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RESEARCH

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# Development and using of some nanopesticide formulations against the conical snail, *Cochlicella acuta,* and the chocolate banded snail, *Massylaea vermiculata*

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## Abstract

**Background:** Nanopesticides are considered promising strategy in pest control. So, this strategy became an important new approach in pesticide formulations production. In this study, three traditional pesticide formulations (chlorfenapyr, imidacloprid and indoxacarb) were developed into nanoformulations. The toxicity of these nanoformulations was evaluated against the adults of the conical snail, *Cochlicella acuta* (Müller, 1774), and the chocolate banded snail, *Massylaea vermiculata* (Mohamed and Ali, Anim Biol J2(4):171–180, 2011).

**Results:** The results revealed that the nanosizes of nanoformulation nanoparticles were ranged between 220 and 534 nm. The loading capacities of the selected pesticides on polymer were 65.3 ± 6.4, 62.8 ± 5.3 and 37.1 ± 0.4%for nanochlorfenapyr, nanoimidacloprid and nanoindoxacarb, respectively. The nanochlorfenapyr was the most toxic against both the *C. acuta* and *M. vermiculata* followed by nanoindoxacarb and nanoimidacloprid. The $LC_{50}$s of the nanoformulations were 6.1, 7.7 and 14.9 ppm for chlorfenapyr, indoxacarb and imidacloprid, respectively.

**Conclusions:** These results cleared that the conical snails were more susceptible to all the tested pesticides than the chocolate banded snail, *M. vermiculata*. The efficacy of nanochlorfenapyr, nanoindoxacarb and nanoimidacloprid on conical snails (6.1, 7.7 and 14.9 ppm, respectively) was about fourfold compared with *M. vermiculata* (23.1, 31.9 and 60.6 ppm, respectively).The results also revealed that the potency of nanoformulation used was not only by direct killing of tested snails but also by repellent effect.

**Keywords:** Nanopesticide formulations, Imidacloprid, Indoxacarb, Chlorfenapyr, *Massylaea vermiculata*, *Cochlicella acuta*

## Background

Currently, there is an urgent need to develop a new approach of pesticide formulations. Therefore, using of alternative formulation may be resolving these side effects. Using of nanopesticide formulations is considered one of these solutions. The benefits of nanopesticides not only improve the pesticide efficacy but also improve the solubility of the formulations used (Bombo et al. 2019). The nanopesticide formulations play an important role in reducing plant injury and economic loss by decreasing the quality and quantity of synthetic chemicals used in pest control (Syafrudin et al. 2021). Nanopesticide formulations can also reduce the environmental pollution and cost of treatment (Sabry and Hussein2021).

Terrestrial snails cause many injuries for important economic plants and field crops by damaging the leaves, fruits, roots, tubers and seeds (El-Deeb et al. 1999; Mohammed 2015; Ali and Robinson 2020; Ali and Ramdane 2020). Recently, the terrestrial snails are considered

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the main agricultural and economic pest in Egypt and causing reduction in crops yield (Nakhla and Tadros 1995; Sallam and El-Wakeil 2012). The conical snail, *Cochlicella acuta*, is considered one of the moderate hazard pests in Egypt. This pest causes damage to infested, grain crops and pastures by chewing or rasping the plant leaves (Hashem and El-Halawany 1996; Baker2002; Eshra 2004), while the chocolate banded snail, *Massylaea vermiculata*, which was previously known as *Eobania vermiculata* (Bouaziz-Yahiatene et al. 2017) is the most serious terrestrial snail to many vegetables fruits and field crops in many governorates in Egypt (Ismail 1997; Eshra 2013; Desoky et al. 2015; Ismail et al. 2017; Desoky 2018). This pest species was recorded in Europe (Puizina et al. 2013; Ronsmans and Van den Neucker 2016), Japan (Ueshima 2006), the USA (Roth and Sadeghian 2003), Australia (Blacket et al. 2016), and Egypt (Herbert 2010).

The conventional formulations of pesticides have many disadvantages such as environmental pollution, pest resistance, and underground water contamination. Nanopesticides formulations can be resolving these problems by reducing the pesticides concentrations and environmental contamination (Sabry and Hussein 2021).

