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Evaluation of conventional and nanoformulations of some pesticides against the adults of chocolate banded snail, *Eobania vermiculata* (O. F. Müller, 1774)

Al-kazafy H. Sabry and Mona A. Hussein*
Pests and Plant Protection Department, National Research Centre, Cairo, Egypt 12622

Abstract

Four pesticides (thiacloprid, etofenprox, spirotetramat and fenbutatin oxide) were evaluated against the adults of chocolate banded snail, *Eobania vermiculata* under laboratory conditions. Two types of pesticide formulations were used; conventional formulations (suspension concentrate and emulsifier concentrate) and nanoformulations (nanoparticles). The size of nanoparticles in tested pesticides ranged between 145 to 587 nm. The obtained results showed that the conventional formulations were more effective than the nanoformulations in all tested pesticides except etofenprox. The efficacy of etofenprox as a conventional formulation was approximately the same in nanoformulation in all concentrations used. The percentage of mortality were 86.7, 66.7 and 46.7; and 81.7, 55.0 and 35.0% with conventional and nanoformulations, respectively. The LC_{50}'s in etofenprox conventional and nanoformulations were 14.9 and 20.3 ppm, respectively. This result showed that etofenprox can be used as an effective nanoparticle pesticide against the adults of chocolate banded snail, *E. vermiculata.* This means that one fifth of etofenprox concentration in nanoformulation was effective as conventional formulation. So, it can be reduced the pesticides hazard on nontarget organisms and reduced the pesticide cost.

Keyword: Nanoformulations, conventional formulations, nanoparticles, chocolate banded snail, pesticides evaluation

Introduction

Recently, the soil snails have become the most serious pests on ornamental plants and vegetables in Egypt [1] (Shahawy, 2013). This pest causes great damage during the young stages and in new growth in spring. Mahmoud et al [2] attempt for the first time in Egypt to control the adults chocolate banded snail, *Eobania vermiculata* under laboratory conditions using a new parasitic nematodes isolated from the same snail and many conventional pesticides were used against this pest, such as methomyl (pesticide belong to carbamates group) [3]. Hartnik et al. [4] evaluated alpha-cypermethrin (synthetic pyrethroids) against the land snail, *Helix aspersa* in Norway. Hussein and Sabry [5] used five common insecticides (Indoxacarb, abamectin, spiromesifan, imidacloprid and fipronil) against the adults and eggs of chocolate banded snail, *E. vermiculata* under laboratory conditions.

The chocolate banded snail, *E. vermiculata* is an air-breathing terrestrial snail and it has a long life cycle (about 2.5 years). This long life cycle makes this pest is more dangerous to vegetables and crops. This pest not only causes damage to plant leaves but also fruits and roots [6].

Thiacloprid is a new chloronicotinyl insecticide that belongs to the neonicotinoid pesticide group. This insecticide uses against many insect pests such as sucking insects and weevils, leafminers and beetles [7]. This pesticide has contact and stomach action. It acts on pests as an Acetylcholine receptor (nAChR) agonist.

The mode of action of etofenprox against pests is similar to that of pyrethroid pesticides, but it is called pyrethroid ethyl not pyrethroid ester. The main action site of this pesticide is the neuronal axon. This pesticide is used against many pests such as mosquitoes [8].

Spirotetramat is a systemic pesticide and widely used against the juvenile stages of sucking insects such as whiteflies and aphids [9]. This pesticide belongs to the lipid biosynthesis inhibitor group.

Fenbutatin oxide is a new pesticide that acts as inhibitor of mitochondrial ATP synthase. It is a non systemic acaricide. Ziad [10] evaluated fenbutatin oxide against *Tetranychus urticae and Olyphagotarso nemuslatus.*

Due to the intensive use of conventional formulations of pesticides, the hazard and residues of pesticides

*Corresponding author e-mail: ma.hussein@nrc.sci.eg (Mona Ahmed Hussein)
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have increased for nontarget organisms such as humans and beneficial insects (insect predators, insect parasites and pollinators). To reduce this hazard, nanotechnology has introduced a new trend in pesticides formulation. This trend is called nanoformulation of pesticides. These nanoformulations include nanoparticles, nanocapsules, nanosuspensions and nanoemulsion pesticides.

