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Potential toxic effects of nano-derived form thiobencarb on two land snails, *Theba pisana* and *Eobania vermiculata*

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Abstract

Potential toxic effects of thiobencarb and its nano-derived form as topical applications against land snails: *Theba pisana* and *Eobania vermiculata* were evaluated under laboratory conditions. Median lethal doses (LD_{50s}) of nano-derived form were 485.3 and 422.8 µg/snail on *E. vermiculata* and *T. pisana*, respectively, inducing toxicity index: 1.45 and 1.86-folds, compared with traditional form. Sublethal doses: 1/10 and 1/50 LD_{50} of the examined pesticide were tested against some biochemical measurements e.g. acetylcholinesterase (AChE), Glutathione-*S*-transferase (GST), acid and alkaline phosphatase (ACP and ALP), aminotransferases (AST/ALT), lactate dehydrogenase (LDH), and malondialdehyde (MDA) in ganglia and digestive glands. All treatments significantly decreased AChE activity, respect to control. Significant increase in MDA level and activities of GST and LDH were noticed. No significant changes in ACP and ALP activities were noticed, but ALT/AST enzymes exhibited significant decline, respect to their controls. These findings may highlight the role of nano-emulsion of thiobencarb as a molluscicide independent on its inhibitory action of AChE and other targets.

Key words: Thiobencarb; nano-emulsion; land snails; toxicity; biochemical responses.

1. Introduction

Snails represent a major proportion of the invertebrate biomass and are suitable organisms for environmental bio-monitoring assessment studies. Most research trails demonstrated the use of biochemical alterations in snail exposed to various toxicants and their implication in the environmental monitoring programs. Thus, it provides sensitive biomarkers for exposure and toxicity in the environmental monitoring [1]. The white garden snail, *Theba pisana* (Müller), belongs to Helicid family (Helicidae), and is distributed all over the world, especially in the Mediterranean region. It became one of the most important agricultural pests causing substantial damages to different crops e.g. vegetables, fruit, ornamental, and others. The brown garden snail, *Eobania vermiculata* is dangerous economic pest to the cultivated crops [2, 3]. The chemical control of snail populations using molluscicides is still the most efficient method, especially in the large areas. So, synthetic molluscicides are still the most effective substances to control terrestrial gastropods [4-7]. Thiobencarb [S-(4-chlorophenyl) methyl N, N-diethyl carbamthioate, IUPAC] (CAS: 28249-77-6) fits to thiocarbamate group [8]. It is widely used for controlling the weeds and grass in rice fields in many countries [9-11]. Its mode of action is inhibition of very long chain of fatty acid synthesis [8]. It is necessary to search new pesticides have molluscicidal potency, because there is limited number of remarkable compounds as molluscicides [12].

Nanotechnology provides green and efficient alternatives for the agricultural pest management with minimized impacts on the environment [13]. It is a very attractive tool to offer both new active ingredients (a.i) and/or formulations [14]. This technology provides protection to the a.i. and enhances the bioavailability to the site of action [15-16]. Nano-pesticides are similar to synthetic conventional

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pesticides in size and surface characters [17]. They are technological established to offer numerous benefits such as reducing concentration of a.i., as well as increase the stability and efficacy [15-16]. This has been shown to bring improvements in terms of better stability of dispersions, slow-or controlled release formulations, and provide a better control during field application [18, 19]. Thus, the present work aims to evaluate the comparative toxic effects of herbicide, thiobencarb and its nano-derived form against two the land snails, *E. vermiculata* and *T. pisana* using topical application technique under laboratory conditions. Also, evaluate biochemical responses to their sub-lethal concentrations.

2. Experimental:

2.1. Technical thiobencarb (95%), and its traditional formulation, Saturn® 50% EC were obtained from Kafr El-Zayat Company for Pesticides & Chemicals Production, Egypt. Acetylthiocholine iodide (ASChI), 5, 5'-dithiobis 2-nitrobenzoic acid (DTNB), sodium pyruvate, α-nicotinamide adenine dinucleotide (NADH), 1-Chloro 2, 4-dinitrobenzene (CDNB), 2-amino-2-hydroxy methyl-propane-1, 3-diol (Tris-HCl), bovine serum albumin (BSA), and reduced glutathione (GSH) were supplied by Sigma Chem. Co. P.O. Box 14508 St. Louis MO 63178, USA. Sodium chloride (NaCl) and phosphate buffer, potassium phosphate monobasic; dibasic and potassium phosphate monobasic; dibasic were supplied by J.T. BAKER Chem. Co, Phillipsburg, N.J. 08865. Trichloroacetic acid (TCA; Cl_3C_3COOH) was obtained from Research Lab. Fine Chem. Indust., Mumbai 400002, India. Thiobarbituric acid (TBA) was supplied by LOBA CHEMIE PVT. Ltd, Mumbai-400005, India.

