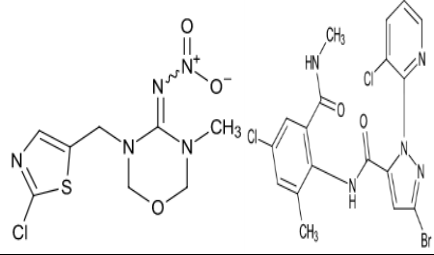
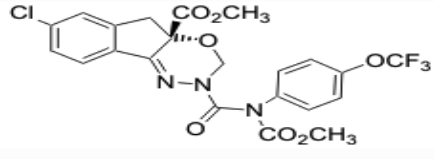
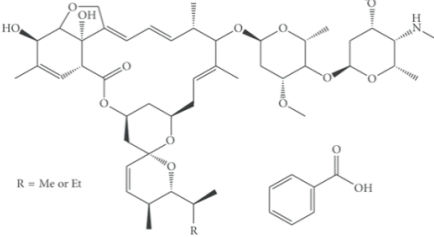
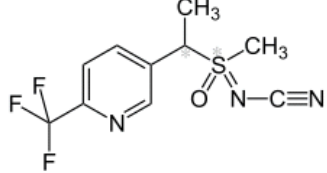
After pupation, pupae were kept in glass jar (30 × 10 cm) every 48 hours till the emergence of PTM moths. Emerged adults were collected in an oviposition cage consisted of plastic container (12 × 9 cm) to lay eggs. The containers were covered with a filter paper and the top of the cage covered by Dapalan cloth to help as deposition sites and the bottom of containers was covered with a filter paper. A Petri dish with 20 mm diameter was placed on the upper outside of the fine gauze as weight on the top to assure contact of the tissue filter paper with gauze inside each cage. However, the filter paper containing PTM eggs was substituted daily by another clean one and placed in 2% sodium hypochlorite and fresh water, respectively, it was kept in clean Petri dish with 12 cm after dried well for 1-3 hours. These egg sheets were kept at a temperature of 4 ± 1 °C to slow its growth until collect enough amount. For moth feeding, a piece of cotton was saturated with 20% sugar solution.

Neonate larvae (<24 hours post hatching) were collected from egg sheets kept at room temperature of 26 ± 1 °C and 60 ± 5% R.H. Under these conditions, hatching occurred after 3 - 4 days which use for experiments. Light microscope was used to verification of vitality of newly hatching larvae. Insects from the colony were used in all bioassays. The rearing cycle was uninterrupted to compound the PTM for providing proper eggs for experiments, so eggs were allowed to hatch for experiments or continue rearing (Maharjan and Jung, 2011).

### Source of the tested pesticides

Recommended concentrations of 5 commercial pesticides belong to different chemical groups were used in this study (Table 1).

**Table 1:** List of tested pesticides that used in this study

Trade Names Formulations	Active ingredient	Chemical structure
Voliam Flexi 30% SC	Thiamethoxam 20% + Chlorantraniliprole 10%	
Dipel 6.4% DF	<i>Bacillus thuringiensis</i> (Kurstaki strain)	-----
Easo plus 30% WG	Indoxacarb	
Egy Chem 5.7% WG	Emamectin benzoate	
Closer 24% SC	Sulfoxaflor	

### Evaluation of effectiveness of certain pesticides against *P. operculella* under laboratory conditions

Pesticides were applied using leaf dipping technique to test the efficiency against larvae of PTM. The tested concentrations were 0, 10, 50, 100, 250, 500, and 1000 µg/ml for thiamethoxam + chlorantraniliprole, 0, 25, 50, 100, 500, and 1000 µg/ml for *Bacillus thuringiensis*, 0, 10, 50, 100, 250, 500, and 1000 µg/ml for indoxacarb, 0, 2, 20, 200, 500, 1000, and 2000 µg/ml for emamectin benzoate and 0, 50, 100, 250, 500, and 1000 µg/ml for sulfoxaflor.