This work aims to evaluate the nanoformulations of some common pesticides and compare the efficacy of these nanoformulations with the conventional formulations of respective pesticides.

Experimental

Tested animal

The adults of chocolate banded snail, *Eobania vermiculata* were collected from the garden of the National Research Centre. These adults reared in plastic cages with moist sandy loam soil and fed on lettuce leaves. These adults were reared under laboratory conditions (27 ± 1 °C and 65 ± 5% RH).

Tested pesticides

Four common pesticides were used, thiacloprid, etofenprox, spirotetramate and fenbutatin oxide (Table 1). These pesticides are divided into two types of formulations; conventional formulations (suspension concentrations and emulsifier concentrate) and nanoformulations (nanoparticles). All tested pesticides belong to different pesticide classes and have different modes of action against the tested pest.

Preparation of nanoformulations

All conventional formulations of tested pesticides were converted to nanoformulations according to Vaezifar et al. [11]. Chitosan with a high molecular weight was used as a carrier or a polymer for all tested pesticides. First, chitosan was dissolved in acetic acid (2% v/v) followed by continuous stirring with the help of magneticstirrer for 25–30 min. After that tripolyphosphate 0.8% (w/v) solution containing the tested pesticide (thiacloprid or etofenprox or spirotetramate or fenbutatin oxide) was added to the chitosan solution. The obtained solution put on magnetic stirring for 10–20 min. The suspension was centrifuged at10,000 rpm for 30 min. The pellet was collected and lyophilized to obtain nanoparticles formulation. This work was carried out on all tested pesticides. Photography of nanoparticles was achieved by scan electronic microscope. The obtained solution of all pesticide nanoparticles was more transparent than the conventional formulation solutions (Fig. 1).

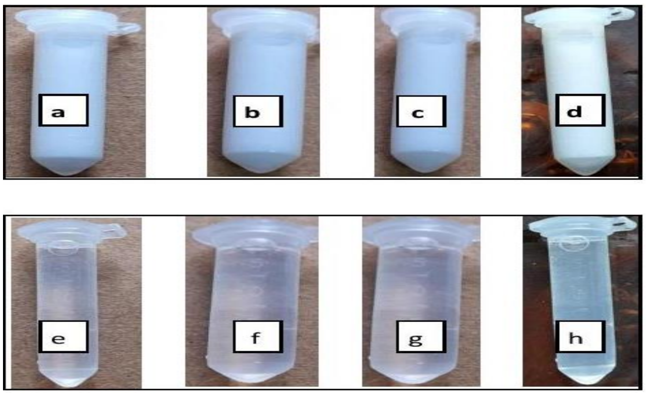

Fig.1. Solutions of conventional formulations (thiacloprid (a), etofenprox (b), spirotetramat (c) and fenbutatin oxide (d)) and nanoformulations (thiacloprid (e), etofenprox (f), spirotetramat (g) and fenbutatin oxide(h).

Loading capacity determination

After preparing nanoformulations for all test pesticides; loading capacity was determined according to He et al. [12]. The loading capacity was determined by about 30 mg of the samples (nanoparticle formulations) being 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. The solution was filtered, the mass concentration of tested pesticide 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 × 250 mm length (Waters®, USA) under a detection wavelength of 278 nm. The loading capacity was calculated by dividing the mass of loading pesticide on the mass of pesticide nanoparticles x 100.

