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Development of imidacloprid and indoxacarb formulations to nanoformulations and their efficacy against *Spodoptera littoralis* (Boisd)

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Abstract

Background: The conventional pesticide formulations have many side effects on environmental. These effects were due to the huge quantity of pesticides used. Using of nanopesticide formulations can be reduced the quantity of pesticides used and subsequently decreased the cost of pest control and environment contamination.

Results: Indoxacarb and imidacloprid nanoparticles were developed. The size of nanoparticles ranged between 200 and 400 nm. The obtained results showed that the percentage of mortalities in second instar larvae of Egyptian cotton leafworm, *Spodoptera littoralis* were 95 and 75% in indoxacarb and imidacloprid nanoparticles, respectively. On the other hand, these percentages were 76.7 and 91.7%, respectively, with conventional formulations. In spite of one fifth of pesticide concentrations were used in nanoformulations, the LC_{50} s in nanoformulations were 2.9 and 15.9 ppm for indoxacarb and imidacloprid, respectively, compared to 34.3 and 66.5 in conventional formulation. The loading capacity in both indoxacarb nanoparticle and imidacloprid nanoparticle were 60.7 and 52.0%, respectively.

Conclusion: These results found that indoxacarb and imidacloprid nanoparticles were 12 and 4 times, respectively, more effective than indoxacarb and imidacloprid conventional formulations against the second instar larvae of *S. littoralis*. These results also showed that indoxacarb and imidacloprid nanoparticles can be used as effective formulations against the cotton leafworm larvae instead of the conventional formulation to reduce the environment contamination, control cost and pest population.

Keywords: Nanoformulations, Conventional formulations, *Spodoptera littoralis* loading capacity

Background

The Egyptian cotton leafworm, *Spodoptera littoralis* was considered the key pest for most main crops in Egypt (Ragaei et al. 2019). This pest has been acquired resistance to many common insecticides. So, it's badly needed to develop a new formulation to suppress the highly population of this pest and reduce the environmental contamination. Recently, the global pesticides consumption is approximately 2 million tonnes of pesticides (De et al. 2014). The intensive use of conventional pesticide

formulations involves the enormous amounts of different chemicals types to control weeds, insects, and pathogens of crops (Arnaud et al. 2005). However, many types of conventional pesticides formulations used were accumulated in biological system, contaminate soil and water environments, harm living organisms, and caused a disturbance in the balance of natural ecosystems (Carvalho 2017). Now there is a new trend to get new formulations for reducing the risk of side effects of conventional pesticide formulations. Nanotechnology is currently an important tool for increasing agricultural productivity. Nanotechnology makes a balance between minimal concentration and maximum pest control, safe concentration

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and reduces the cost of pest control (Oliveira et al. 2019). So, using of nanopesticides is very important in modern agriculture (He et al. 2019). There are many types of nanopesticide formulation such as nanoparticle pesticide formulations, nanosuspension pesticide formulations, nanoemulsion pesticides formulations and nanocapsules pesticide formulations (Sabry 2020).

Imidacloprid is the promising insecticide in pest control. The mechanism of action of this insecticide concerned on the central nervous system of insects, with low side effect on mammals. The action of this insecticide is caused by interfering with the transmission of stimuli in the insect nervous system. This mechanism leads to blockage of the nicotinic neuronal pathway. By blocking nicotinic acetylcholine receptors, imidacloprid prevents acetylcholine from transmitting impulses between nerves, resulting in the insect's paralysis and eventual death. El-Sheikh et al. (2018) used that imidacloprid against the second and forth instar larvae of *S. littoralis*. On the other hand, Sabry et al. (2013) used imidacloprid against the forth instar larvae *S. littoralis*. The LC_{50} was 0.22 g/l.

Indoxacarb has also a new mode of action against insect pests. It works as a sodium channel blocker resulting in paralysis and death of targeted pests. This insecticide has been reported to have a good field activity against umber of Lepidoptera as well as certain Homoptera and Coleoptera insects. Also, these insecticide formulations are reducing of the pesticide risk on mammalian (Wing et al. 2000; McKinley et al. 2002).

This work aims to develop nanoparticle pesticide formulations of both imidacloprid and indoxacarb as new pest control trend and using it against the second instar larvae of cotton leafworm control.