For experiments, leaf discs of cabbage (*Brassica pekinensis*) with 40 mm diameter were selected using leaf dipping technique and were determined by LC<sub>50</sub> and LC<sub>90</sub> values (Symington and Catherine 2003). Cabbage leaf was dipped for 30 seconds in pesticide concentrations. The leaf discs were then permissible to dry for one hour at room temperature, ten neonate larvae (<24 hours post hatching) were separately placed on each disc. Three replicates were used for each concentration in all treatments. Bioassay containers were kept in an incubator at 27±1 °C, the survived and dead larvae at each replicate were determined at 24, 48 and 72-h after treatment. The treated leaf discs were examined under a dissecting microscope to count the total numbers of them. Dead larvae were considered dead if they did not move when touched or if larvae took longer than five seconds to roll over when arranged with their dorsal surface confronting the pesticide. Leaves immersed in water served as control.

### Statistical analysis

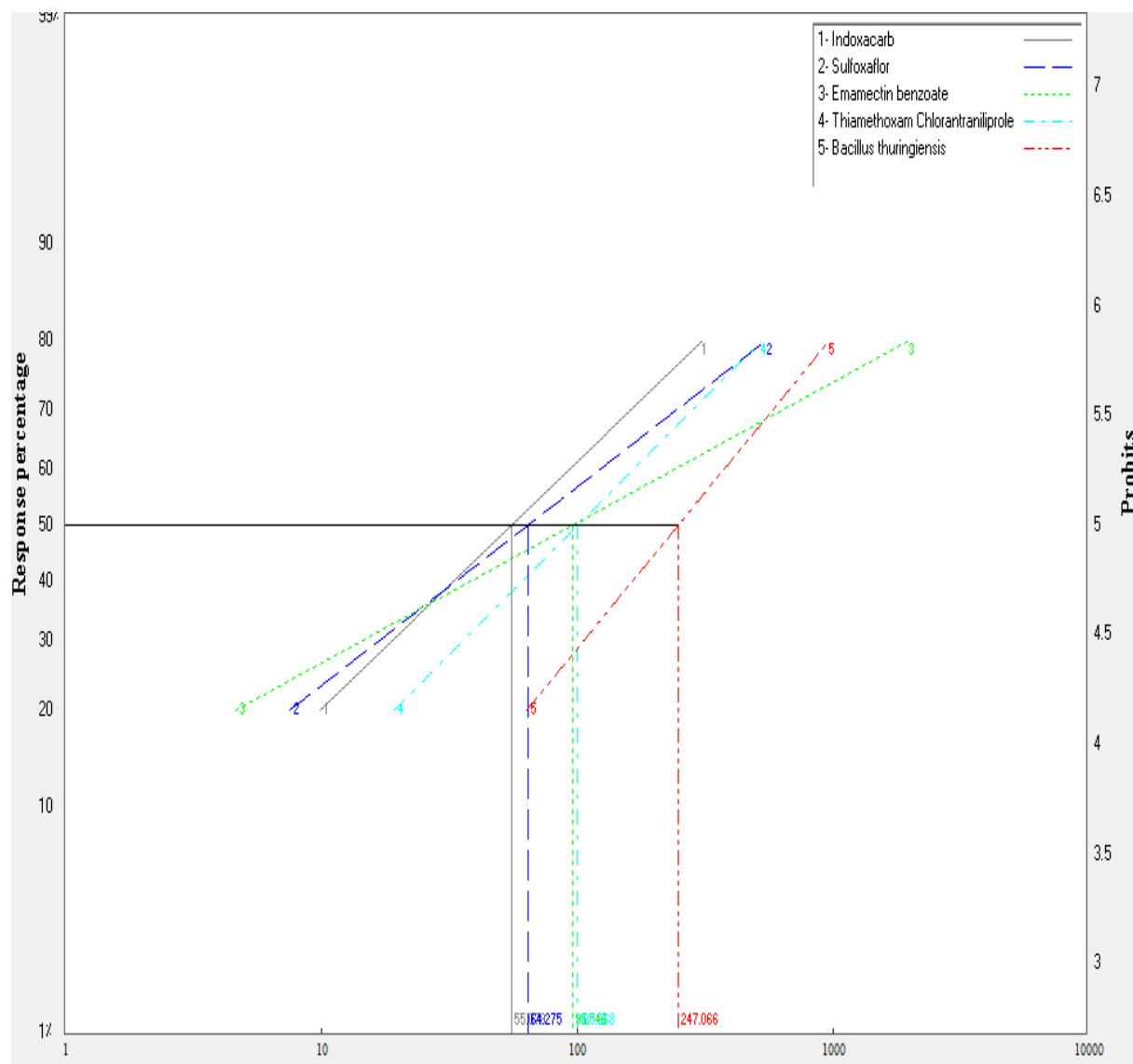
Data were corrected for control mortality (Abbott 1925) and square-root transformed when required to gather analogy of normality and homogeneity of variances. Probit analysis was done to estimate LC<sub>50</sub> and LC<sub>90</sub> values, confidence limits, and slope values of LCP lines for the five pesticides using SPSS analysis program version 20. LCP lines were drawn by using the program Sigma plot 8.02 system software.

