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(RESEARCH ARTICLE)

Comparative toxicity of abamectin and nano-derived form on land snail, *Helix aspersa* in attributing to cytotoxicity and biochemical alterations

Khaled Yassin Abdel-Halim [1,*], Safaa Ramadan Osman [1], Heba Mohamed El-Danasoury [2] and Gihan Fathy Aly [3]

[1] *Mammalian & Aquatic Toxicology Department, Central Agricultural Pesticides Laboratory (CAPL), Agricultural Research Center (ARC), 12618-Dokki, Giza, Egypt.*
[2] *Department of Plant Protection, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt.*
[3] *Central Agricultural Pesticides Laboratory (CAPL), Agricultural Research Center (ARC), Sabahia, Alexandria, Egypt.*

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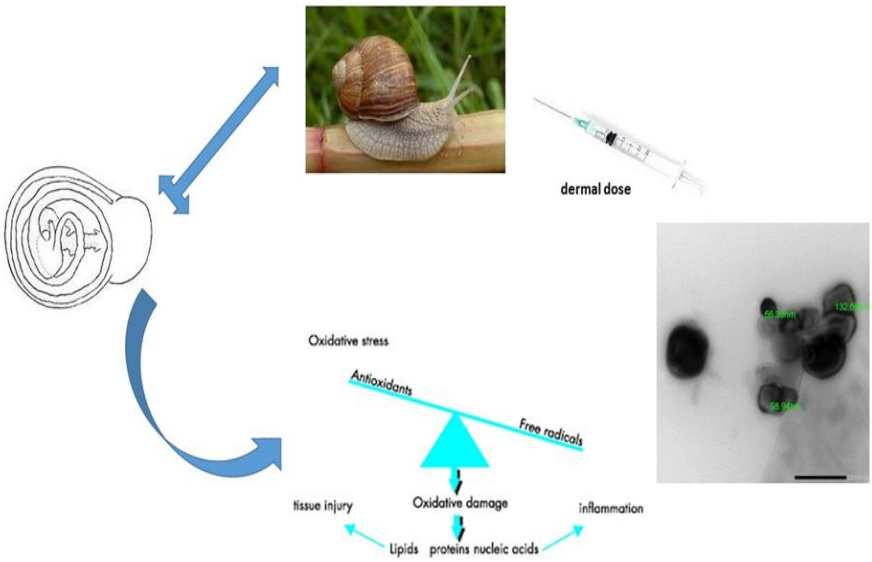
Abstract

The comparative toxic effect of Vertimec® 1.8% EC, Fast Max Super® 8.4% SC and nano-derived form of abamectin (ABM) (1% nano-emulsion) as a dermal contact for 48 h against land snail, *Helix aspersa* was evaluated at laboratorial trail. Acute toxicity values (LD_{50}) were 6.45, 11.97, and 45.95 µg $snail^{-1}$ for nano-derived form of ABM, Fast Max Super® and Vertimec®, respectively. Nano-derived form exhibited the highest toxic effects (1.86 and 7.12-folds), respect to Fast Max Super® and Vertimec®. Sublethal doses: 1/10 and 1/100 LD_{50s} of the examined compounds were applied to evaluate some biochemical alterations e.g. acetylcholinesterase (AChE), malondialdhyde (MDA), lactate dehydrogenase (LDH), glutathione-*S*-transferase (GST), acid phosphatase (ACP), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), respectively, in haemolymph and digestive glands homogenates. The all treatments significantly decreased AChE activity in ganglia homogenate, respect to control group (untreated). All treatments exhibited MDA level and LDH activity greater than the control in both haemolymph and digestive gland. This concept recognizes the cytotoxic effect of ABM on gastropods. Significant declines in GST, ACP, and ALP activities were exhibited in homogenate of digestive gland for the all treatments. However, AST/ALT activities exhibited increase greater than untreated group. These findings may explain the role of these doses of ABM for dysfunction in organs of *H. aspersa*. Thus, prepared nano-emulsion was more potent toxic on land snails. However, *H. aspersa* is considered a useful tool to assess ecotoxicological impact of pesticides.

Keywords: Abamectin; Nano-emulsion; Land snail; Biochemical alterations; Cytotoxicity

* Corresponding author: Khaled Yassin Abdel-Halim
Mammalian & Aquatic Toxicology Department, Central Agricultural Pesticides Laboratory (CAPL), Agricultural Research Center (ARC), 12618-Dokki, Giza, Egypt.



1. Introduction

The terrestrial snails become an economic serious pest in Egypt. They cause serious economic damage, especially in horticulture and ornamental plants [1]. The garden snail *Helix aspersa* (Müller) is a terrestrial gastropod mollusk, fits to helicid family (Helicidae), known as European brown snail, and dispersed in the world, especially the Mediterranean region. Also, it is used as a bio-indicator for toxicant pollution [2-4].

Recently, nanotechnology has a promising area of research in dependence on particles with special properties of size and morphology than the primary matrix. Nanoparticles (NPs) have a size in range of 1-100 nm, and increased surface area leading to the enhancement of their biological activity. However, there are many challenges in field of NPs synthesis e.g. vital need to develop non-toxic, cost effective and environmentally safe method matching with high yields. Nanomaterials have roles to enhance agricultural production providing nano-porous materials with slow release and efficient dosage of pesticides and fertilizers suitable for pest management. Nanoparticles (NPs) have remarkable characteristics (chemical, physical and biological) compared to larger particle of bulk material. For example, metallic NPs have unique catalytic activity for removal of both organic and inorganic contaminants. Using nanotechnological methods in crop protection is still in the early stages for various agricultural purposes such as development of the nano-formulations of pesticides, fungicides and herbicides used in the agricultural sector. Similarly, NPs help in gene transfer which acts as ideal tool to assess crops and plants resistant to pathogens and pests. Nanomaterials (NMs) can be used as a carrier for the agrochemicals or used as biopesticides or biofertilizers [5-6].