Bioassay

Two types of pesticide formulations were used. Conventional formulations such as suspension concentrations with thiacloprid, spirotetramate and fenbutatin oxide; and emulsifier concentrate with etofenprox. The other type of formulation was nanoparticles of all test pesticides. Three concentrations of each pesticide were used. With the conventional formulations the recommended field rate was used as a first concentration and other two lower concentrations (half of the recommended field rate and one fourth the field rate) (Table 2). With the nanoparticles one fifth of field rate was used as a first concentration and other lower two concentrations (Table 2). Each concentration has three replicates and other three replicates were treated with water only as a control. Each replicate has ten healthy adults of chocolate banded snail, *E. vermiculata*.

Lettuce leaves were used as natural diet to all adults. The dipping technique was used with the adult treatment. The percentages of adults mortality were recorded after one, three, five, seven and ten days. After ten days of adults treatment the lethal concentration of 50% (LC_{50}) of adult population was calculated according to Finney 1971. Photographs of dead adults were taken.

Statistical analysis

Data were subjected to the analysis of variance (one way classification ANOVA) followed by a least significant difference (LSD) at 5% (Costat Statistical Software [13]).

Results and Discussion

All tested pesticides were evaluated in conventional and nanoformulations against the adults of chocolate banded snail, *Eobania vermiculata*.

Table 1. The tested pesticides used against the adults of chocolate banded snail, *E. vermiculata.*

Pesticide common names	Trade name	Producer	Recommended field concentration
Thiacloprid	Blanch 48%SC	Flag Chem. China	120 ml/Feddan
Etofenprox	Primo 10% SC	Beta Chem. China	200 ml/Feddan
Spirotetramat	Movento 10% SC	Bayer Crop Science Germany	160 ml/Feddan
Fenbutatin Oxide	Dumper 55% SC	Indus. Quimica Key, Spain	400 ml/Feddan

Table 2. The concentrations used in both conventional and nanoformulations

Pesticides	Conventional formulations concentrations (ppm)			Nanoformulations concentrations (ppm)		
	C1	C2	C3	C1	C2	C3
Thiacloprid	144	72	36	28	14	7
Etofenprox	50	25	12.5	10	5	2.5
Spirotetramat	40	20	10	8	4	2
Fen. Oxide	550	275	137.5	110	55	27.5

C1: The first concentration is recommended field rate against the chocolate banded snail, *E. vermiculata*

Table 3. Toxicity of the conventional formulations of the tested pesticides against the adults of chocolate banded snail, *E. vermiculata*

Pesticides	Percentages of mortalities			Slope ± SE	LC_{50} and fudicial limits
	C1	C2	C3		
Thiacloprid	86.7±11.5[a]	66.7±11.5[a]	46.7±11.5[b]	2.1± 0.3	41.7(31.0 – 50.7)
Etofenprox	86.7±11.5[a]	66.7±11.5[a]	46.7±11.5[b]	2.1 ± 0.3	14.9(11.2-18.1)
Spirotetramat	100± 0.0[a]	86.7±11.5[a]	66.7±11.5[ab]	3.1 ± 0.5	7.7(5.5-9.3)
Fenbutatin Oxide	100± 0.0[a]	86.7±11.5[a]	80.0 ± 0.0[a]	2.1 ± 1.2	61.5 (55.3-75.4)
Control	3.3± 5.8[b]	3.3± 5.8[b]	0.0[c]		
F-values	83.2***	31.1***	34.5***		
L.S.D	14.1	19.4	16.3		

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

Table 4. Toxicity of nanoformulations of the tested pesticides against the adults of chocolate banded snail, *E. vermiculata*

Pesticides	Percentages of mortalities			Slope ± SE	LC_{50} and fudicial limits
	C1	C2	C3		
Thiacloprid	41.7±2.9[c]	25.0±5.0[d]	11.7±2.9[c]	1.7±0.3	173.2 (128.4-317.3)
Etofenprox	81.7±2.9[a]	55.0±5.0[a]	35.0±5.0[a]	2.2±0.3	20.3 (16.6 – 23.7)
Spirotetramat	53.3±5.8[b]	36.7±2.9[c]	18.3±5.8[bc]	1.6±0.3	35.0 (27.7 – 52.4)
Fenbutatin Oxide	75.0±5.0[a]	45.0±5.0[b]	21.7±2.9[b]	2.5±0.3	299.1 (259.2 – 384.1)
Control	1.0±2.9[d]	0.0[e]	1.7±2.9[d]		
F-values	210.4***	80.7***	27.4***		
L.S.D	7.1	7.4	7.4		