## RESULTS AND DISCUSSIONS

Toxicity of selected pesticides on 1<sup>st</sup> instar larvae of *P. operculella* under laboratory conditions after 24-h exposure exhibited in table 2 and figure 1. The pesticide, indoxacarb, was the most potent one among the selected pesticides tested with LC<sub>50</sub> value of 55.08 µg/ml. However, sulfoxaflor, emamectin benzoate, and the mixture of thiamethoxam + chlorantraniliprole

**Table 2:** Toxicity of selected pesticides on 1<sup>th</sup> instar larvae of *P. operculella* under laboratory conditions after 24-h exposure

Pesticides	LC <sub>50</sub> (µg/ml) (95% CL)	LC <sub>90</sub> (µg/ml) (95% CL)	Slope ± SE
Indoxacarb	55.08 (31.30-84.76)	757.68 (421.16-1999.13)	1.13 (0.09)
Sulfoxaflo	64.28 (32.57-106.62)	1682.13 (750.25-7728.81)	0.90 (0.09)
Emamectin benzoate	82.77 (37.36-161.80)	587.29 (150.77-956.24)	0.68 (0.06)
Thiamethoxam + Chlorantraniliprole	100.27 (63.61-149.67)	237.88 (669.75-3448.14)	1.17 (0.12)
<i>Bacillus thuringiensis</i>	247.07 (169.77- 381.83)	1964.89 (1044.85-5666.84)	1.25 (0.11)

**Figure 1:** Toxicity lines of five selected pesticides against *P. operculella* after 24-h of treatment using method of dipping under laboratory conditions.