Abamectin (ABM) is a compartment of large ring lactone disaccharide compounds and the natural ferment product of the soil dwelling actinomycete *Streptomyces avermitilis* [7]. The main target of ABM is believed to affect the gamma-amino butyric acid (GABA) system of animal cells [8], in which the GABA receptor is responsible for regulating the neutral basal tone [9]. Abamectin (ABM) consists of avermectin BIa, and avermectin BIb, where they are macrocyclic lactones extracted from the fermentation process of soil bacteria, *S. avermitilis*. It is a nerve poison used as an insecticide, acaricide, nematicide, and a veterinary antihelmintic [10]. This group stimulates the gamma-amino butyric acid (GABA) at nerve endings, and inhibits both nerve to nerve and nerve to muscle communication. The affected pest becomes paralyzed, stops feeding and dies after few days. Abamectin is broken down quickly in the soil *via* photodegradation at the soil surface and microbial degradation in dark, aerobic conditions. Its half-life is about 1 week on an unshaded soil surface and about two weeks to two months underneath the soil surface [11].

Snails represent a major proportion of the invertebrate biomass and are used as suitable organisms for environmental bio-monitoring assessment. Most research trails demonstrated the use of glutathione-*S*-transferase (GST), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and lipid peroxidation (LPO) in snail exposed to various toxicants and their implication in the environmental monitoring programs [12]. Thus, it provides sensitive biochemical markers for exposure and toxicity in the environmental monitoring. Among antioxidant parameters e.g. SOD, CAT, GST, GPx and LPO are extensively studied [2, 13-14]. Recently, antioxidant enzymes and the contents of malondialdehyde (MDA) have been used as biomarkers of pollutants that generate oxidative stress in aquatic animals [15-16]. Huang et al. (2019) [17] found that, ABM exposure to the Chinese Mitten Crab, *Eriocheir sinensis* caused significant decreases in antioxidants e.g. SOD, CAT and total antioxidant capacity (T-AOC) with dose-and time-

dependent responses. Meanwhile, levels of MDA and carbonyl protein (CP) significantly increased. GST belongs to the phase II detoxication enzyme of animal liver and plays an important role in metabolizing and detoxication of chemicals [18-19]. Acetylcholinesterase (AChE) is an important enzyme that plays a crucial role in catalyzes the hydrolysis of the acetylcholine (ACh) neurotransmitter to maintain the normal conduct of nerve impulses [20]. Now, a lot of reports have approved that AChE is the main target of organophosphate and carbamate pesticides and it can be used as an important biomarker of pesticide toxicity to snails [21-22].

The present study aims to indicate if the ABM besides the NPs formulation of pesticide exposure alerts the activities of AChE, and other biochemical quantifications in haemolymph, digestive gland and ganglia homogenates of snail *H. aspersa*.

2. Material and methods

2.1. Chemicals

Tested pesticides: Vertimec® 1.8% EC and technical ABM 98.0% were obtained from Syngenta Company for Agrochemicals. Fast Max Super® 8.4% SC was obtained from Zhejang Qianjiang Biochemical Company, Ltd-China. The chemicals: potassium phosphate mono and dibase salts, trichloroacetic acid (TCA), hydrochloric acid (HCl), Tris HCl, and sodium chloride (NaCl) were obtained from J.T. Baker Chemical Co., Philipsburg, N.J. 08865. Acetylthiocholine iodide (ASChI), 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB), sodium pyruvate, α-nicotinamide adenine dinucleotide (NADH), 1-Chloro, 2-4 dinitrobenzene (CDNB), glutathione (GSH; reduced form), and bovine serum albumin (BSA) were supplied by Sigma Chem. Company, P.O box 14508 St., Louis, Mo 63178 USA.

2.2. Tested animals

Land snail, *H. aspersa* was selected for experimental treatments. Healthy individuals weighing 3.0±0.7 g were collected from some gardens in Ismailia governorate, Egypt. The individuals were maintained for 14 d in wood aerated cages (40x40x40 cm; 100 individuals each) under laboratory conditions (25±2 °C; 63±2% relative humidity and 12:12 h light/dark). The animals were fed on lettuce leaves *ad libitum*. The experiments have been carried out in accordance with the European Ethical Guidelines [23].

2.3. Nano-emulsion assessment

2.3.1. Preparation

Nano-emulsion of ABM 1% was prepared under high energy mode using sonication technique [24]. The active ingredient (a.i) was dissolved in a vegetable oil and employed to dispersion of liquid (water) with surfactant and co-surfactant to generate homogenous solution (o/w).

2.3.2. Characterization

Nano-derived form of ABM was employed to various storage conditions of temperature and humidity to assess emulsion stability according to ICH guidelines Q1A [25]. The prepared formulation was centrifuged at 3500 rpm for 30 min to observe the phase separation. Ten ml of prepared formulation were diluted to 100 ml with distilled water in graduated cylinder and employed for shaken 30 times from top to bottom continuously. At the end, the jar was allowed for 10 min and observed for oil separation: creaming, and sedimentation. On the other hand, an aliquot of the formulation was taken for heating and cooling cycle. Six cycles between refrigerator temperature of 4 and 48 ˚C for 48 hr were done. So, it was employed to freeze-thaw cycle test. Three cycle were done between -21 and 25 ˚C.

Morphology and structure of the prepared nano-form were determined by Transmission Electron Microscopy (TEM) (JOEL 1400 Plus, Japan) at filament at 80 Kev to observe the form and size of it. So, an aliquot of the formulation was diluted with deionized water (1/100 v/v), sonicated for enough time, drop of the diluted solution deposited on the film grid, dried and observed [26]. A combination of bright-field imaging at increased magnification with diffraction modes was used for optimal visualization.

Either conventional or nano-form of ABM were achieved on Fourier Transform Infrared (FTIR) instrument (TENSOR 27 Bucker, Germany-FTIR L203/1 2887). The observation was in a range from 4000 to 400 cm^{-1}. The run was conducted with sensitivity range 50 and absolute threshold level 6.00.

2.4. Acute toxicity

The contact toxicity of the examined pesticides against snail, *H. aspersa* was carried out by using topical application method to determine the median lethal and the sub-lethal doses [27-28]. The tested concentrations of Vertimec®, Fast Max Super® and nano-ABM were 1, 2, 4, 8, 40; 1, 2, 4, 20, 40 and 1, 2, 8, 20, 40 µg snail^{-1}, respectively. Three replicates (10 individuals, each) were used for each treatment and the animals were caged in plastic boxes. The pesticides were slightly loaded on the surface of the snail body inside the shell using micropipette containing 10 µl of vehicle. All boxes were sprayed with water to provide suitable conditions for snail activity. Percentages of mortality were estimated after 48 h of dosage.