Chlorfenapyr is a new pesticide that act as disruptor to the oxidative phosphorylation process and inhibit the (Adinosin triphosphate enzyme) ATP synthesis in respiration process in the target pest (Hollingworth and Gadelhak 1998). This pesticide was used against wide range of pests. Nanoformulation of this pesticide was developed and used against the adults of glossy clover snail, *Monacha cartusiana* (Sabry and Hussein 2021).

Imidacloprid is a neonicotinoids that acts on the nicotinic acetylcholine that are receptors in the nervous system (Buckingham et al. 1997). The nanoformulation of this pesticide was used against the Egyptian cotton leafworm, *Spodoptera littoralis* (Sabry et al. 2021), and glossy clover snail, *Monacha cartusiana* (Sabry and Hussein 2021).

Indoxacarb is a new pesticide used against many pests which acts by blocking of sodium channel this blocking leads to paralysis and death of treated pests (Wing et al. 2000). Nanoformulation of this pesticide was used against the Egyptian cotton leafworm, *S. littoralis* (Sabry et al. 2021).

This work aims to evaluate the efficacy of nanoformulations of chlorfenapyr, imidacloprid and indoxacarb against the adults of the conical snail, *C.* a*cuta,* and the chocolate banded snail, *M. vermiculata*.

## Methods

### Selected animal

1. The adults of chocolate banded snail, *Massylaea vermiculata*, were collected from the farm belongs to Faculty of Agriculture, Moshtohor, (Toukh Directorate), Banha University, Qalyubia Governorate (coordinates*:* 30° 21′ N 31° 13′ E) (Fig. 1).
2. The adults of the conical snail, *Cochlicella* a*cuta*, were collected from crop field farm at Belbeis, Sharkia Governorate (coordinates: 30° 25′ N 31° 38′ E) (Fig. 2).

**Fig. 1** Collecting of *M. vermiculata* adults

**Fig. 2** Collecting of *C. acuta* adults

All collected samples of adult snails were transferred to the laboratory and then reared in plastic boxes (30 × 20 × 10 cm) with moist sandy loam soil. These adult snails were fed on lettuce, *Lactuca sativa* L. (Asteraceae), leaves and reared under laboratory conditions (25 ± 1 °C and 75 ± 5% RH).

### Selected pesticides

1. Chlorfenapyr (Challenger®36% SC) belongs to chemical family "pyrroles" and it is the first pyrrole submitted for US registration. This pesticide was obtained from Huaian Glory Chemical Co. Ltd. China. One-fifth of the recommended field rate of

nanoformulation and two lower concentrations were used (Table 1).

2. Imidacloprid (Command°® 35% SC) produced by Vapco Company Jordan. This insecticide is related to neonicotinoids group. The recommended field rate is 250 ml/feddan (4200 $^{m2}$). One-fifth of the recommended field rate of nanoformulation and two lower concentrations were used (Table 1).
3. Indoxacarb (Avaunt 15% EC) produced by Du Pont De Nemours. Indoxacarb is related to a new insecticide class called oxadiazine and it acts as a sodium channel blocker. One-fifth of the recommended field rate of nanoformulation and two lower concentrations were used (Table 1).

#### Preparation of the selected pesticide nanoformulations

All tested pesticides formulations were converted to nanoformulations. Chitosan with a high molecular weight (850 KDa) was used as a carrier (polymer) for active ingredient of all selected pesticides. All nanoformulations were developed according to Vaezifar et al. (2013). One gram of chitosan highly molecular weight was weighted and dissolved in acetic acid (2% v/v). The obtained mixture was transferred to magnetic stirrer for 15–20 min. After that 0.8% (w/v) of tripolyphosphate (TPP) solution containing one-fifth of the field concentration of each tested pesticide individually was added to the chitosan mixture (chitosan + acetic acid) followed by 5–10 min of stirring. The obtained mixture was centrifuged at 10,000 RPM for 30 min. The obtained pellet was collected and lyophilized to obtain nanoparticles in all selected pesticides. Photography of nanoparticles was achieved by scan electronic microscope (SEM) (Fig. 3).