Methods

Test insect

Laboratory colony of Egyptian cotton leafworm, *Spodoptera littoralis* was reared under laboratory conditions ($26 \pm 1^\circ\text{C}$ and 70 ± 5 RH) for many generations. The second instar larvae of *S. littoralis* were used as a target test against both indoxacarb and imidacloprid conventional and nanoformulations.

Test chemicals

Two common insecticides were used.

1. Indoxacarb (Avaunt 15% EC), produced by Du Pont De Nemours. Indoxacarb belong to a new class of insecticides called oxadiazine and it works as a sodium channel blocker. The recommended field rate is 200 ml/feddan (4200 m^2)

2. Imidacloprid (Trade name is Commando 35% SC) produced by Vapco Company Jordan. This insecticide belongs to neonicotinoids group. The recommended field rate is 250 ml/feddan (4200 m^2)

Preparing of imidacloprid and indoxacarb nanoparticles

Chitosan with a high molecular was used as a carrier for active ingredient of imidacloprid and indoxacarb. Both imidacloprid and indoxacarb nanoparticles were prepared according to Vaezifar et al. (2013). The chitosan was taken and dissolved in the acetic acid (2% v/v) followed by continuous stirring with the help of magnetic stirrer for 15–20 min. The 0.8% (w/v) tripolyphosphate solution containing insecticides (imidacloprid or indoxacarb) were added to the chitosan solution (chitosan + acetic acid) followed by 5–10 min of stirring. The suspension was centrifuged at 10,000 RPM for 30 min. The pellet was collected and lyophilized to obtain imidacloprid and indoxacarb nanoparticles. Photography of nanoparticles was achieved by scan electronic microscope (Fig. 1a, b).

After the imidacloprid and indoxacarb nanoparticles were prepared the loading capacity of both imidacloprid and indoxacarb were calculated according to He et al. (2017):

Loading capacity LC

$$= \frac{\text{Mass of loaded insecticide}}{\text{Mass of insecticide nanoparticles}} \times 100$$

Loading capacity is defined as the mass percentage of the loaded LC to the total solids in the imidacloprid or indoxacarb/chitosan nanoparticles. This loading capacity was determined by about 30 mg of the samples (indoxacarb or imidacloprid nanoparticles) were weighed and dissolved in 50 ml of acetonitrile, and the mixture remained in a shaking tank overnight at a constant temperature to completely dissolve the carrier material. After the solution was filtered, the mass concentration of indoxacarb or imidacloprid in acetonitrile was examined by HPLC (The HPLC system was equipped with an XTerra RP18 column, 5 μm particle size, 4.6 mm internal diameter \times 250 mm length (Waters®, USA) under a detection wavelength of 278 nm (He et al. 2017) (Fig. 2). The loading capacity of imidacloprid was calculated by division of $2.600/5.000 \times 100 = 52\%$. The loading capacity of indoxacarb was $3.083/5.000 \times 100 = 60.8\%$.

Bioassay

Three concentrations of both indoxacarb and imidacloprid were used; the field rate and other two lower concentrations (Table 1).