2.5. Sub-acute toxicity

Independent on acute LD_{50} values of the examined pesticides, sub-lethal doses; 1/10 and 1/100 LD_{50} with levels; 1.2, 0.12 µg snail^{-1} for Vertimec®; 2.6, 0.26 µg snail^{-1} for Fast Max Super® and 0.65, and 0.065 µg snail^{-1} for nano-ABM, respectively, were used as described above in acute toxicity experiment. Control groups were injected with vehicle (as a reference group). After 48 h of dosage, the live animals were taken for analysis and haemolymph was collected carefully by inserting syringe under the shell from the hemocoel along the right side of the head. The fluid was withdrawn in anticoagulant's vails and stored at -20 °C until used. Then, the animals were dissected to remove digestive glands and ganglia and stored as described above.

2.6. Biochemical quantifications

2.6.1. Sample preparation

One g of ganglia gland or digestive gland was homogenized in potassium phosphate buffer pH 6.5 (1/10 w/v) using mechanical polytron for 15 s. The samples were centrifuged at 5000 rpm for 10 min. An aliquot of haemolymph was diluted with buffer (1/10 v/v). The supernatant of ganglia gland was used for AChE activity. However, supernatant of digestive gland was used for acid (ACP) and alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and GST. The homogenate was used for lactate dehydrogenase (LDH), MDA quantifications. The diluted haemolymph was used for quantifications as above.

2.6.2. AChE

The enzymatic activities were determined by using acetylthiocholine iodide (ASChI) as a substrate. The optical density of the developed yellow colour was recorded after 10 min against blank, which contained the entire reagent, expect the substrate at 412 nm. The activity was calculated as µM of substrate hydrolysed per mg protein per min [29].

2.6.3. MDA

The barbituric acid reactive substances (TBARS) were used as an index of lipid peroxidation according to Rice-Evans et al. (1991) [30]. TBARS was determined by spectrophotometric quantification of MDA content in tissues. An aliquot (250 µl) of tissue homogenate was mixed with 1 ml of 15% (w/v) trichloroacetic acid (TCA) in 25 mM HCl and 2 ml of 0.37% (w/v) thiobarbituric acid (TBA). The samples were boiled for 10 min, quickly cooled, and immediately centrifuged at 5000 rpm for 5 min. The absorbance was determined at 535 nm. MDA was quantified using an extinction coefficient of 156 mM^{-1} and its concentration was expressed as µM g^{-1} tissue.

2.6.4. LDH

An aliquot (100 µl) of tissue homogenate was added to 1 ml of working solution prepared by mixing 4 volumes of (80 mM Tris buffer pH 7.4, sodium pyruvate 1.6 mM, and 200 mM NaCl) with one volume of α-nicotinamide adenine dinucleotide (NADH) (240 µM). The change of absorbance was recorded every min at 340 nm for 3 min. The enzyme activity was expressed as U L^{-1} [31].

2.6.5. GST

The activity was determined by spectrophotometric method of Habig and Jakoby (1981) [32] by using 1-chloro, 2-4 dinitrobenzene (CDNB). Enzyme source was mixed with 500 µl of potassium phosphate buffer (50 mM; pH 6.5). Incubation was done at 25 °C for 5 min, followed by adding of 100 µl of 0.2 M CDNB and 150 µl of 10 mM reduced glutathione (GSH). After 1 min, the change of absorbance was recorded every 30 sec for 6 min at 340 nm. The enzyme activity was expressed as nM mg^{-1} min^{-1}.

2.6.6. ACP

The enzyme activity was measured by using specific kits (Bio Diagnostic Co., Germany). Absorbance of the sample and standard against the blank were recorded at 510 nm. The activity was expressed as U L^{-1} [33].

2.6.7. ALP

The enzyme activity was measured by using phenyl phosphate as a substrate (N.S. Bio-Tec., kits, UK). So, the complex colour of *P*-nitrophenyl phosphate was measured at 405 nm against the blank. The activity was expressed as U L^{-1} [34].

2.6.8. AST/ALT

The enzyme activities were measured according to the method of Gello et al. (1985) **[35]** by using specific kits (Bio system Kits, Spain). The activity was expressed as U L^{-1}.

2.6.9. Protein content

Protein level was determined according to the method of Lowry et al. (1951) [36]. The intensity of the developed blue colour was measured at 750 nm against the blank. Bovine serum albumin (BSA) was used as a standard.

2.7. Statistical analysis

LD_{50} values were expressed as µg $snail^{-1}$ with confidence limit (CL) and slope for the examined pesticides which computed using probit analysis [37]. All data presented as mean ± SE were subjected to analysis of variance (ANOVA) and means were compared to significance by Student-Newman Keuls at the probability of 0.05 [38].

3. Results

3.1. Characterization of nano-abamectin

The prepared nano-emulsion was stable during freeze-thaw cycle's storage. No Creaming or floating phases were made up. Also, no separated phase was formed after centrifugation process. TEM image showed that, NPs of ABM appeared as spherical shapes, and the size was mainly in the range (65-132 nm). The above results demonstrated that, this method could effectively construct the compound as a nano-emulsion (**Fig. 1**). On the other hand, **Fig. 2** illustrates FTIR patterns of conventional and nano-form of ABM, where the typical absorption peaks at 1742 cm^{-1} for C=O, 3423 cm^{-1} for OH, and 1618 cm^{-1} for benzene skeleton vibration which appeared in the spectra of ABM. Regarding nano-form, 1742 and 1092 cm^{-1} are attributed to C=O and C-O-C stretching vibration peaks. The broad peak at 3567 cm^{-1} may be attributed for C=O group of the used co-surfactant (polymer).

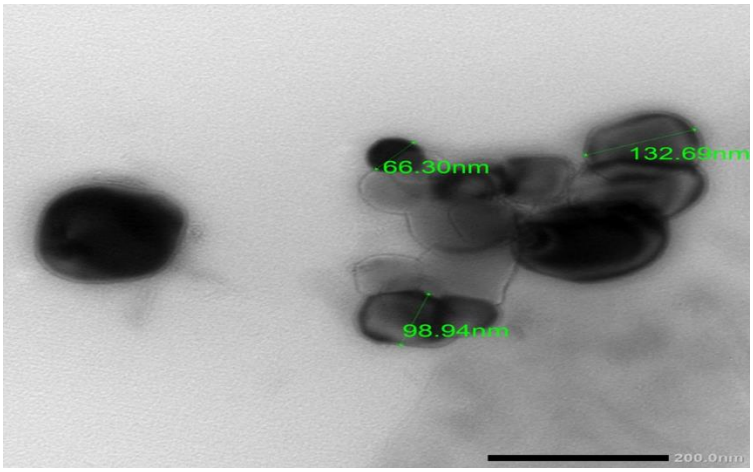


Figure 1 Electron photograph of prepared nano-emulsion of abamectin 1% a.i. visualized at 30000X.