**Table 1** The concentrations of nanopesticide formulations used against *C. acuta* and *M. vermiculata* adults

| Pesticides | Nanoformulations concentrations (ppm) | | |
|---|---|---|---|
| | C1[a] | C2 | C3 |
| Chlorfenapyr | 20 | 10 | 5 |
| Imidacloprid | 36 | 18 | 9 |
| Indoxacarb | 30 | 15 | 7.5 |

[a] C1: first concentration (one fifth of recommended field rate), C2: the second concentration, C3: third concentration

After all tested pesticides were converted to nanoparticles, loading capacity (LC) of these nanoparticles calculated by (He et al. 2017) (Fig. 4):

$$\text{Loading Capacity(LC)} = \frac{\text{Mass of loaded pesticide}}{\text{Mass of pesticide nanoparticles}} \times 100$$

Loading capacity means that the mass percentage of the selected pesticides that loaded on carrier used (polymer) compare with the active ingredient in conventional formulation.

The method of loading capacity determined is carried out by 30 mg of the obtained nanosamples (all selected pesticides nanoparticles) were weighed and dissolved in 50 ml of acetonitrile. This mixture was shaking overnight at a constant temperature to completely dissolve the chitosan nanoparticles. After that, the obtained solution was filtered, and the mass concentration of each pesticide nanoformulation in acetonitrile was examined by HPLC (Agilent 1260 system with a WATO45905 C18 column) under a detection wavelength of 278 nm. The loading capacity of each nanoparticle was calculated by division of the mass of loaded pesticides on mass of pesticide nanoparticles.

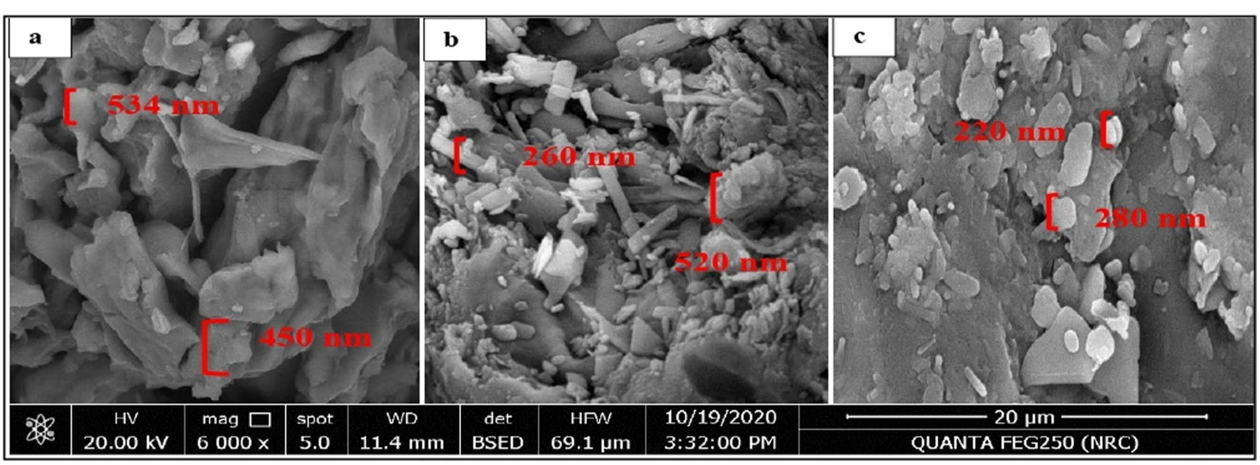


**Fig. 3** The nanoparticles of chlorfenapyr (**a**), imidacloprid (**b**) and indoxacarb (**c**) under scan electronic microscope (SEM)