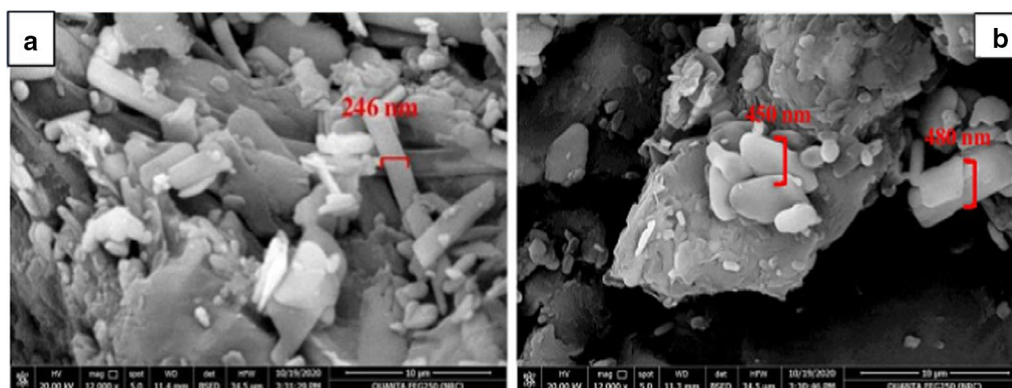


Fig. 1 Imidacloprid (a) and indoxacarb (b) nanoparticles

Each concentration has three replicates and ten healthy larvae per replicate were used. Other three replicates sprayed by water as a control. Lettuce leaves were used as natural diet to larvae. Deeping technique was used with larvae treatment. The percentages of mortalities were recorded after one, two four and seven days. After seven days the lethal concentrations for 50% of population LC_{50} s were recorded and the dead larvae were photographed.

Statistical analysis

Data were subjected to the analysis of variance test (ANOVA) via Randomized Complete Block Design (RCBD) (F test) and analysis of variance (one ways classification ANOVA) followed by a least significant difference (LSD) at 5% (Costat Statistical Software 1990).

Results

To make sure that the size of insecticide particles in nano size, scanning electron microscope (SEM) (JSM-7401 F, JEOL Ltd., Akishima-shi, Japan) was used to determine the size of pesticide particles (Fig. 1). Also, to make sure that the pesticide particles are deposited on polymer particles (chitosan), loading capacities of both tested insecticides are determined (Fig. 2). The obtained results showed that the loading capacities of both imidacloprid and indoxacarb were 52 and 60.8%, respectively.

Using of imidacloprid and indoxacarb conventional formulations

Imidacloprid and indoxacarb are promising insecticides belong to different insecticide groups. Imidacloprid and indoxacarb conventional formulations are used against the second instar larvae of Egyptian cotton leafworm under laboratory conditions (Table 2). As clear in Table 2 the first concentration (recommended field rate) for imidacloprid is more effective than indoxacarb (the percent of larvae mortalities are 91.7 and 76.7%, respectively.

With the second and the third concentration imidacloprid also is more effective than indoxacarb. The statistical analysis shows that there is a significant difference between the first concentrations in both imidacloprid and indoxacarb but there is no difference in the second and third concentrations. The LC_{50} s of imidacloprid is less than and indoxacarb. The LC_{50} s are 66.5 and 34.3 ppm, respectively.

Using of imidacloprid and indoxacarb nanoparticles

The efficacy of imidacloprid and indoxacarb nanoparticles against the second instar larvae of *S. littoralis* are examined (Table 3). One fifth of the concentrations which used with conventional formulations are used (Fig. 1).

The efficacy indoxacarb nanoparticles are more than imidacloprid nanoparticles with all concentrations used. The percent of mortalities with the first, second and third concentrations are 95, 80, 58.3 and 75, 50, 35 in indoxacarb and imidacloprid, respectively. The LC_{50} s are 2.9 and 15.9 ppm, respectively (Table 3) (Fig. 3). The statistical analysis shows that there are significant differences between indoxacarb and imidacloprid nanoparticles.

Discussion

The intensive use of conventional formulations of pesticides caused many problems to environment. Using of nanoparticles formulations may be reducing these problems. Using of imidacloprid and indoxacarb nanoparticles against the second instar larvae of *Spodoptera littoralis* reduces the amount of insecticides uses and increases the efficacy of pest control. The quality of insect control by nanoparticles formulation was better than conventional formulations. The LC_{50} s of both indoxacarb and imidacloprid nanoparticles were 2.9 and 15.9 ppm, respectively. While the LC_{50} s of indoxacarb and imidacloprid conventional formulations were 34.3 and 66.5 ppm, respectively. This means that the