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

Table 5. Comparison between the efficacy of conventional and nanoformulations of the tested pesticides against the adults of chocolate banded snail, *E. vermiculata*

Tested pesticides	Percentages of mortalities											
	C1				C2				C3			
	Conv.	Nano.	*F*values	L.S.D	Conv.	Nano.	*F*values	L.S.D	Conv.	Nano.	*F* values	L.S.D
Thiacloprid	86.7±11.5[a]	41.7±2.9[b]	42.9***	19.1	66.7±11.5[a]	25.0±5.0[b]	32.9**	20.2	46.7±11.5[a]	11.7±2.9[b]	25.9***	19.1
Etofenprox	86.7±11.5[a]	81.7±2.9[a]	0.5[ns]	19.1	66.7±11.5[a]	55.0±5.0[a]	2.6[ns]	20.2	46.7±11.5[a]	35.0±5.0[a]	2.6[ns]	20.2
Spirotetramat	100± 0.0[a]	53.3±5.8[b]	196.1***	9.2	86.7±11.5[a]	36.7±2.9[b]	52.9**	19.1	66.7±11.5[a]	18.3±5.80[b]	42.1***	20.7
Fen. Oxide	100± 0.0[a]	75.0±5.0[b]	75.0***	8.0	86.7±11.5[a]	45.0±5.0[b]	32.9**	20.2	80.0 ± 0.0[a]	21.7±2.9[b]	1225.0***	4.6

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

Efficacy of conventional formulations of the tested pesticides against *E. vermiculata*

All tested pesticides are evaluated under laboratory conditions (Table 3). The percentages of mortalities were reached 100% with the recommended field rate in both spirotetramate and fenbutatin oxide. The results showed that spirotetramat is the most effective against the adults of *E. vermiculata* followed by etofenprox, thiacloprid and fenbutatin oxide (Table 3 and Fig.2). The LC_{50}'s were 7.7, 14.9, 41.7 and 61.5 ppm, respectively. The statistical analysis showed that no significant difference among all tested pesticides. Although the percentage of mortality in fenbutatin oxide and thiacloprid recommended field rates were 100 and 86.7%, respectively, the LC_{50}'s was the lowest among all tested pesticides. This is due to the high percentage of active ingredients (50% and 48%, respectively) in these pesticides compared with the 10% in both etofenprox and spirotetramat..

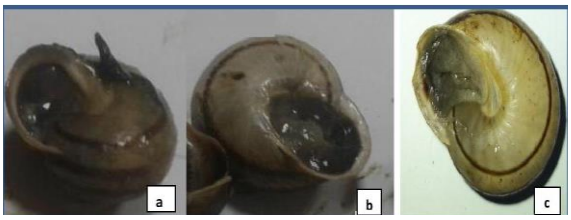

Fig. 2. Mortality of *Eobania vermiculata* adults by etofenprox (a) and spirotetramat (b) compared with control (c)

These results were agreed with Vehovszky et al. [14]. The authors found that thiacloprid (0.01 mg/ml) blocked almost 90% of excitatory postsynaptic potentials (EPSPs) in a molluscan, *Lymnaea stagnalis.* Eshra et al. [15] evaluated two neonicotinoids (imidacloprid and acetamiprid) against *E. vermiculata.* The obtained results showed that the percentages of mortalities ranged between 65.30-89.28% for imidacloprid and 76.25-85.51% for acetamiprid. Hussein and Sabry [5] found that spiromesifen (lipid synthesis inhibitor as spirotetramat) was the less toxic pesticide against *E. vermiculata.* The LC_{50} was 280.9 ppm. Shirley et al. [16] found that spirotetramat reduced the number of *Meloidogyne incognita* (Plant-parasitic nematode) to 62%.