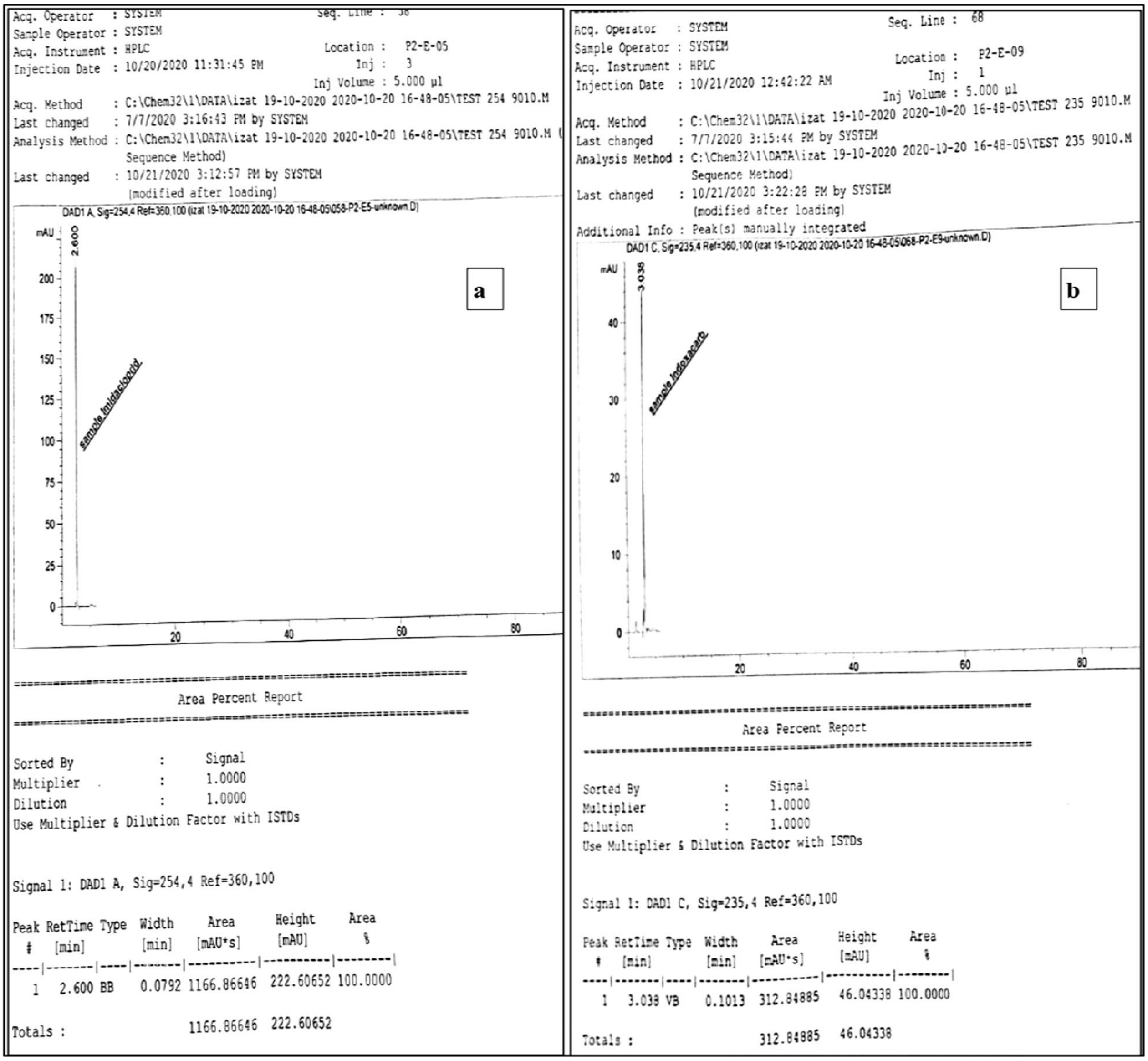


**Fig. 4** Loading capacity of imidacloprid (**a**) and indoxacarb (**b**)

## Bioassay

Three concentrations of each nanoformulation were used (Table 1). Every concentration has three replicates. Every replicate includes ten healthy and starved adults of the selected snails in plastic box. The bioassay test was carried out by dipping test that the fresh lettuce leaves were dipped in each concentration and then the health adult snails were fed on. All tested snails were kept under laboratory conditions (25 ± 1 °C and 75 ± 5% RH). The fresh leaves were checked daily and replaced with fresh one. The percentages of mortality were evaluated after 1, 3, 5 and 10 days of treatment. The lethal of 50% of tested population ($LC_{50}$) was calculated by Finney (1971)

## Statistical analysis

The data were analyzed by 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

The activities of chlorfenapyr, imidacloprid and indoxacarb against both the adults of *Cochlicella acuta* and *Massylaea vermiculata* were evaluated under laboratory condition. The efficacy of all tested pesticides showed differences between the *C. acuta* and *M. vermiculata*.