2.2. Tested animals.

Land snails, *E. vermiculata* and *T. pisana* were selected for experimental treatments. Healthy individuals weighing 2.0±0.4 g were collected from some fields in El-Beheira governorate, Egypt. The animals were maintained for 14 d in plastic aerated cages (40x40x40 cm; 100 individuals each) under laboratory conditions (25±2 °C; 63±2% relative humidity and 12:12 h light/dark). They were fed on lettuce leaves *ad libitum*.

2.3. Nano-emulsion.

2.3.1. Preparation

Nano-derived form of thiobencarb 50% was prepared under high energy mode using sonication technique [20]. The active ingredient (a.i) was dissolved in a vegetable oil and employed for dispersion of liquid (water) with surfactant and co-surfactant to generate homogenous solution (o/w) during 10 min.

2.3.2. Characterization

Nano-derived form was achieved at various storage conditions of temperature and humidity to evaluate emulsion stability according to ICH guidelines Q1A [21]. The formulation was centrifuged at 3500 rpm for 30 min to observe any phase separation. Ten ml of the prepared formulation were diluted to 100 ml with distilled water and shaken for 30 times from top to bottom continuously. At the end, the solution was settled 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, where six cycles between refrigerator temperature of 4 and 48℃ for 48 h were done. So, it was employed to freeze-thaw cycle test, where three cycle were done between -21 and 25℃.

Morphology and size of the prepared nano-form were examined by Transmission Electron Microscopy (TEM) (JOEL 1400 Plus, Japan) at filament of 80 Kev to observe them. An aliquot of the formulation was diluted with deionized water (1/100 v/v), sonicated for enough time, deposited on the film grid, dried and observed [22]. A combination of bright-field imaging at increased magnification with diffraction modes was used for optimal visualization.

Conventional thiobencarb and its nano-derived forms were examined on Fourier Transform Infrared (FTIR) instrument (TENSOR 27 Bucker, Germany-FTIR L203/1 2887). The absorbance was done in the range (4000 to 400 cm^{-1}). The run was conducted with sensitivity range 50 and absolute threshold level 6.00.

2.4. Acute toxicity

Contact toxicity of the examined pesticide against *E. vermiculata* and *T. pisana* snails was carried out by using topical application method [6, 23]. The tested doses of thiobencarb and its nano-derived form were 40, 80, 200, 400, 800, 1200 and 40, 80, 200, 400, 800 µg/snail, for *E. vermiculata* and *T. pisana*, respectively. Three replicates (10 individuals for each) were used and the animals were caged in plastic boxes. The pesticide was 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.

2.4.1 Sub-acute toxicity

The acute LD_{50} values for thiobencarb and its nano-derived form, sub-lethal doses: 1/10 and 1/50

LD_{50} with values: 70.5 and 14.1 µg/snail for thiobencarb and 48.5 and 9.7 µg/snail for its nano-derived form, respectively, were used as described above in acute toxicity experiment for *E. vermiculata*. While, 78.5, 15.7 µg/snail of thiobencarb and 42.3, 8.5 µg/snail of its nano-derived form were used for *T. pisana*. Control group was injected with vehicle (as a reference group). After 48 h of dosage, the live animals were taken for analysis. Then, they were dissected to remove ganglia and digestive glands and stored at -20 °C until used.

2.5. Biochemical quantifications.

2.5.1. Sample preparation.

One g of ganglia or digestive gland was homogenized in potassium phosphate buffer pH 6 .5 (1/10 w/v) using a homogenizer (Polytron TEMPEST-VIRTIS Comp. Inc., model Gardiner, N.Y. 12525, USA) for 15 sec. The samples were centrifuged at 5000 rpm for 10 min. The supernatant of ganglia gland was used for acetylcholinesterase (AChE) and Glutathione-*S*-transferase (GST) activities. The supernatant of digestive gland was used for acid (ACP), alkaline phosphatase (ALP), alanine amino transferase (ALT), and aspartate aminotransferase (AST). Homogenate was used as a source for lactate dehydrogenase (LDH), and malondialdehyde (MDA) assays.

2.6. AChE

The enzymatic activity was determined according to Ellman et al. (1961)[24] using acetylthiocholine iodide (ASChI) as a substrate. In a 5 ml test tube, 2.9 ml of 0.1M phosphate buffer pH 8.0 and 0.1 ml of 0.075 mM 5, 5'-dithiobis, 2-nitrobenzoic acid (DTNB) were added. Then, 20 µl of the prepared tissue supernatant added. To the above mixture, same volume of ASChI (0.075 M) was added. The optical density of the developed yellow color 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 hydrolyzed per mg protein per min.