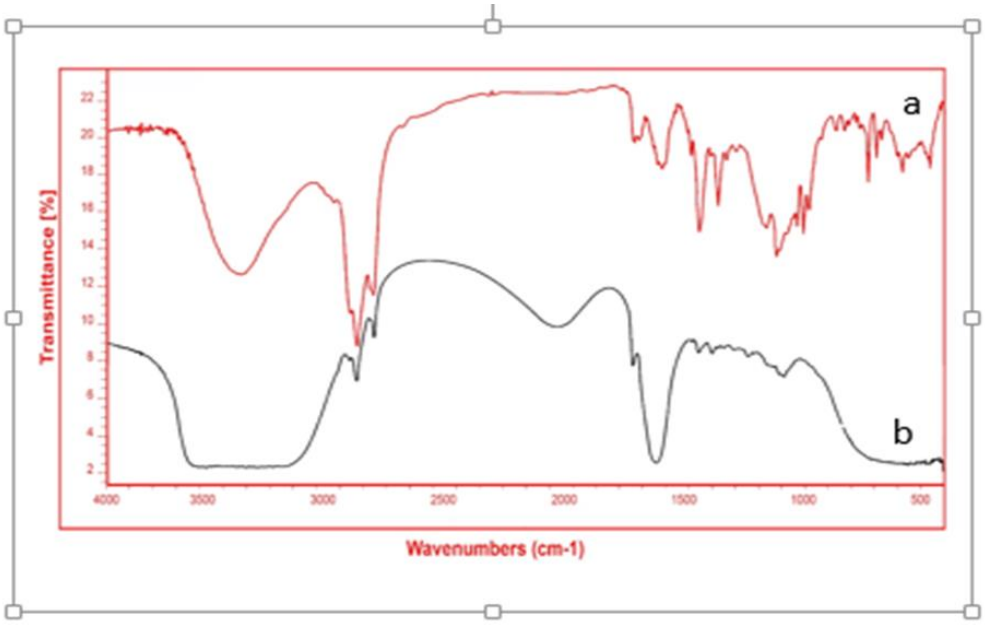


Figure 2 FTIR pattern of insecticides (a) Vertimec and (b) prepared nano-emulsion of ABM 1% a.i.

3.2. Acute toxicity

The acute toxicities of the examined pesticides on *H. aspersa* are listed in **Table 1**. Nano-ABM exhibited the greatest toxic effect (6.45 µg snail^{-1}), followed by Fast Max Super® (11.97 µg snail^{-1}), and Vertimec® (45.95 µg snail^{-1}). Compared with nano-ABM as the highest toxic, it was greater than others with 1.86 and 7.12 folds for Vertimec® and Fast Max Super®.

Table 1 The relative toxicities of the examined pesticides on land snail *Helix aspersa*.

Pesticide	**LD_{50} (µg snail^{-1})**	**Lower limit**	**Upper limit**	**Index (%)**	**Folds**	**Slope**	**LD_{90} (µg snail^{-1})**
Nano-ABM	6.45	4.57	9.06	100	1	0.80	262.56
Vertimec®	11.97	6.75	52.79	52.88	1.86	1.18	144.94
Fast Max Super®	54.93	25.67	121.99	14.04	7.12	0.65	4458.81

3.3. Biochemical responses

For biochemical quantifications, two doses (1/10 and 1/100 of LD_{50}) for each pesticide were used for 48 h building on LD_{50} value.

The activity of AChE in ganglia homogenate exhibited the greatest activity (0.244 µM mg^{-1} min^{-1}) for 1/10 LD_{50} treatment of Vertimec®, followed by (0.156 µM mg^{-1} min^{-1}) for 1/100 LD_{50} treatment of nano-ABM and (0.104 µM mg^{-1} min^{-1}) for 1/100 LD_{50} treatment of Vertimec®, respectively. Fast Max Super® treatments (1/10 and 1/100 LD_{50}) exhibited the activities: 0.079 and 0.091 µM mg^{-1} min^{-1} compared with the control (0.248 µM mg^{-1} min^{-1}) (Fig. 3).

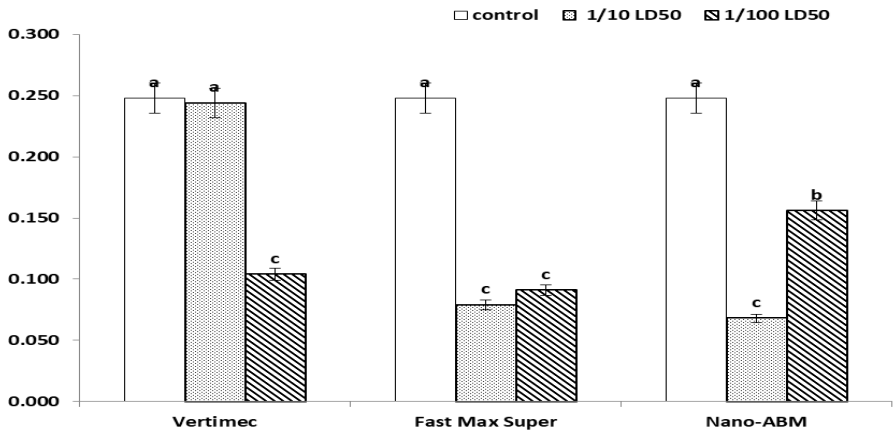


Figure 3 Activity of AChE (μM mg^{-1} min^{-1}) in ganglia homogenate of land snail, *H. aspersa* treated with two doses of different pesticides.