### Activity of chlorfenapyr, imidacloprid and indoxacarb nanoformulations against the adults of conical snails, *C. acuta*

Data in Table 2 show that the first concentration (the highest concentration that equal one-fifth of the recommended field rate) of chlorfenapyr is the most effective against *C. acuta* followed by imidacloprid and indoxacarb.

The percentages of mortality are 93.3, 83.3 and 73.3, respectively (Table 2). These results also are obtained with the second concentration (the half of first concentration). With the third concentration (one-fourth of the first concentration), chlorfenapyr is the most effective followed by indoxacarb and imidacloprid (Fig. 5).

This difference leads to the $LC_{50}$s of the tested pesticides are 6.1, 7.7 and 14.9 ppm for chlorfenapyr, indoxacarb and imidacloprid, respectively (Table 2). The statistical analysis shows that there is no significant difference between chlorfenapyr and imidacloprid with all concentrations. The results also revealed that there is a significant difference between chlorfenapyr and indoxacarb. The less significant difference are 10.9, 10.9 and 12.2 with the first, second and third concentrations.

### Efficacy of chlorfenapyr, imidacloprid and indoxacarb nanoformulations against the adults of chocolate banded snail, *M. vermiculata*

Data in Table 3 show that all tested pesticides have medium effects against *M. vermiculata* adults (Table 3 and Fig. 6). The percentages of mortality with first concentration (the highest concentration) in all tested

**Table 2** Toxicity of some nanopesticide formulations against the conical snail, *C. acuta*

| Pesticides | Concentrations and percent of mortalities ± SE | | | Slope ± SE | $LC_{50}$ and fiducial limits |
|---|---|---|---|---|---|
| | First concentration | Second concentration | Third concentration | | |
| Chlorfenapyr | $93.3 \pm 5.8^{a}$ | $66.7 \pm 5.8^{a}$ | $43.7 \pm 5.8^{a}$ | 2.6 ± 0.4 | 6.1 (5.0–7.0) |
| Imidacloprid | $83.3 \pm 5.8^{ab}$ | $56.7 \pm 5.8^{ab}$ | $30.0 \pm 0.0^{ab}$ | 2.5 ± 0.3 | 14.9 (12.7–17.2) |
| Indoxacarb | $73.3 \pm 5.8^{b}$ | $46.7 \pm 5.8^{b}$ | $33.3 \pm 5.8^{a}$ | 1.7 ± 0.3 | 7.7 (6.2–9.4) |
| Control | $6.7 \pm 5.8^{c}$ | $3.3 \pm 5.8^{c}$ | 0.0 | | |
| *F* values* | 138.3*** | 70.0*** | 25.1*** | | |
| LSD | 10.9 | 10.9 | 12.2 | | |