2.7. MDA

Thiobarbituric acid reactive substances (TBARS) were used as an index of lipid peroxidation by spectrophotometric quantification of MDA content in the tissues [25]. An aliquot (250 µl) of tissue homogenate was mixed with 1ml 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 expressed as µM/g tissue.

2.8. 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 chance in absorbance was measured at 340 nm for 3 min. The enzyme activity was expressed as U/L [26].

2.9. GST

The activity was determined by spectrophotometric method of Habig and Jakoby (1981)[27] 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 s for 6 min at 340 nm. The enzyme activity was expressed as nM/mg/min. The unit will reduce 1.0 µM of oxidized glutathione per min at pH 7.6 and 25 ℃.

2.10. ACP

The enzyme activity was measured according to the method of Kind and King (1954) [28] by using specific kits (Bio Diagnostic Co., Germany). The absorbance of sample and standard against reagent blank was recorded at 510 nm. The activity was expressed as U/L.

2.11 ALP

The enzyme activity was measured by using phenyl phosphate as a substrate (N.S. Bio-Tec., kits, UK). So, the complex color of *P*-nitrophenyl phosphate was measured at 405 nm against the blank. The activity was expressed as U/L [29].

2.12. AST/ALT

The enzyme activities were measured according to the method of Gello *et al.* (1985) [30] by using specific kits (Bio system Kits, Spain). The activity was expressed as U/L.

2.13. Statistical analysis

LD_{50} values were expressed as µg/10 µl/snail with confidence limit (CL) and slope for the examined pesticide which computed using Probit analysis [31]. 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 [32].

3. RESULTES

3.1. Characterization of nano-derived form

Nano-derived form of thiobencarb exhibited stable form during freeze-thaw cycle's storage. No creaming or floating phases were made up as well as no separation phase was formed after centrifugation or shaken processes. Concerning TEM examination, nano-derived form appeared spherical shape and dimension mainly ranged from 46 to 69 nm (Figure 1A).

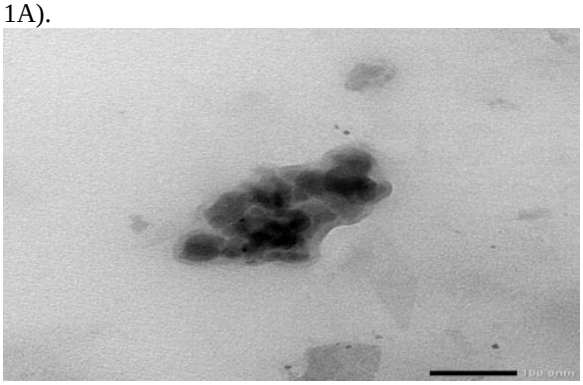
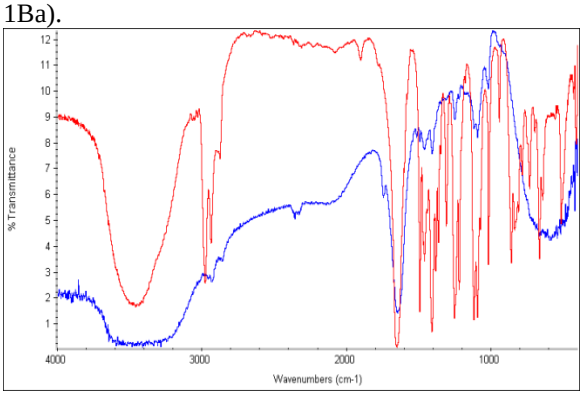


Figure 1: (A) TEM micrograph of nano-derived form of thiobencarb visualized at 30000X and (B) FTIR profiles of [a] conventional thiobencarb and [b] its nano-derived form at absorbance range 4000-400 cm^{-1}.

FTIR pattern of traditional thiobencarb was formed in similar profile with its nano-derived form (Figure 1B). Traditional pattern obtained aromatic ring stretching at 1410 cm-1, but nano-form obtained shifting of aromatic C-H stretching at 1550 cm-1. There is potentially broad number of molecular fragments of conventional thiobencarb that can be considered functional groups attached to an organic structure. For example, the two forms exhibited C-N stretching (primary amine) at 1020-1090 cm-1. Otherwise, C-O stretching for epoxy or ethoxy rings showed absorbed peaks at 1250 cm-1. Significant different was obtained in nano-form pattern concern polymeric-OH stretching at 3200-3400 cm-1 which not obtained in case of the traditional formula (Figure 1Ba).



(Figure 1Ba)

3.2. Acute toxicity

The acute toxicities of the examined pesticide on *E. vermiculata* and *T. pisana* after 48 h of dermal dosage are illustrated in Figure 2. Thiobencarb exhibited LD_{50} values: 704.9 µg/snail (CL 142.99-1062.06; slope 1.132) and 784.5 µg/snail (CL 318.73-601.06; slope 1.068), while its nano-derived form exhibited the values: 485.5 µg/snail (CL 84.42-2383.04; slope 1.018) and 422.8 µg/snail (CL 113.53-627.59; slope 0.908) on *E. vermiculata* and *T. pisana*, respectively. Nano-derived form was more toxic than traditional form arising toxicity index, 1.86-folds for *T. pisana* and 1.45-folds for *E. vermiculata*.