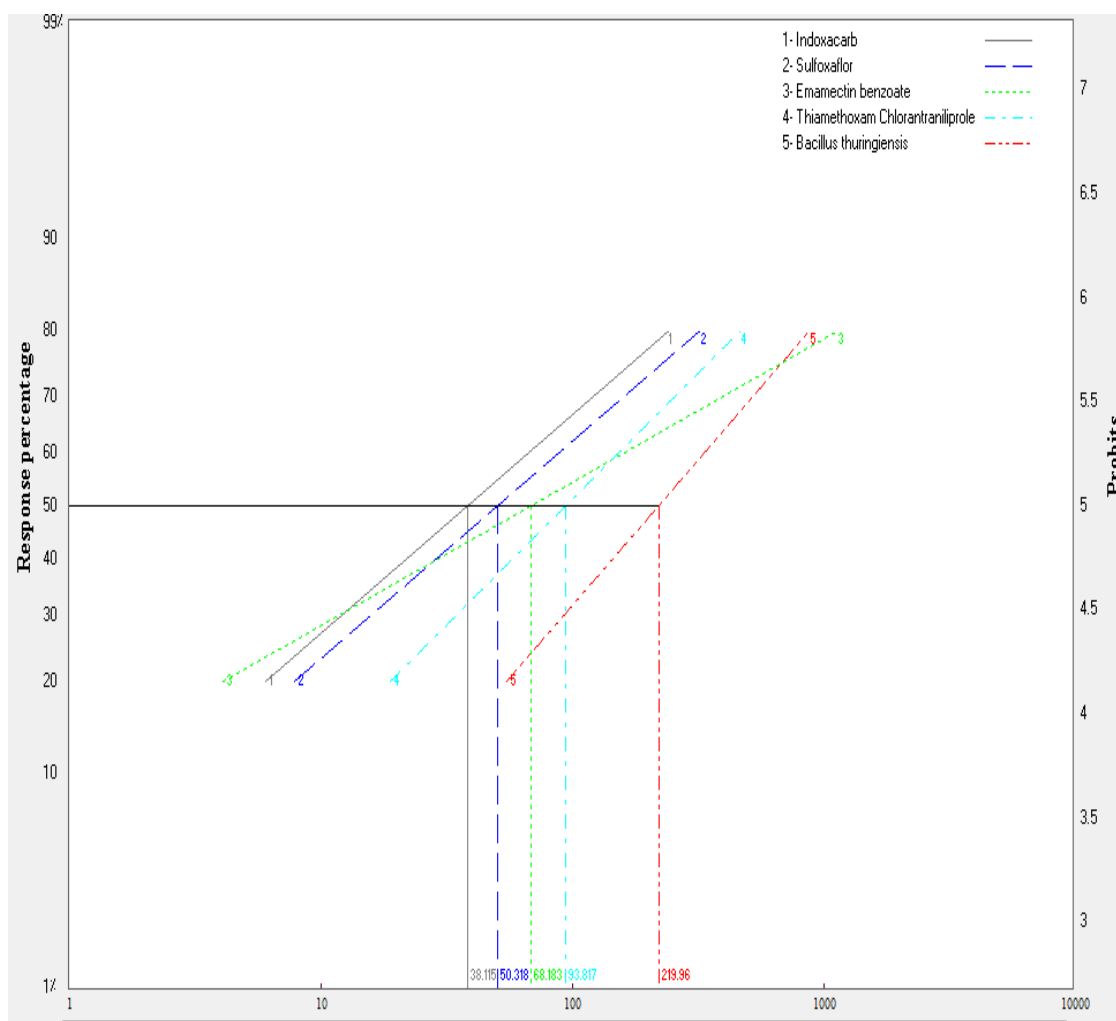
exhibited moderate toxic pesticides (LC<sub>50</sub> values were 64.28, 82.77, and 100.27 µg/ml, respectively). Furthermore, Bt was the lowest effective pesticide among the selected pesticides and the LC<sub>50</sub> value was 247.07 µg/ml. After 48-h of treatment, the same trend of the toxicity was continuous (Table 3 and figure 2). Indoxacarb was the most potent pesticide among the selected pesticides with LC<sub>50</sub> value of 38.30 µg/ml. Moreover, sulfoxaflo, emamectin benzoate, and the mixture of thiamethoxam + chlorantraniliprole were the moderate toxic pesticides (LC<sub>50</sub> values were 50.33, 68.26, and 93.83 µg/ml, respectively). However, Bt was the lowest effective pesticide among the selected pesticides and LC<sub>50</sub> value was 219.97 µg/ml.

Based on the toxicity after 72-h exposure, similar direction of the toxicity was enhanced (Table 4 and figure 3) except that emamectin benzoate was the most potent pesticide among the selected pesticides and the LC<sub>50</sub> value was 22.96 µg/ml. Moreover, indoxacarb, sulfoxaflo, and the mixture of thiamethoxam + chlorantraniliprole were the moderate toxic pesticides (LC<sub>50</sub> values were 29.65, 45.42, and 76.01 µg/ml, respectively). However, Bt was the lowest effective pesticide among the selected pesticides and the LC<sub>50</sub> value was 133.35 µg/ml.

According to the slope values of the selected pesticides, *P. operculella* larvae demonstrated a relative high homogeneity response to Bt, the mixture of thiamethoxam + chlorantraniliprole, and indoxacarb pesticides (1.25, 1.17 and 1.13, respectively) after 24-h exposure. They showed heterogeneity to emamectin benzoate and sulfoxaflor (0.68 and 0.90).