All treatments exhibited MDA levels greater than control in both haemolymph and digestive gland homogenate (**Fig. 4**). Levels of MDA in digestive gland homogenate were greater than in haemolymph. In haemolymph samples, 1/10 LD_{50} treatment of Fast Max Super® exhibited the greatest level (1.69 μM g^{-1} tissue), followed by 1/10 LD_{50} treatment of Vertimec® (1.61 μM g^{-1} tissue), and 1/100 LD_{50} treatment of Vertimec® (1.57 μM g^{-1} tissue). Treatments of nano-ABM (1/10 and 1/100 LD_{50}) exhibited 1.23 and 1.05 μM g^{-1} tissue, respectively, compared with the control (0.88 μM g^{-1} tissue) (**Fig. 4a**). Regarding digestive gland, the treatments exhibited MDA levels in the following order: Fast Max Super® > Vertimec® > nano-ABM with values: 2.89, 2.71; 2.66, 2.51 and 2.09, 2.07 μM g^{-1} tissue for 1/10 and 1/100 LD_{50}, respectively. Control group did not exceed 1.83 μM g^{-1} tissue (**Fig. 4b**).

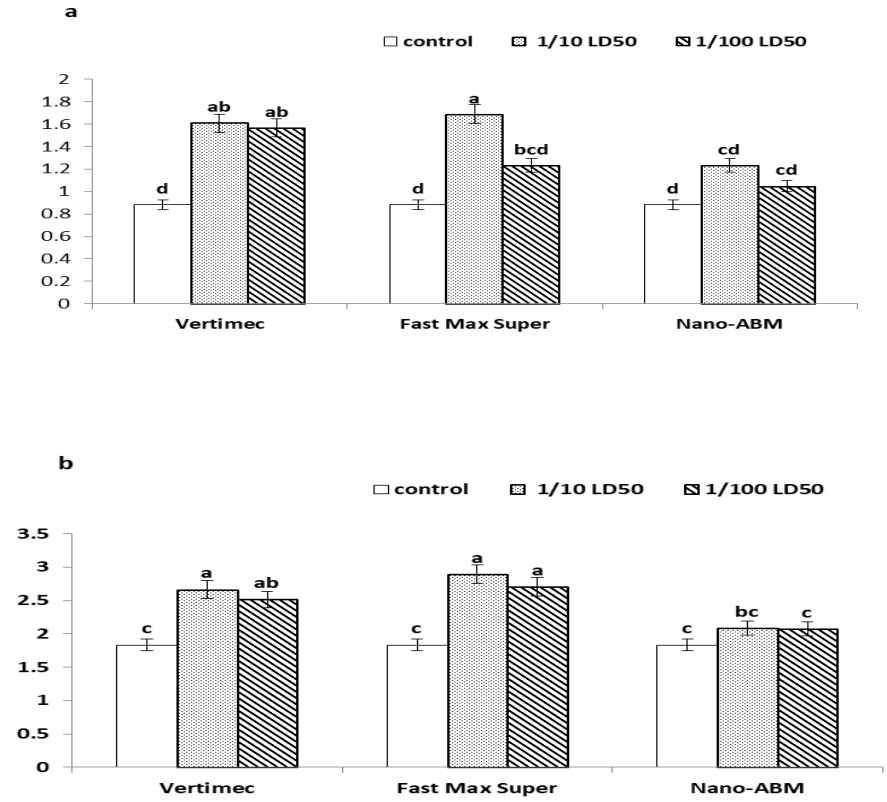


Figure 4 MDA levels (μM g^{-1} tissue) in (a) haemolymph and (b) digestive gland of land snail, *H. aspersa* treated with two doses of different pesticides for 48 h.

LDH activity is considered a good biomarker for cell damage. The all treatments exhibited enzyme activities greater than control in both haemolymph and digestive gland homogenate (**Fig. 5**). Activity of LDH in digestive gland homogenate was greater than in haemolymph. 1/10 LD_{50} treatment of Vertimec® exhibited the greatest activity (30.83 U L^{-1}), followed by nano-ABM (29.98 U L^{-1}), and 1/100 LD_{50} of nano-ABM (28.78 U L^{-1}). No significant difference was observed in Fast Max Super® treatments. 1/100 LD_{50} treatment of Vertimec® exhibited the least activity (23.11 U L^{-1}) compared with the control (10.85 U L^{-1}) (**Fig. 5a**). In case of digestive gland, 1/10 LD_{50} of Fast Max Super® exhibited the greatest activity (52.07 U L^{-1}), followed by 1/10 LD_{50} treatment of Vertimec® (44.59 U L^{-1}), and 1/100 LD_{50} of Fast Max Super® (43.99 U L^{-1}). Nano-ABM exhibited low activities: 33.77 and 40.37 U L^{-1} for 1/10 and 1/100 LD_{50} treatments, compared with the control (19.50 U L^{-1}) (**Fig. 5b**).

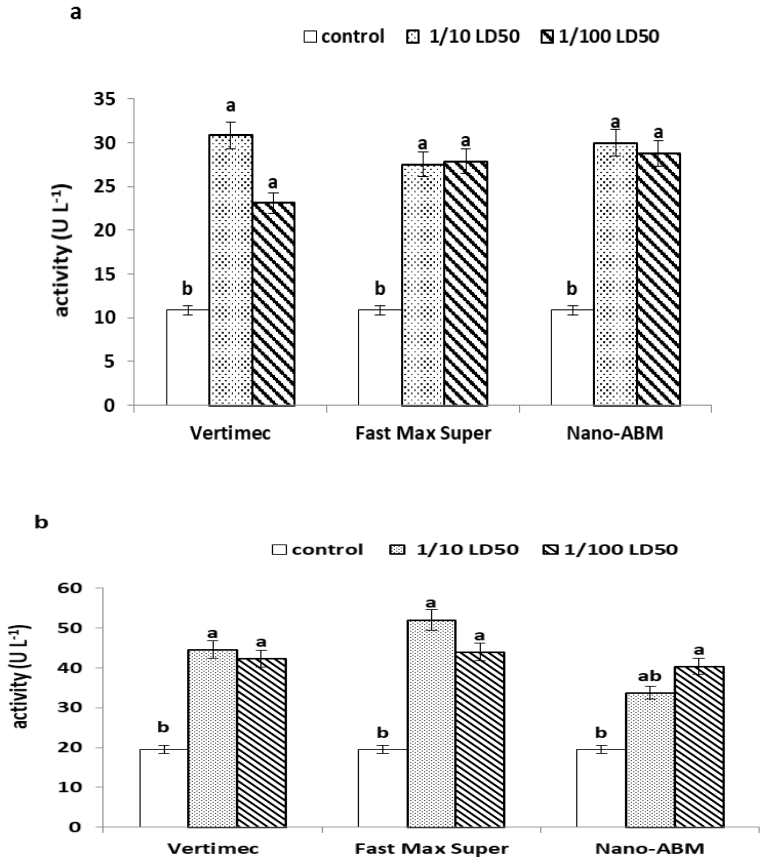


Figure 5 Activity of LDH (U L^{-1}) in (a) haemolymph and (b) digestive gland of land snail, *H. aspersa* treated with two doses of different pesticides for 48 h.