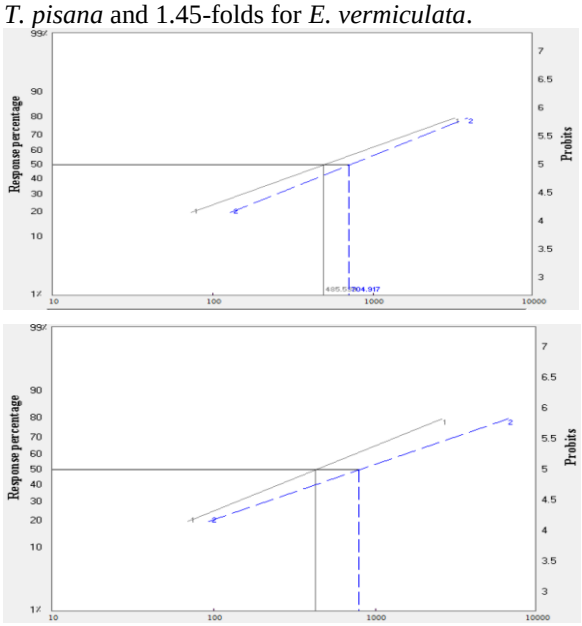


Figure 2: Lpd Lines of relative toxicity for (a) nano-derived form of thiobencarb and (b) thiobencarb on land snails: (a) *E. vermiculata* and (b) *T. pisana*, respectively, for 48 h by using contact toxicity technique.

3.3. Biochemical responses

For biochemical quantifications, two doses of 1/10 and 1/50 of LD_{50} for thiobencarb and its nano-derived form were used for 48 h independent on LD_{50} value. The activity of AChE in ganglia homogenate of *E. vermiculata* exhibited the greatest activity (0.050 µM/mg/min) for dose, 1/50 LD_{50} of nano-derived form of thiobencarb, followed by (0.031 µM/mg/min) for 1/10 LD_{50} of thiobencarb. Dose, 1/10 LD_{50} of nano-form exhibited the least activity (0.007 µM/mg/min), respect to control (0.149 µM/mg/min). In case of *T. pisana*, the least activity (0.050 µM/mg/ min) was recorded for the 1/50 LD_{50} of thiobencarb and 1/10 LD_{50} of its nano-derived form, respect to the control (0.140 µM/mg/min). Dose, 1/50 LD_{50} of nano-form exhibited the greatest activity (0.090 µM/mg/min), followed by 1/10 LD_{50} of thiobencarb (0.080 µM/mg/min) (Figure 3).

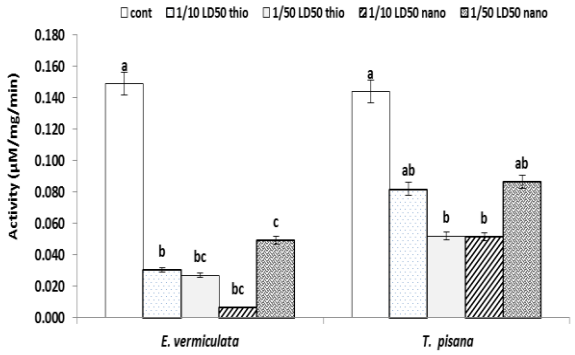


Figure 3: AChE activity (µM/mg/min) in ganglia homogenate of land snails, *E. vermiculata* and *T. pisana* treated with thiobencarb and its nano-derived form for 48 h.

Increased levels of MDA represent a good marker for cellular membrane damage. All treatments significantly exhibited increases in MDA levels in digestive glands greater than control group (Table 1). Doses: 1/10 and 1/50 LD_{50} of thiobencarb exhibited the greatest values (1.128 and 0.799 µM/g tissue) in *E. vermiculata*, representing % of control, 119.88 and 55.75%, followed by 1/10 LD_{50} of its nano-form (0.636 µM/g tissue; % of control 23.98%) and 1/50 LD_{50} (0.611 µM/g tissue; 19.11%), respectively, respect to control (0.513 µM/g tissue). In case of *T. pisana*, 1/10 LD_{50} of thiobencarb exhibited the greatest value (1.710 µM/g tissue; 441.14%), followed by 1/50 LD_{50} of nano-form (1.192 µM/g tissue; 277.22%), and its value 1/10 LD_{50} (1.098 µM/g tissue; 247.47%). Dose (1/50 LD_{50} of thiobencarb) exhibited the least value (0.761 µM/g tissue; 140.51%), respect to control (0.316 µM/g tissue).