**Table 3:** Toxicity of selected pesticides on 1<sup>th</sup> instar larvae of *P. operculella* under laboratory conditions after 48-h exposure

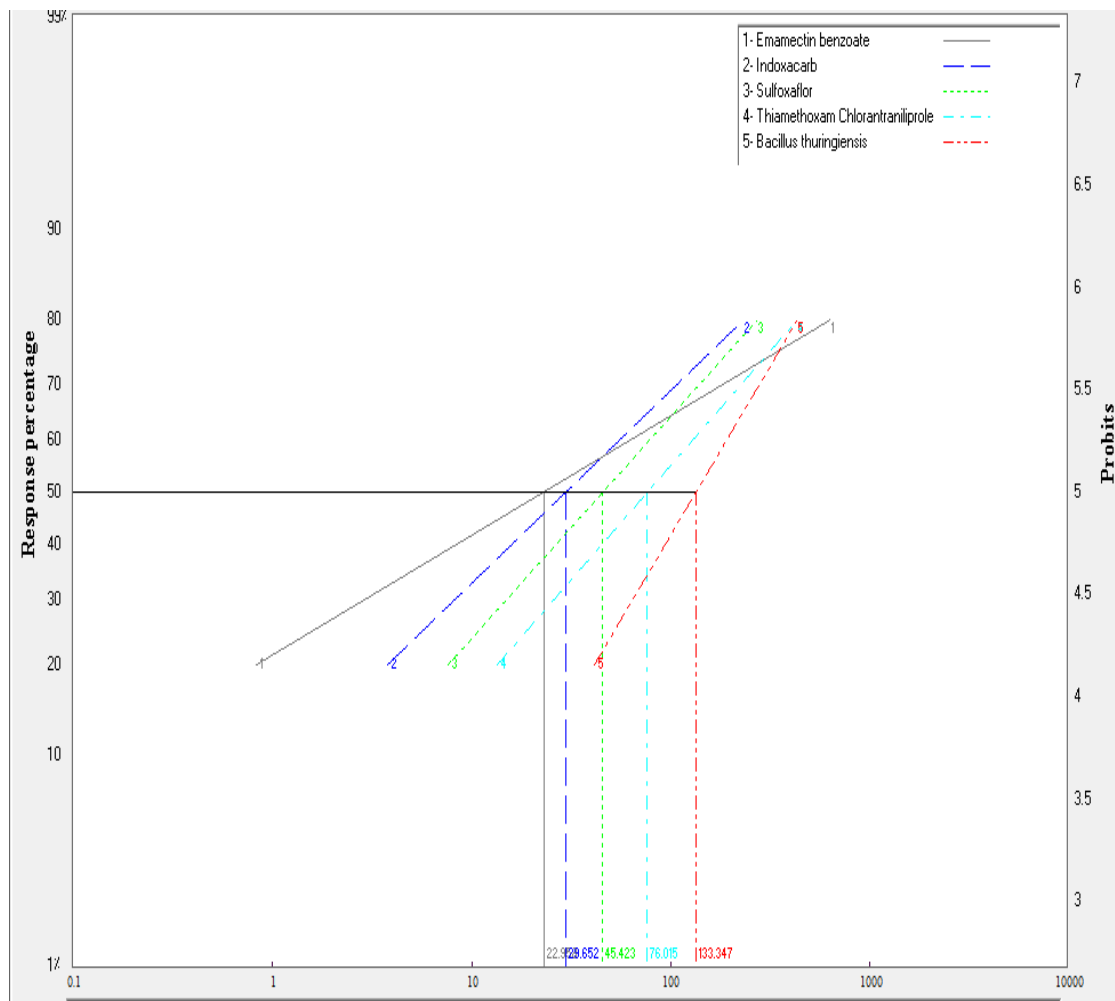
Pesticides	LC <sub>50</sub> (µg/ml) (95% CL)	LC <sub>90</sub> (µg/ml) (95% CL)	Slope ± SE
Indoxacarb	38.30 (20.91-59.42)	469.78 (274.59-1108.60)	1.18 (0.18)
Sulfoxaflor	50.33 (26.97-79.84)	856.15 (449.39-2591.43)	1.04 (0.17)
Emamectin benzoate	68.26 (30.30-134.23)	443.67 (218.74-2391.24)	0.69 (0.11)
Thiamethoxam + Chlorantraniliprole	93.83 (59.78-138.90)	1085.13 (603.20-2843.49)	1.21 (0.18)
<i>Bacillus thuringiensis</i>	219.97 (150.58-339.19)	1821.72 (964.01-5329.14)	1.40 (0.21)