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

***Means the difference between these data is very significant

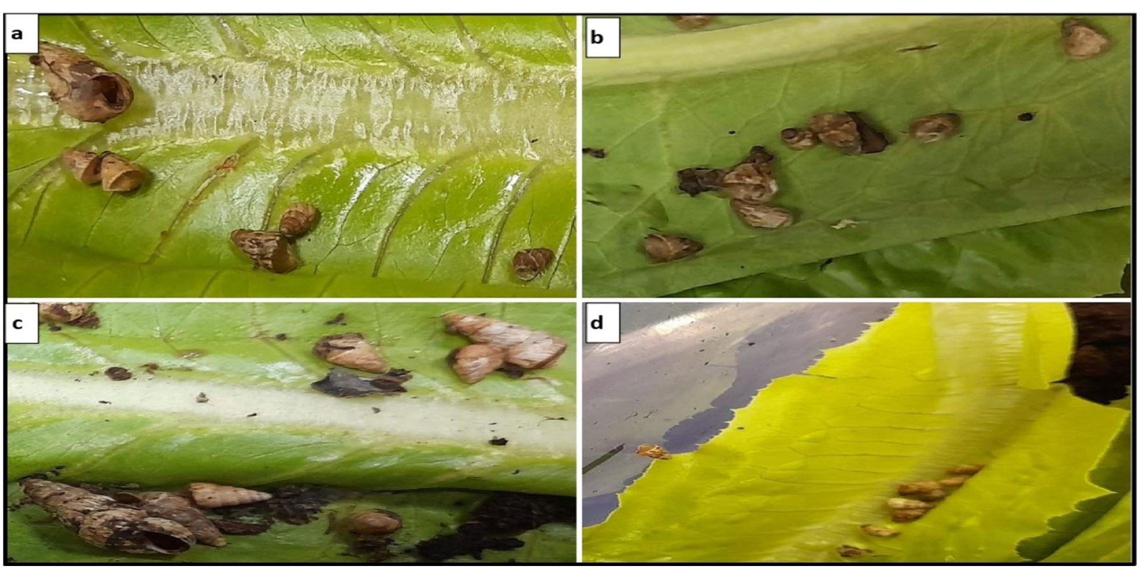


**Fig. 5** Effect of nanoformulations of chlorfenapyr (**a**), imidacloprid (**b**), indoxacarb (**c**) compared with untreated (control) (**d**) on the conical snails adults

**Table 3** Toxicity of some nanopesticide formulations against the chocolate banded snail, *M. vermiculata*

| Pesticides | Concentrations and percent of mortalities ± SE | | | Slope ± SE | $LC_{50}$ and fiducial limits |
|---|---|---|---|---|---|
| | First concentration | Second concentration | Third concentration | | |
| Chlorfenapyr | 46.7 ± 5.8[a] | 23.3 ± 5.8[a] | 13.3 ± 5.8[a] | 1.8 ± 0.3 | 23.1 (17.7–37.9) |
| Imidacloprid | 33.3 ± 5.8[b] | 23.3 ± 5.8[a] | 10.0 ± 10.0[a] | 1.6 ± 0.4 | 60.6 (41.5–143.9) |
| Indoxacarb | 36.7 ± 5.8[b] | 16.7 ± 5.8[a] | 10.0 ± 0.0[a] | 1.4 ± 0.3 | 31.9 (19.7–119.4) |
| Control | 0.0[c] | 3.3 ± 5.8[b] | 0.0[b] | | |
| *F* values** | 49.2*** | 8.0** | 3.1[ns] | | |
| LSD | 9.4 | 10.9 | 10.9 | | |

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

***Means the difference between these data is very significant

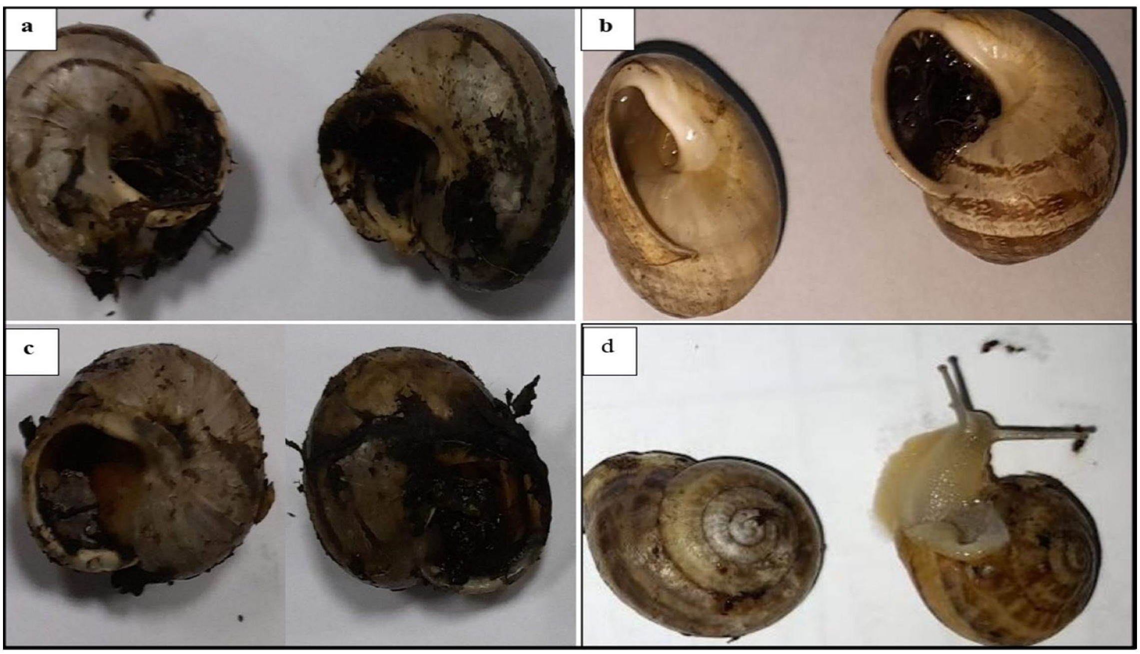


**Fig. 6** Effect of chlorfenapyr (**a**), imidacloprid (**b**) and indoxacarb (**c**) on the adults of *M. vermiculata* compared with the control (**d**)

pesticides are 46.7, 33.3 and 36.7% with chlorfenapyr, imidacloprid and indoxacarb, respectively.