Table 1: MDA levels (µM/g tissue) in digestive gland homogenates of *E. vermiculata* and *T. pisana* treated with thiobencarb and its nano-derived form.

Treatment	MDA			
	E. vermiculata		*T. pisana*	
	MDA (µM/g tissue)	% of control	MDA (µM/g tissue)	% of control
1/10 LD_{50} thiobencarb	1.128^{a} ±0.06	119.88	1.710^{a} ±0.06	441.14
1/50 LD_{50} thiobencarb	0.799^{b} ±0.08	55.75	0.761^{c} ±0.15	140.51
1/10 LD_{50} nano-form	0.636^{c} ±0.11	23.98	1.098^{b} ±0.10	247.47
1/10 LD_{50} nano-form	0.611^{c} ±0.11	19.11	1.192^{b} ±0.09	277.22
Control	0.513^{c} ±0.13	100.00	0.316^{d} ±0.35	100.00

Each value is the mean of 3 replicates ± SE. No significant differences were obtained for the same letters at 0.05 levels.

Excess of LDH activity in the cells indicates cellular damage and apoptotic effect of the xenobiotic. All treatments exhibited significant increases in enzyme activity of *E. vermiculata* compared to control, but no significant differences were obtained for activity in samples of *T. pisana* (Table 2). Dose, 1/10 LD_{50} of nano-form exhibited the greatest activity (42.50 U/L) representing % of control (322.37%), followed by 1/50 LD_{50} (27.29 U/L; 171.15%) and 1/50 LD_{50} of thiobencarb (27.17 U/L; 169.99%), respect to control (10.06 U/L). Dose, 1/10 LD_{50} of thiobencarb exhibited the least value (17.05 U/L; 69.73%). In case of *T. pisana*, 1/50 LD_{50} of nano-form exhibited the greatest value (35.24 U/L), representing 39.07% of control. Other treatments exhibited activities lower than control which did not exceed 25.34 U/L.

Table 2: LDH activity (U/L) in digestive gland homogenates of *E. vermiculata* and *T. pisana* treated with thiobencarb and its nano-derived form.

Treatment	**LDH**			
	E. vermiculata		***T. pisana***	
	Activity (U/L)	**% of control**	**Activity (U/L)**	**% of control**
1/10 LD_{50} thiobencarb	17.06^{c} ±0.21	69.73	23.51^{b} ±0.11	-7.22
1/50 LD_{50} thiobencarb	27.17^{bc} ±0.13	169.99	24.78^{b} ±0.11	-1.86
1/10 LD_{50} nano-form	42.50^{a} ±0.08	322.37	22.71^{b} ±0.12	-10.38
1/10 LD_{50} nano-form	27.29^{b} ±0.13	171.15	35.24^{a} ±0.08	39.07
Control	10.06^{a} ±0.35	100.00	25.34^{b} ±0.11	100.00

Each value is the mean of 3 replicates ± SE. No significant differences were obtained for the same letters at 0.05 levels.

Activity of GST displayed changes in homogenates of digestive gland and ganglia of the treated animals, respect to control (Figure 4). Dose, 1/10 LD_{50} of nano-form exhibited the greatest activity in digestive gland homogenate of *E. vermiculata* (16.06 µM/mg/min), followed by 1/50 LD_{50} of thiobencarb (10.67 µM/mg/min), respect to control (2.73 µM/mg/min). Dose, 1/10 LD_{50} of thiobencarb

exhibited the least value (2.46 µM/mg/min). In ganglia homogenate, dose 1/50 LD_{50} of thiobencarb exhibited the greatest activity (1.96 µM/mg/min), followed by 1/10 LD_{50} (0.96 µM/mg/min), respect to control (1.56 µM/mg/min). Dose, 1/10 LD_{50} of its nano-form exhibited the least value (0.24 µM/mg/min). Regarding *T. pisana*, all treatments exhibited decreases in enzyme activity compared to control group (6.58 µM/mg/min). The activity was found in the following order: 1/50 LD_{50} thiobencarb> 1/10 LD_{50} thiobencarb> 1/10 LD_{50} of nano-form> 1/50 LD_{50} of nano-form with mean values: 4.02, 1.84, 1.04 and 0.65 µM/mg/min, respectively. However, the activity in ganglia homogenate displayed the following order: 1/50 LD_{50} thiobencarb> 1/10 LD_{50} thiobencarb> 1/50 LD_{50} of nano-form> 1/10 LD_{50} of nano-form with mean values: 3.25, 2.44, 2.28, and 1.96 µM/mg/min, respectively.