Efficacy of nanoformulations of the tested pesticides against *E. vermiculata*

All tested pesticide formulations are converted to nanoparticles formulations and evaluated against the adults of *E. vermiculata* (Table 4 and Fig. 3). Results showed that etofenprox nanoparticle is the most effective against the *E. vermiculata* adults followed by spirotetramat, thiacloprid and fenbutatin oxide. The LC_{50}'s were 20.3, 35.0, 173.2 and 299.1 ppm, respectively (Table 4). The statistical analysis showed that there is a significant difference between etofenprox and other tested pesticides. The statistical analysis also showed thatno significant difference between the first concentrations of etofenprox and fenbutatin oxide. This may be due to the high concentration of active ingredient in fenbutatin oxide (55%) compared with (10%) in etofenprox.

The obtained results showed that the efficacy of etofenprox nanoparticles (81.7%) have the same efficacy as etofenprox conventional formulations (86.7%) (Table 4). The results with other tested pesticides showed that there are significant differences between the efficacy of nanoparticles and conventional formulations. The percent of mortality in spirotetramate conventional formulation is 100% with the recommended field rate (C1) compared with 53.3% with nanoformulation. So, there is a difference between spirotetramat conventional formulation and spirotetramat nanoparticles. These results are also found in both thiacloprid and fenbutatin oxide. The obtained results cleared that etofenprox nanoparticle formulations can be used against the chocolate banded snail, *E. vermiculata.* This means that it can be used one fifth of etofenprox concentration instead of the conventional formulation. This can be led to decrease in the pesticide concentration, the cost of pesticide application, soil contamination and the toxicity on nontarget organisms. These results disagreed with Muneer et al. [17]. The authors found that the nanothiacloprid was more effective than normal formulations against the apple aphid (*Aphis pomi*). The LC_{50}'s were 1.02 and 6.05 ppm for nano and conventional thiacloprid formulation, respectively.

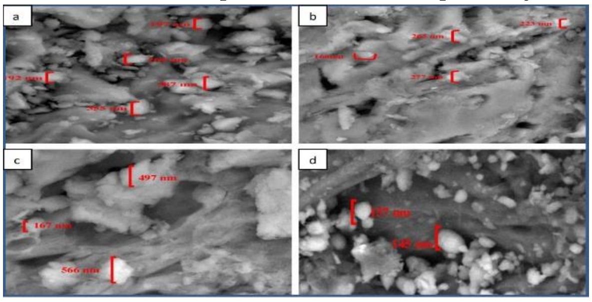

Fig. 3. Nanoparticles of thiacloprid (a), etofenprox (b), spirotetramat (c) and fenbutatin oxide (d) under Electronic microscope

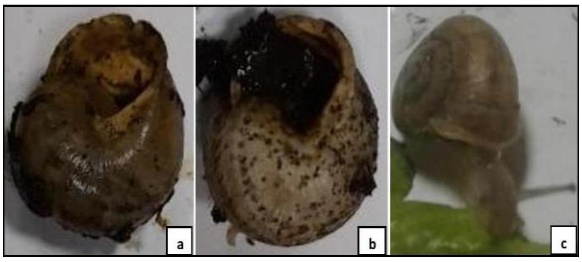

Fig. 4. Effect of etofenprox (a) and etofenprox nanoparticles (b) compared to control (c) on *E. vermiculata*

Conclusion

Nano formulations of thiacloprid, etofenprox, spirotetramat and fenbutatin oxide were used against the adult of chocolate banded snail, *E. vermiculata* in comparison with conventional formulations. The obtained results showed that etofenprox and spirotetramat nanoparticles were promising against *E. vermiculata* compared to other pesticides. These results need to be carried out on field-scale.

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