All treatments exhibited decline in GST activity in both haemolymph and digestive gland homogenate (**Fig. 6**). In haemolymph samples, GST activity was 2.42, 0.31; 3.59, 0.53 and 0.94, 3.72 µM mg^{-1} min^{-1} for 1/10 and 1/100 LD_{50} treatments of Vertimec®, Fast Max Super® and nano-ABM, respectively (**Fig. 6a**). Regarding digestive gland, 1/10 and 1/100 LD_{50} treatments of Fast Max Super® exhibited high activities: 18.26 and 11.74 µM mg^{-1} min^{-1}. Treatments (1/10 and 1/100 LD_{50}) of nano-ABM exhibited activities: 7.35 and 11.27 µM mg^{-1} min^{-1}. The same treatments of Vertimec® exhibited low activities: 3.09 and 2.28 µM mg^{-1} min^{-1} compared with the control (20.08 µM mg^{-1} min^{-1}) (**Fig. 6b**).

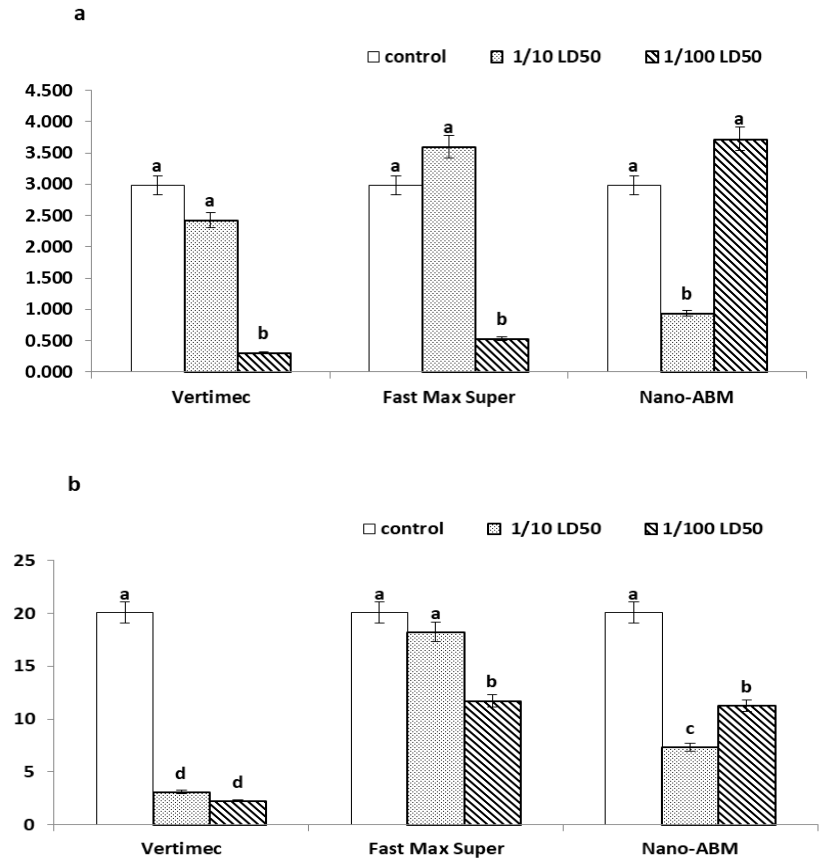


Figure 6 Activity of GST (μM mg^{-1} min^{-1}) in (a) haemolymph and (b) digestive gland of land snail, *H. aspersa* treated with two doses of different pesticides for 48 h.

No significant difference was obtained in activities of ACP for the all treatments in digestive gland homogenate (**Fig. 7**). The activity ranged from 5.18 to 5.22 U L^{-1} for 1/10 LD_{50} treatment of the examined pesticides, but it ranged from 5.43 to 5.31 U L^{-1} for 1/100 LD_{50} treatment. The control group did not exceed 5.95 U L^{-1}. In case of ALP, the activities significantly declined in comparison with control (**Fig. 8**). Fast Max Super® treatments (1/10 and 1/100 LD_{50}) significantly decreased enzyme activity (138.50 and 165.10 U L^{-1}) compared with the control (374.23 U L^{-1}). Nano-ABM decreased activity (328.33 and 199.80 U L^{-1}) for 1/10 and 1/100 LD_{50} treatments, but the same treatments of Vertimec® exhibited the activities: 318.63 and 325.42 U L^{-1}.

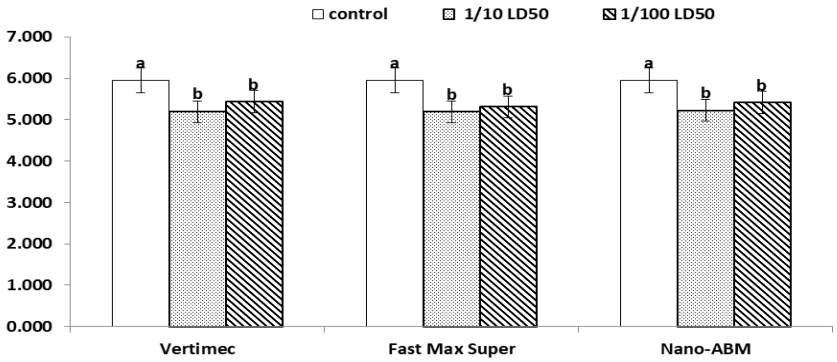


Figure 7 Activity of ACP (U L^{-1}) in digestive gland samples of land snail, *H. aspersa* treated with two doses of different pesticides for 48 h.