This means that the highest percent of mortality did not exceed 46.7%. According to the $LC_{50}$ chlorfenapyr is the most effective pesticide against *M. vermiculata* followed by indoxacarb and imidacloprid. The $LC_{50,s}$ are 23.1, 31.9 and 60.6 ppm, respectively. The statistical analysis shows that there is a significant difference between the first concentration (one-fifth of recommended field rate) of chlorfenapyr and the first concentration in other tested pesticides.

The LSD is 9.7. On the other hand, there is no significant difference among all tested pesticides with the second and third concentrations. All tested pesticides showed a significantly different effect compared with the control.

## Discussion

*Cochlicella acuta* snails are considered one of the most destructive pest causing damage to palm trees, citrus orchards and ornamental plants (Mohamed and Ali 2011). The toxic action of all tested nanopesticides was very fast against the adult of *C. acuta* snail species. The adults of *C. acuta* were died after 1 h of treatment and the survived adults were stopped feeding (Fig. 5). The similar result was found by Sabry et al. (2021). The obtained results found that the nanoformulation of indoxacarb was more effective than nanoimidacloprid against the second instar larvae of *Spodoptera littoralis*. Sabry and Hussein (2021) found that the nanochlorfenapyr was more effective than nanoimidacloprid against the glossy clover snails, *Monacha cartusiana*. Chlorfenapyr was the most effective compared with other used pesticides this may be due to the nature of mechanism of action of chlorfenapyr. This pesticide inhibits the adinosin triphosphate enzyme synthesis and therefore inhibits the respiration process.

The *Massylaea vermiculata* adults were moderately affected by the nanoformulations of the tested pesticides. These results were consistent with Ali et al. (2014). The obtained results found that the silver nanoparticles reduced the *M. vermiculata* by 20% under laboratory conditions. Khidr (2018) evaluated the efficacy of nanochitosan against the adults of *M. vermiculata*. The $LC_{50}$ was 1.4 ppm. Mohamed et al. (2021) used the nanosilica against the adults of *M. vermiculata*. The nanosilica reduced the population of *M. vermiculata.* Hussein and Sabry (2019) found that indoxacarb was more effective against *M. vermiculata* adults than abamectin and spiromesifen. The lethal concentration for 50% of population ($LC_{50}$) of indoxacarb was 58.6 ppm.

## Conclusions

The obtained results showed that the adults of *C. acuta* were more susceptible to the tested nanopesticides than the adults of *M. vermiculata*. The $LC_{50}$ of chlorfenapyr was 6.1 ppm with the *C. acuta* adults compared with 23.1 ppm with the *M. vermiculata* adults. This means that the susceptibility of *C. acuta* was 4-times approximately. These results also found with both imidacloprid and indoxacarb nanoparticles. Hussein and Sabry (2019) found that the $LC_{50}$ of indoxacarb normal formulation was 58.6 ppm. The obtained results showed that the indoxacarb nanoformulation was 7.7 ppm. This result means that the nanoformulation (7.7 ppm) was more effective against the adults of *M. vermiculata* than the normal formulation (58.6 ppm). Consequently, using of nanopesticides can reduce the pesticides risk in the environment by reducing the concentrations used.

### Abbreviations
$LC_{50}$: Lethal concentrations for 50% of pest population; LSD: Less significant difference; TPP: Tripolyphosphate; RCBD: Randomized Complete Block Design.

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### Authors' contributions
SKH designed the workplan, carried out the laboratory evaluation, data analysis and writing the manuscript; ARF collecting the samples from different places, laboratory experiment and writing the paper. Both authors read and approved the final 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.

## Declarations

### Ethics approval and consent to participate
Not applicable.

### Consent for publication
Not applicable.

### Competing interests
The authors declare that they have no competing interests.

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## Publisher's Note