**Figure 2:** Toxicity lines of five selected pesticides against *P. operculella* after 48-h of treatment using method of dipping under laboratory conditions

**Table 4:** Toxicity of selected pesticides on 1<sup>th</sup> instar larvae of *P. operculella* under laboratory conditions after 72-h exposure

Pesticides	LC <sub>50</sub> (µg/ml) (95% CL)	LC <sub>90</sub> (µg/ml) (95% CL)	Slope ± SE
Emamectin benzoate	22.96 (8.97-76.14)	282.67 (179.08-2101.44)	0.58 (0.06)
Indoxacarb	29.65 (12.12-51.89)	680.37 (338.72-2459.21)	0.94 (0.11)
Sulfoxaflor	45.42 (24.16-71.77)	695.01 (381.43-1896.37)	1.08 (0.14)
Thiamethoxam + Chlorantraniliprole	76.01 (45.43-115.79)	1072.51 (573.96-3073.11)	1.11 (0.09)
<i>Bacillus thuringiensis</i>	133.35 (94.99-188.94)	796.29 (488.30-1715.67)	1.65 (0.13)



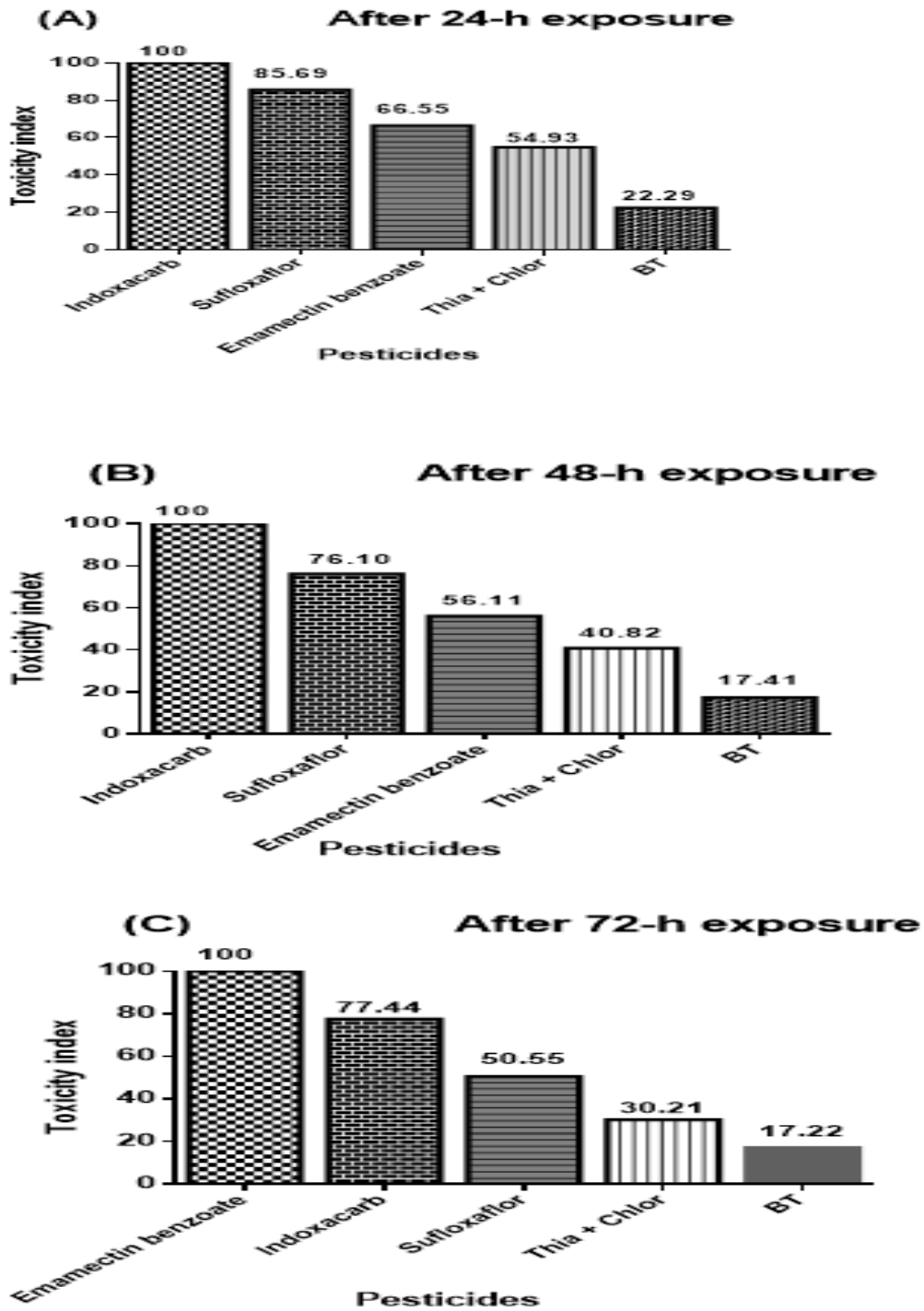
**Figure 3:** Toxicity lines of five selected pesticides against *P. operculella* after 72-h of treatment using the method of dipping under laboratory conditions