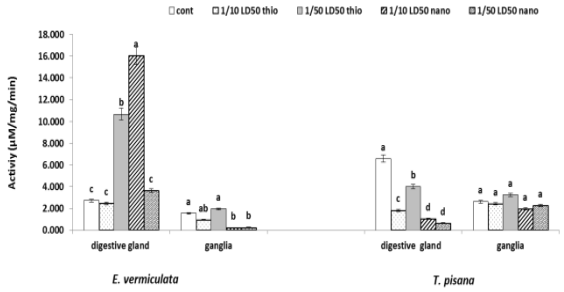


Figure 4: Activity GST (µM/mg/min) in ganglia and digestive gland homogenates of land snails: *E. vermiculata* and *T. pisana* treated with thiobencarb and its nano-derived form for 48 h.

No significant differences were obtained in ACP activity in digestive gland homogenates of both tested snails (Figure 5). In *E. vermiculata* homogenate, 1/50 LD_{50} of thiobencarb exhibited the greatest activity (5.49 U/L), while 1/50 LD_{50} of its nano-form exhibited the least activity (4.76 U/L), respect to control (5.92 U/L). Regarding *T. pisana*, the above doses exhibited activities: 5.35 and 4.65 U/L, respect to control (6.12 U/L). On the other hand, significant differences were obtained in ALP activity in both snails. In case of *E. vermiculata*, the activity displayed the following order: 1/50 LD_{50} thiobencarb> 1/50 LD_{50} nano-form> 1/10 LD_{50} thiobencarb> 1/10 of nano-form with mean values: 149.98, 141.71, 116.26, and 76.74 U/L, respectively, compared with control (219.64 U/L). Regarding *T. pisana*, the activity displayed the order: 1/10 LD_{50} thiobencarb> 1/50 LD_{50} nano-form> 1/50 LD_{50} thiobencarb> 1/10 of nano-form with mean values: 235.52, 183.52, 163.44, and 124.29 U/L, respectively, compared with control (264.34 U/L).

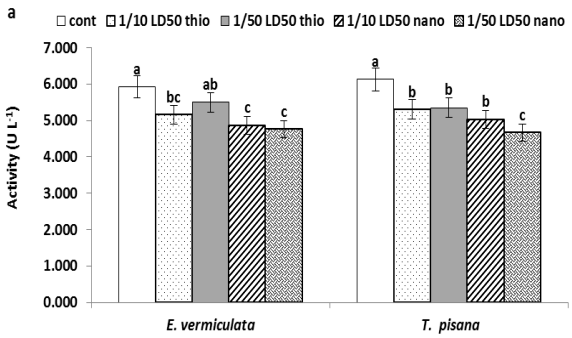


Figure 5: Activities of (a) ACP and (b) ALP (U/L) in digestive gland homogenates of *E. vermiculata* and *T. pisana* treated with thiobencarb and its nano-derived form for 48 h.

Enzymes, ALT and AST displayed changes in their activities in treated animals compared with the control group (Figure 6). Activity of ALT in *E. vermiculata* displayed values lower than control, where 1/50 LD_{50} of thiobencarb exhibited the greatest activity (7.53 U/L), but 1/10 LD_{50} of its nano-form exhibited the least value (6.33 U/L), respect to control (9.00 U/L). Regarding *T. pisana*, dose 1/50 LD_{50} of thiobencarb exhibited the greatest activity (19.33 U/L), while 1/10 LD_{50} of its nano-form exhibited the least one (8.57 U/L), respect to control (16.70 U/L). On the other hand, AST activity in *E. vermiculata* displayed the least value (0.82 U/L) for dose, 1/10 LD_{50} of thiobencarb, but 1/50 LD_{50} of its nano-form exhibited the greatest activity (12.27 U/L), respect to control (12.27 U/L). Regarding *T. pisana*, dose 1/50 LD_{50} of thiobencarb exhibited the greatest activity (30.77 U/L), followed by 1/10 LD_{50} of thiobencarb (11.57 U/L), respect to control (8.40 U/L). While, the least activity (1.07 U/L) was recorded for dose, 1/50 LD_{50} of its nano-form.

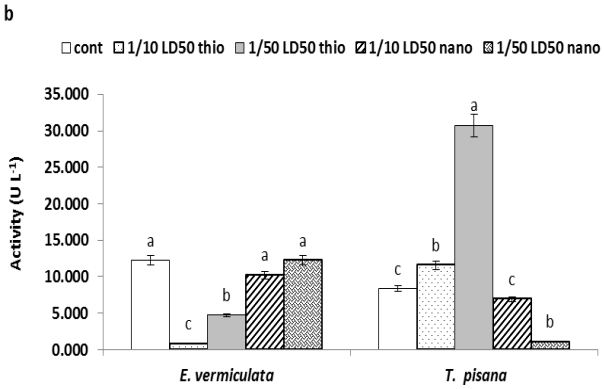


Figure 6: Activities of (a) ALT and (b) AST (U/L) in digestive gland homogenates of *E. vermiculata* and *T. pisana* treated with thiobencarb and its nano-derived form for 48 h.