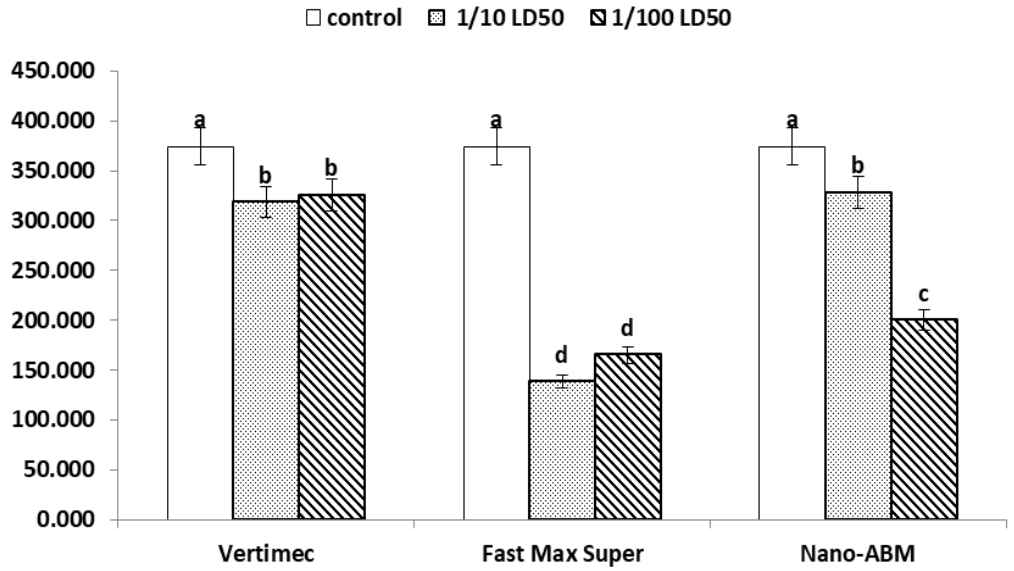


Figure 8 Activity of ALP (U L^{-1}) in digestive gland samples of land snail, *H. aspersa* treated with two doses of different pesticides for 48 h.

The activity of ALT was greater than control group in both haemolymph and digestive gland homogenate (**Table 2**). The activity for all treatments in digestive gland samples was greater than haemolymph. In haemolymph, 1/10 LD_{50} treatment exhibited activity in the following order: Fast Max Super® >Vertimec® >nano-ABM with values: 1.69, 1.61 and 1.23 U L^{-1} compared with the control (0.83 U L^{-1}). However, 1/100 LD_{50} treatment was found in the order: Vertimec® > Fast Max Super® > nano-ABM with values: 1.57, 1.23 and 1.05 U L^{-1}, respectively. In digestive gland homogenate, 1/10 LD_{50} treatment of Fast Max Super® exhibited the greatest activity (2.89 U L^{-1}), followed by 1/100 LD_{50} treatment of Fast Max Super® (2.71 U L^{-1}), and 1/10 LD_{50} treatment of Vertimec® (2.66 U L^{-1}). 1/100 LD_{50} treatment of nano-ABM exhibited the least activity (2.07 U L^{-1}) compared with the control (1.83 U L^{-1}). Regarding AST activity, 1/10 LD_{50} treatment was found in the following order: Vertimec® ≥ Fast Max Super®> nano-ABM with values: 1.52, 1.52 and 0.93 U L^{-1}, respectively, in haemolymph samples. However, 1/100 LD_{50} treatment was found in the order: nano-ABM> Vertimec®> Fast Max Super® with values: 1.40, 1.17 and 1.10 U L^{-1}, respectively, compared with the control (0.92 U L^{-1}). In digestive gland homogenate, 1/10 LD_{50} treatment of Fast Max Super® exhibited the greatest activity (30.00 U L^{-1}), followed by 1/10 LD_{50} treatment of nano-ABM (26.43 U L^{-1}), and 1/100 LD_{50} treatment of Fast Max Super® (22.53 U L^{-1}). The treatment (1/100 LD_{50} of Vertimec®) exhibited the least activity (7.67 U L^{-1}) compared with the control (24.57 U L^{-1}).

Table 2 Activities of AST and ALT enzymes (U L^{-1}) in land snail, *Helix aspersa* treated with different pesticides for 48 h.

Treatment	AST						ALT					
	Haemolymph			Digestive gland			Haemolymph			Digestive gland		
	Vert.	Fast M.	Nano-ABM	Vert.	Fast M.	Nano-ABM	Vert.	Fast M.	Nano-ABM	Vert.	Fast M.	Nano-ABM
1/10 LD_{50}	1.52± 0.29^{a}	1.52± 0.29^{a}	0.92± 0.48^{a}	17.25± 0.10^{d}	30.00± 0.06^{a}	26.43± 0.06^{b}	4.90± 0.17^{ab}	3.03± 0.27^{bc}	4.77± 0.17^{ab}	10.63± 0.08^{c}	16.93± 0.05^{a}	18.03± 0.04^{a}
1/100 LD_{50}	1.16± 0.38^{a}	1.10± 0.41^{a}	1.40± 0.32^{a}	7.67± 0.22^{f}	22.53± 0.08^{c}	14.19± 0.12^{e}	3.37± 0.24^{bc}	0.76± 1.08^{d}	2.43± 0.34^{c}	9.33± 0.09^{c}	14.57± 0.06^{d}	14.07± 0.06^{b}
Control	0.92 ± 0.48^{a}			27.57 ± 0.07^{bc}			5.37± 0.15^{a}			16.90 ± 0.05^{a}		

4. Discussion

Abamectin (ABM) has potential effect as anthelminthic, insecticidal, acaricidal, and nematocidal, respectively. It consists a mixture of 80% B1a and 20% B1b avermectins with actual comparable biological and toxicological properties [39]. The present data of ABM toxicity on snail, *H. aspersa* are in accordance with some previous studies. There is an information of the toxic effects of ABM against land snails, where it had lethal toxic actions on the freshwater snails, *Physa acuta* [40], *Biomphalaria alexandrina* [41], land snails: *Monacha contiana* [42] and *M. obstructa* [43]. In addition, ABM was found to be more toxic on chocolate snail *Eobania vermiculata* [21, 44-45]. On the same species, assessment of pesticides: indoxacarb, ABM, and spiromesifen for 24 h obtained that, indoxacarb was the most toxic, followed by ABM and spiromesifen. The LC_{50} values were 58.3, 83.3 and 280.9 ppm, respectively [46]. However, biopesticides: Biovar® (*Beauveria bassiana*), Andros® (ABM derivative), and Radiant® (Spinosad derivative) were assayed on snail, *M. obstructa* fed on treated lettuce for 1, 2, and 5 d. The mean values of LC_{50} were 5.49×10^3 viable spores ml^{-1}, 58.2 and 59.6 ppm, respectively [47].