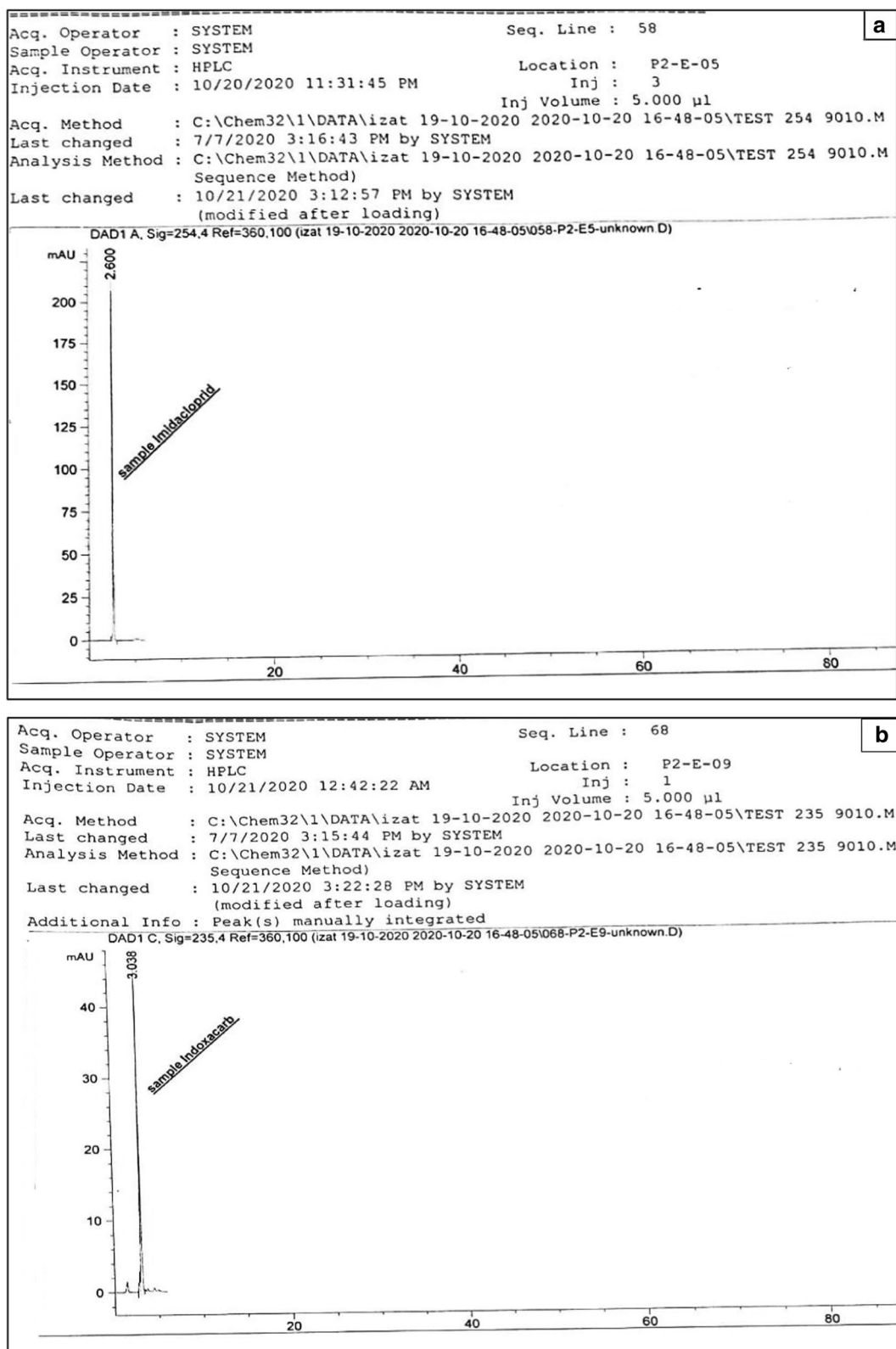


Fig. 2 The loading capacities of imidacloprid (a) and indoxacarb (b)

Table 1 Concentrations of indoxacarb and imidacloprid conventional and nanoparticles formulation

Formulations	Conventional formulations (ppm)			Nanoparticles formulations (ppm)		
	C1*	C2	C3	C1	C2	C3
Insecticides						
Imidacloprid	180	90	45	36	18	9
Indoxacarb	80	40	20	14	7	3.5

C1: First concentration per ppm (field rate)

Table 2 Effect of the conventional formulations of imidacloprid and indoxacarb against the second instar larvae of *S. littoralis*