4. Discussion

The present data showed that, nano-derived form of thiobencarb was more toxic than traditional form on both land snails. This finding was confirmed with its ability to inhibit AChE in ganglia homogenate, respect to control group and other biomolecules as targets in the snails. In addition, efficient of nano-form of any substance may be attributed to chemical in nano scale has the ability to be more stable, penetrate and good sticking with treated parts of organism leading to more efficient and long-term effect [33]. Surface properties of nano-formulation play a critical role in its uptake and translocation into the body. There is a substantial relation between the physico-chemical properties e.g. size, shape, charge and its toxicological effects in the organism [34].

Land snails cause great economic losses worldwide; however, there is no specific compound available to control these pests without being harmful to the non-target organisms [2]. The highly toxic (to mammals and insects) organophosphorus and pyrethroid pesticides are not effective against land snails and are not used to combat them. Methomyl and methiocarb, carbamate pesticides, and metaldehyde are used to control land snails in the form of baits containing high concentrations of the active ingredients; however, these conventional pesticides have the previously mentioned disadvantages [12, 23]. Two cardenolide extracts from *Adenium arabicum* and *Calotropis procera* exhibited contact LD_{50} values on the harmful land snail, *Monacha cantiana* (Montagu) 12.62, and 34.63 mg/kg of body weight, respect to insecticide, methomyl (116.62 mg/kg), The plant extracts indexed 9.24 and 3.37-folds than methomyl [35]. Most pervious investigations focused on the efficacy of traditional formulations and some natural extracts against land snails. For example, abamectin had lethal toxic action on freshwater snails, *Physa acuta* [36], *Biomphalaria alexandrina* [37], land snail, *M. contiana* [36] and *M. obstructa* [38]. On the other hand, efficacy of indoxacarb, abamectin and spinomesifen was examined against *E. vermiculata* for 24 h. Indoxacarb was the most toxic, followed by abamectin and spinomesifen with LC_{50} values: 58.3, 53.3 and 280.9 ppm, respectively [39]. However, some biopesticides such as Biovar® (*Beaveria bassiana*), Andros® (abamectin derivative) and Radiant® (spinosad derivative) were effective on *M. obstructa* with LC_{50} values: 5.49×10^3 spores/ml, 58.3 and 59.2 ppm, respectively [40]. There is a lack concern efficacy of nano-pesticides against land snails. However, nano-derived form of abamectin (1%) as nano-emulsion exhibited the highest toxic effects (1.86 µg/snail; 7.12-folds), respect to conventional formulations of the compound: Fast Max Super® and Vertimec® as a dermal contact for 48 h against land snail *H. aspersa* [41].

Pesticides are considered one of the major causes of oxidizing stress. In the invertebrates, snails were largely used like model of study to highlight biomarkers of environmental pollution. Measurement of antioxidant enzymes activity thus constitutes a marker of the disordered state of the biological systems [42]. Glutathione (GSH) is the major non-enzymatic antioxidant in the animal cells, it is the most abundant cellular thiol, oblique in the metabolism and the processes of transport and in the protection of the cells against the toxic effects of the endogenous and compounds including ROS and heavy metals [43]. Enzyme, GST has a critical role of biotransformation in phase II, combines molecule of glutathione with large variety of substrates e.g. pesticides to allow their elimination. It indeed has an interesting activity as a biomarker of contamination [44]. Our results highlight a reduction in the rate of the GSH, and an increase in activity GST this increase is a response to the oxidative stress caused by the presence of spinosad in the body [45]. The strong reduction in GSH content could be explained by a direct réaction/liaison of the pesticide with the glutathione, indeed the carboxyl groups: amine group, and sulfhydrile group (-SH) of GSH [46]. This interaction glutathione-pesticide takes place by the intervention of GST which allows the conjugation of xenobiotic or its metabolites with GSH during phase II of the metabolism. On the other hand, reduction in GSH content can be also explained by increased consumption of it by GST in reaction of the conjugation process. This concept was confirmed by the results which indicated an induction of GST was made up in the presence of Spinosad [47]. This finding was confirmed by Canesi *et al.* (1999) [48] and Regoli *et al.* (1998). [49] Also, Radwan *et al.* (1992) [50] highlighted an induction of GST activity after exposure of land snail, *T. pisana* to carbamates and those of Pandey *et al.* (2005) [51] at gastropod, *C. penctatus.*