The same trend was observed after 48-h exposure. *P. operculella* larvae revealed relative high homogeneity response to Bt, mixture of thiamethoxam + chlorantraniliprole, and indoxacarb pesticides (1.40, 1.21, and 1.18, respectively) after 48-h exposure, larvae showed heterogeneity to emamectin benzoate and sulfoxaflor (0.69 and 1.04). However, the trend was changed after 72-h of treatment, *P. operculella* larvae revealed relative high homogeneity response to Bt, mixture of thiamethoxam + chlorantraniliprole, and sulfoxaflor pesticides (1.65, 1.11 and 1.08, respectively) and showed heterogeneity to emamectin benzoate and indoxacarb (0.58 and 0.94).

Figure 5 presents the time-dependent changes of the  $LC_{50}$  values as affected by the selected pesticides. However, the most noticed trends were 1) all time-dependent were increased based on the  $LC_{50}$  values; 2) there were noticeable time-dependent increases in  $LC_{50}$  value on emamectin benzoate and, to a lesser extent thiamethoxam + chlorantraniliprole; 3) relatively consistent levels of  $LC_{50}$  were found on indoxacarb and Bt.

The toxicity index of selected pesticides is presented in Figure 4. In this regard, for  $LC_{50}$  values of selected pesticides after 24-h exposure were 100, 85.69, 66.55, 54.93, and 22.29 for indoxacarb, sulfoxaflor, emamectin benzoate, thiamethoxam + chlorantraniliprole, and Bt, respectively, whereas after 48-h exposure were 100, 76.10, 56.11, 40.82, and 17.41, respectively. However, after 72-h of treatment, the values were 77.44, 50.55, 100, 30.21, and 17.22, respectively.