Insecticides	The percentages of mortalities				
	C1	C2	C3	Slope \pm SE	LC ₅₀ and fudicial limits
	Means \pm SE	Means \pm SE	Means \pm SE		
Imidacloprid	91.7 \pm 2.9 ^a	61.7 \pm 2.9 ^a	31.7 \pm 2.9 ^a	3.1 \pm 0.4	66.5 (57.7–75.2)
Indoxacarb	76.7 \pm 2.9 ^b	58.3 \pm 7.6 ^a	30.0 \pm 5.0 ^a	2.1 \pm 0.3	34.3 (28.4–40.3)
Control	1.7 \pm 2.9 ^c	0.0 ^b	1.7 \pm 2.9 ^b		
F values*	837.14***	162.3***	61.4***		
LSD _{5%}	5.77	9.4	7.4		

*Means under each variety sharing the same letter in a column are not significantly different at $P < 0.05$ **Table 3 Effect of imidacloprid and indoxacarb nanoparticles against the second instar larvae of *S. littoralis***

Insecticides	The percentages of mortalities				
	C1	C2	C3	Slope \pm SE	LC ₅₀ and fudicial limits
	Means \pm SE	Means \pm SE	Means \pm SE		
Imidacloprid	75.0 \pm 5.0 ^b	50.0 \pm 5.0 ^b	35.0 \pm 5.0 ^b	1.8 \pm 0.3	15.9 (12.7–19.4)
Indoxacarb	95.0 \pm 5.0 ^a	80.0 \pm 5.0 ^a	58.3 \pm 2.9 ^a	2.3 \pm 0.4	2.9 (2.0–3.6)
Control	1.7 \pm 2.9 ^c	0.0 ^c	1.7 \pm 2.9 ^c		
F values*	372.5***	294.0***	175.2***		
LSD _{5%}	8.8	8.2	7.4		

*Means under each variety sharing the same letter in a column are not significantly different at $P < 0.05$

indoxacarb nanoparticles was most effective than the conventional formulations by 12 times. And also, imidacloprid nanoparticles were more effective than the conventional one by 4 times.

The insecticide concentrations in nanoparticles were one fifth of the concentrations in conventional formulations. This result also showed that the nanoparticles formulations were less toxic to nontarget organisms such as human and natural enemies of tested pest. The same result was found by Assemi et al. (2014). The authors

found that the nanoimidacloprid was 8 times more effective than imidacloprid conventional formulations against tobacco aphids, *Myzus persicae*. Rouhani et al. (2012) found that the imidacloprid nanoparticles were more effective than Ag and Ag-Zn nanoparticles against *Aphis nerii*. Ahmed et al. (2019) used lambda-cyhalothrin nanoparticles against the second instar larvae of *S. littoralis*. The authors found that the tested concentrations of lambda-cyhalothrin nanoparticles decreased the insect population to 37 times compare with the



Fig. 3 Effect of indoxacarb (a) and imidacloprid (b) nanoparticles against the 2nd instar larvae of *S. littoralis* in compared with control (c)

conventional formulations. Memarizadeh et al. (2014) used nano indoxacarb against *Glyphodes pyloalis*. The obtained results showed that the efficacy of indoxacarb nanoparticles were very effective against *G. pyloalis* and reduces the pesticides residues. Bilal et al. (2020) developed indoxacarb nanoparticles against the diamond back moth, *Plutella xylostella* to overcome on insecticides resistance. The loading capacity was 24%. The obtained results showed that the indoxacarb nanoparticles were effective in insecticides resistance management.

Conclusion

The conventional formulations of pesticides have many side effects on environment due to their residues in soil and plants. Nanopesticide formulations are the best solution for reducing the pesticide residues in soil, human and plants and also reduces the cost of pest control. In this work this strategy was used with indoxacarb and imidacloprid pesticides. The one fifth concentration of nanopesticide was more effective than the conventional formulation. So, this work recommended that the using of nanotechnology in pesticide formulations development to reduce the side effects of pesticides. This strategy also can be used as a part of integrated pest management (IPM) program.

Abbreviations

LC₅₀: Lethal Concentrations for 50% of pest population; LSD: Less Significant Difference.

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Authors' contributions

KHS participated in scientific idea, laboratory experiment, analysis data and writing the paper; HAS carried out the bioassay and participates in writing the paper; HMM collected the data, data analysis and participated in writing the paper. All authors have read and approved the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The manuscript does not contain studies involving human participants, human data or human tissue.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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