Malonedialdehyde (MDA) is a breakdown product of the reactions of lipid peroxidation which are formed at the time of the attack of phospholipids by ROS generated by the contaminants. It is a powerful agent able to react with the biological macromolecules. It was used as indicator of oxidative stress induced by some contaminants at the bivalve's sailors [52]. When oxidative stress immerses the protective forces like catalase activity, a destructive effect on the membranes can be observed via an increase in content of MDA. The present finding is in

accordance with that obtained by Nawel *et al.* (2012) [47], where high level of MDA was induced in gland digestive of molluscs, *H. aspersa* treated by spinosad. Also, El-Gendy *et al.* (2009) [53] studied the toxic effects of a pesticide containing copper on *T. pisana*, where they highlighted a significant level of MDA after treatment of the animals. Moreover, Shwela *et al.* (2010) [54] highlighted a significant increase in MDA level in the marine snail, *Lymnaea natalensis* exposed to environmental pollutants. Another work by Salama *et al.* (2005) [55] highlighted an induction in MDA level after exposure to certain pesticides. Also, lipid play important role in normal function of the cell, not only lipids serve as highly reduced storage forms of energy, but also play an intimate role in the structure of cell membrane and the organelles found in the cell [56].

The elevations of AST and ALT activities was done by carbamate pesticides treatment in *T. pisana*. Also, the deviation of both enzyme activities out of normal range could lead to biochemical impairment and lesions of the tissues and cellular function [50]. The present data are in accordance with that obtained by El-Gohary and Genena (2011) [57] who reported that, Gastrox®, Molatov®, and Mesurol® when examined on land snail, *M. contiana* caused slight increase in AST and ALT activities, but they caused a significant decrease in ALT activity of *E. vermiculata*. Also, Lannat® may cause alterations in the activities of AST, ALT, ALP and LDH [58]. In fact, aminotransferases are very active in the liver and their activities can be detected in small amounts. The significant changes in their activities in land snails pointed out to functional disorder of the digestive system [59].

Enzyme, AChE represents a biomarker of neurotoxicity usually employed to assess exposure to organophosphate and carbamate pesticides [60]. Inhibition of AChE was frequently employed in toxicology to diagnose certain environmental contaminants such as the complex mixtures of pollutants, detergents and heavy metals [61]. It is implied in the transmission systems of the nerve impulse through the organization. The inhibition of the enzyme by the many neurotoxic ones involves an accumulation of the transmitter substance, the acetylcholine (ACh) in the synaptic space, which maintains of this fact a permanent transmission of the nerve impulse leading to dead of the individual [62]. In the invertebrates, the existence of motoneurones cholinergic as that of receivers specific to ACh was highlighted at mollusks and the gastropods [63]. Activity of AChE is used as biomarker of exposure to inhibiting pesticides in mollusks. The present work seems to be in adequacy with those quoted in the literature, indeed, the activity of AChE decreased gradually following the exposure of snails to the various concentrations of Spinosad [47]. This work was supported with that obtained by Radwan *et al.* (2008)[6], Coeurdassier (2001) [64], and Salama *et al.* (2005) [55] who highlighted an inhibition of AChE activity after exposure of the terrestrial gastropods to the pesticides. The various responses of AChE which appearances in different species may be common to organophosphorus and carbamate pesticides. For examples, El-Deeb *et al.* (1999) [65] found that carbamate insecticide, Osbac® elevated AChE activity in land snail, *M. contiana* after 24 h of treatment and was reduced after 3 days of treatment. Also, insecticide Kuik® (rotam) showed significant declines in AChE activity in *Helicella vestalis* with levels 62.9, 64.2 and 83.5% after 1, 3, and 7 days of treatment, respect to control [66]. However, other groups of pesticides have ability to inhibit AChE. Thiobencarb is one of thiocarbamate group which has herbicidal activity, but when its bioaccumulation increases, becomes able to induce toxic responses in fishes and other bioindicators for ecosystem health [67]. For example, Elias *et al.* (2020) [68] showed that, significant decline ($P<0.01$) in AST and ALT activities was showed in African catfish, *Clarias gariepinus* after exposure to 0.72 ppm of thiobencarb for periods: 3, 9, and 15 days. As documented in the literature, thiocarbamate pesticides are classified as ChE inhibitors and have other effects e.g. reproductive or developmental effects, thyroid toxicity and neuropathic effects [69]. Abdel-Halim and Massoud (2014) [70] showed that, AChE and butyrylcholinesterase (BuChE) activities in fish, *Gambusia affinis* represented 55.3 and 48.62% of control after exposure to 0.019 ppm of thiobencarb for 96 h.

5. Conclusion

The present finding provides a prominent toxic effects of thiobencarb and its nano-derived form on both land snails. The inhibitory effect of the pesticide on AChE and GST enzymes may represent main targets on the pest. Thus, further investigations may be done to assess its potential for pests control and ecofriendly regulations.

6. Conflicts of interest

"There are no conflicts to declare".

7. Acknowledgment

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