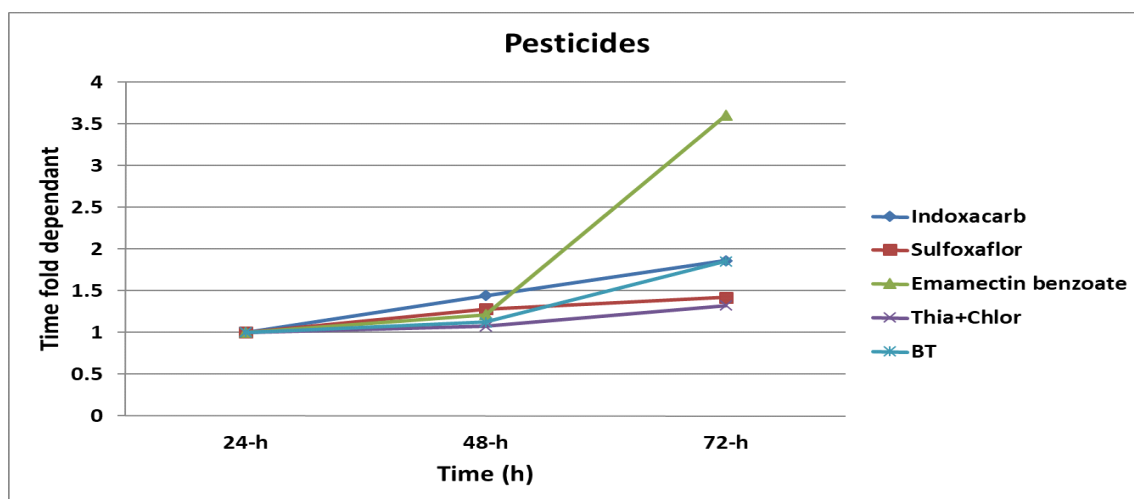
In general, indoxacarb proved to be the most potent pesticide among the selected one, especially after 24 and 48-h after exposure. In agreement with this finding, [Dastjerdi et al. \(2019\)](#) demonstrated that indoxacarb was the most toxic pesticide that tested and the  $LC_{50}$  value of indoxacarb on first instar larvae of *P. operculella* was 93 mg (a.i.)/l.



**Figure 4:** Toxicity index of the selected pesticides (A, B, and C) on 1<sup>st</sup> instar larvae of *P. operculella* after 24, 48, and 72-h exposure

$$\text{Toxicity index} = \left[ \frac{\text{LC}_{50} \text{ of the most toxic tested pesticide}}{\text{LC}_{50} \text{ of the tested pesticide}} \times 100 \right]$$





**Figure 5:** Time-dependent changes in the LC<sub>50</sub> values from Tables 2-4 for as assessed after 24, 48, and 72-h exposure

Further, Abdelmonem *et al.* (2018) stated that indoxacarb was considered the most toxic pesticide among the tested pesticides and the LC<sub>50</sub> value was 59.40 ppm for newly hatched larvae of *P. operculella*. Furthermore, Erdogan and Hassan (2018) found that all of the doses of indoxacarb evaluated (0.10, 0.15, and 0.25 ml/L) effectively controlled *P. operculella* for up to 112 days. Vaneva-Gancheva and Dimitrov (2013) stated that indoxacarb and acetamiprid achieved a high mortality rate of > 90%, especially after 7 and 14 days of treatments. However, the effect was slower than other pesticides tested.

On the other side, emamectin benzoate was the most potent selected pesticide after 72-h of exposure. In line with this finding, Basnet *et al.* (2022) found that emamectin benzoate was the most toxic pesticide among the selected pesticides and the mortality was 100% of *P. operculella*. Also, Saour *et al.* (2014) emphasized that emamectin benzoate is considered the most potent selected pesticide, especially with the three concentrations (5, 10, and 15 mg/l) on hatchability of *P. operculella*. Furthermore, according to the feeding and contact toxicology experiment, An *et al.* (2013) found that emamectin benzoate was the most toxic pesticide among the tested pesticides, especially after 48-h. The LC<sub>50</sub> values were 0.05 and 0.53 ppm, respectively.

## CONCLUSION

In general, the potato crop is an essential crop with a high export advantage in Egypt. It is infected with many agricultural pests. The most important of them is *P. operculella*. In this study, the efficacy of 5 compounds was evaluated and indoxacarb was the most potent compound after 24 and 48-h of exposure. However, after 72-h of treatment, emamectin benzoate was the most potent compound. It is necessary to conduct field experiments using these compounds to determine their effectiveness under field conditions and to integrate them through integrated management programs for pest control in Egypt.

## CONFLICT OF INTEREST

As the authors of this manuscript, we certify that we have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in the manuscript.

## COMPETING INTERESTS

None: There is none competing interests.

## ETHICS COMMITTEE

None.

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