Generally, the differentiation of toxicity values for the examined pesticides on *H. aspersa* may be recognized to physico-chemical properties, concentration and by-products or type of additives in conventional formulation recently effect on potential toxic effects and a.i uptake. The particles size or droplet size of NPs govern their stability. Surface properties of nano-formulation play a critical role in its uptake and translocation into the organism's body. There is a substantial relation between the physico-chemical properties e.g. size surface, shape of NPs dispersion and its toxicological impact on the organisms [48].

From the present findings, the pesticides caused alterations in some biochemical targets which could lead to serious metabolic and cellular damages. They affected the activities of vital enzymes in *H. aspersa,* where significantly imposed inhibition of AChE in ganglia homogenate. This finding was confirmed by some previous investigations. For example, Ma et al. (2014) [49] showed that, ABM-exposure to snail *Physa acuta* induced inhibition in AChE activity at different concentrations. On rodents, Nassar (2016) [50] showed that, ABM was able to inhibit AChE with percent 19% compared with a specific inhibitor, sumithion (83%). Also, Nasr et al. (2016) [51] showed that, oral gavage of ABM (30 mg kg^{-1}) for 30 d in rats significantly decreased AChE activity in brain and kidney organs. On the other hand, exposure to high doses of it led to cholinergic-like neurotoxic effects. In fact, use of experimental and computational studies proved evidence that, ABM is a potent (IC_{50}=10.6 μM) inhibitor of horse serum butyryl cholinesterase (BuChE) and interacted with the enzyme in a reversible, competitive manner predictively to block the mouth of the active-site gorge of the enzyme and to bind to several critical residues that normally bind/hydrolyze choline esters [52].

Lipids are considered a main target of oxidative stress. Lipid peroxidation (LPO) provides rise to a number secondary, highly damaging products, known to further impact of ROS production. MDA is important secondary decomposition product of polyunsaturated fatty acid (PUFA) [53]. Phospholipid of membranes in aerobic organisms is usually employed to a huge number of oxidants from exogenous sources [54-55]. For this reason, MDA concentrations can involve the rate and intensity of LPO within the organism. In the present study, the examined pesticides significantly increased MDA levels greater than control. This finding is accordance with that obtained by Bakry et al. (2013) **[56],** where exposure of *Bulinus truncates* to sublethal concentrations of herbicide, glyphosate for two weeks increased the level of MDA. Previous studies investigated increase of MDA in NPs-treated terrestrials. For example, Fahmy et al. (2014) [57] recorded a significant increase in the haemolymph and tissue of freshwater snail, *B. alexandrina* exposed to zinc oxide NPs (ZnONPs). Grera et al. (2012) [58] showed induction of MDA in snail, *H. aspersa* exposed to metallic dust. On the other hand, MDA level in digestive gland and kidney homogenates was dose-related increased at different concentrations of titanium dioxide NPs (TiO_2NPs) on *H. aspersa* [59]. Abdel-Halim et al. (2020) [60] showed that MDA levels significantly increased in haemolymph and digestive gland of snail, *M. cartusiana* exposed to ZnONPs for 14 d.

LDH is one of the cellular death biomarkers that can be measured in many cases, where its release is associated with cell and membrane damage [61-62]. In the present study, coupling of increased MDA level and LDH activity in haemolymph and digestive gland homogenate of snail, *H. aspersa* is considered a good biomarker for cytotoxic effect of these pesticides.

GST enzyme involves in catalyzing the conjugation of a variety of electrophilic substrates to reduce glutathione and protect the cell against the effects of xenobiotic [63]. In addition, thiol compounds such as reduced and oxidized GSH represent the initial protective substances against pesticides and other pollutants [64]. The present study showed a significant decrease in GST activities of pesticides-treated snail compared with control group. In a previous study, ABM induced increase in GST activity in freshwater snail, *P. acuta* after 96-h exposure to some pesticides. However, MDA levels of the snail soft tissues increased in all treatments (3.4, 9.6, 19.2 and 27.4 μg L^{-1}) indicating that LPO significantly occurred in comparison with the control [49]. However, increased GST activity in snails exposed to lead (Pb)

reproduced and displayed that defense system of the animal achieved to positively respond by trying to protect itself against xenobiotic inducing toxicity [65].

Both ACP and ALP are hydrolyzed enzymes, where they are liable to remove phosphate group from some molecules e.g. nucleotide, proteins, and alkaloids [66]. The present data proved a decline in activity of theses enzymes resulting in dysfunction for digestive gland of *H. aspersa*.

The results of this study were also supported by that obtained by Radwan et al. (1992) [67], where some carbamate pesticides induced significant elevation in AST and ALT activities when applied against land snail, *Theba pisana*. In fact, the deviation of both enzyme activities out of the normal range could lead to biochemical impairment and lesions of the tissues and cellular functions. Generally, transaminase enzymes: AST and ALT are not solely located in hepatocytes, but also in many body organs. In fact, AST/ALT ratio is commonly measured as a biomarker for liver health status [68]. Also, the elevation in their activities could be due to a variety of conditions including muscle damage, intestinal and hepatic injury [69]. Similarly, Felix Wróblewski et al. (1956) [70] found that AST activity increased 20 times following acute myocardial infarctions. As documented by Sharaf et al. (2015) [71], sublethal concentrations of pesticides: diazinon, lambda-cyhalothrin and methomyl induced increases in CAT, AST, ALT, ALP activities and MDA level in tissue homogenate of *M. cantiana*.

Some xenobiotic e.g. pesticides are able to enhance the risk factor, reactive oxygen species (ROS) inducing injury concluded reduced gastric mucus synthesis, which is considered a potent scavenger of ROS [72]. These concepts are in accordance with that obtained in this study, where ABM in the examined forms was able to generate ROS in the selected tissues and fluid.

5. Conclusion

In this study, a prominent toxic effect of ABM and nano-derived form on *H. aspersa* was detected for the measured biochemical parameters in selected orangs. So, the prepared nano-ABM is critical to be used as an acaricide against harmful pests after further toxicological studies to arise its safety to human and non-target organisms. Moreover, *H. aspersa* is considered a useful tool to assess ecotoxicological impact of pesticides.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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