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Biocontrol potential of Chitosan extracted from *Procambarus clarkii* (*Crustacea: Cambaridae*) against *Eobania vermiculata* snails (Muller 1774) in Egypt

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Abstract

Background: Land snails, especially the chocolate banded snails, *Eobania vermiculata* (Muller 1774) are destructive pests of a wide range of field and vegetable crops, and biological treatment appears to be better alternative to the chemical snail control. Therefore, the goal of this work was to assess the molluscicidal activity of chitosan extracted from the crawfish *Procambarus clarkii* against *E. vermiculata* using oxidative stress, histopathological and genotoxic biomarkers.

Results: Exposure of snails to LC_{50} (222.4 mg/l) chitosan for 1, 3 and 7 days induced a significant increase in glutathione S-transferase and catalase levels then decline in reduced glutathione content after 1 and 3 days as well as a slight decrease in CAT levels, GSH content and GST of the treated snails after 7 days exposure. Histologically, the stress induced by chitosan exposure leads to deformation of cells, dilatation of the intertubular spaces, and destruction of tubules with increase in lumen size, necrosis of digestive cells with rise in vacuoles number and increase in calcium cells number. Considerably, a great damage was observed with increasing time of exposure. Furthermore, genotoxicity was assessed using RAPD-PCR technique and the results revealed that change in RAPD profiles of *E. vermiculata* following chitosan treatment included loss of normal DNA bands and appearance of new one compared to control snails. The genomic template stability was 63.6, 36.4 and 18.2% 1, 3 and 7 days of exposure, respectively. The apparent of new bands increased as time of exposure decreased, while GTS values decreased confirming the effect of chitosan-induced DNA damage.

Conclusion: Chitosan may be an ecofriendly acceptable alternative pesticide for snail control.

Keywords: *Eobania vermiculata*, Chitosan, Oxidative stress, Histopathological, Genotoxicity

Background

Land-dwelling snails caused serious problems to variety of crops (De Ley et al. 2020). These dangerous pests could transmit diseases to many fields of agriculture and caused great economic losses as they invaded vegetables, tree fruits and ornamental crops (Shahawy 2019). *Eobania vermiculata* snail (Muller 1774) was recorded in Egyptian localities attacking many ornamental plants and causing considerable damage to all plant parts (Radwan et al. 2008). Controlling land snails by chemical molluscicides remained the most effective method, particularly over large areas (De Ley et al. 2020). Due to the direct and latent hazards of the chemical pesticides and their toxic effect on the ecosystem, it is always advised to use safe biopesticides for controlling pests. In addition, the recent strategies of Integrated Pest Management (IPM) have mainly concentrated on the use of safe control method

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(Kares et al. 2012) for reaching to a minimum residue of pesticides in food (Vu et al. 2007).

Conventional pesticides, especially carbamates, were successfully used for controlling these pests (Abdel-Gawad et al. 2004). Hamed et al. (2007) used methomyl and methiocarb to control *E. vermiculata* snails and concluded that low concentrations of these molluscicides were effective, more feasible and less harmful to nontarget species like vertebrate animals and human beings. However, their use in high concentrations has a harmful effect and leads to pollution of environment (Radwan et al. 2008). Chitosan is a naturally occurring polymer, which is derived from the arthropod exoskeletons (Wuana et al. 2019) by deacetylazation of chitin. It considered as biodegradable polymer, nontoxic, bioactive, environmental-friendly material and biocompatible polymer (Aam et al. 2010) and exhibits various biological activities including, antioxidant, anti-inflammatory, antibacterial, antifungal, anti-tumor, anti-obesity, anti-diabetic and immunological capacity enhancing properties (Walsh et al. 2013). Also, it has many functions in fields of food preservation and microbial mitigation (Vinsova and Vavrikova 2008). Khidr (2018) stated that chitosan and nano-chitosan could be used as promising molluscicidal agents against the land snails *E. vermiculata* and *Monacha obstructa*.

Therefore, the present study aimed to assess the molluscicidal activity of chitosan as a natural-product extracted from the exoskeleton of crawfish *Procambarus clarkii*, against *E. vermiculata* snails, throughout various biomarkers, oxidative stress, histopathological and genotoxicity using RAPD-PCR technique.

Methods

Snails' collection

Two hundred fifty adult *E. vermiculata* land snails were collected from infested fields in Kerdasa city, Giza Governorate, Egypt, during the spring of 2019. The collected snails were transferred to the Laboratory of Invertebrates, Zoology Department, Faculty of Science, Cairo University, Giza. Snails were reared in plastic containers ($20 \times 15 \times 15$ cm) with soil base (10 snails/ container) and f d on green fresh lettuce for 2 weeks to be acclimatized. After the experimental period snails were crushed between two slides, and their soft tissues were withdrawn from the shell using forceps and divided for biochemical analysis, histopathological examination and genotoxicity studies.

Preparation of Chitosan

Procambarus clarkii (Crustacea: Cambaridae) were collected from the River Nile at Giza Governorate, Egypt, packed in plastic bags and transported to the Laboratory of Invertebrates, Zoology Department, Faculty of Science, Cairo University. Samples were dissected and scraped to discard the sticky tissue, then exoskeletons were separated, washed, and boiled for 15 min on the heater, then dried for 6 h. at 60 °C in an oven and grinded by grindery. Samples were kept in transparent Nylon bags with silica preserving sacks and kept away from light. The product blended with an electric blender to get crawfish exoskeleton powder that passes through 300μ sieve (Hamdi 2019). The processed powder was treated by different methods to obtain chitosan powder using the methodology described by (Hadi 2013). First, the powder deproteinized by 3.5% (w/v) NaOH solution with 10 ml/gm solvent to solid ratio, for 3 h. at 65 °C with constant stirring, then demineralized by 1 N HCl at room temperature and constant stirring for 60 min then left for 3 h. to dry in the oven. The extracted chitin was decolorized with acetone for 10 min and dried for 3 h. at 30 °C then bleached with 0.315% (v/v) sodium hypochlorite (NaOCl) solution (containing 5.25% available chlorine) for 5 min at ambient temperature. Chitin was washed and left to dry for 2 h. at 65 °C, then were soaked in 50% sodium hydroxide solution (NaOH) for 24 h., autoclaved for 1 h. and washed till neutrality with tap water. Finally, Chitosan was obtained from deacetylation of chitin.

Molluscicidal activity

Chitosan was dissolved in 1 ml 1% glacial acetic acid. A stock solution was prepared as 1gm and series of concentrations (100, 200, 250, 350 and 500 mg/l) were prepared to calculate the mortality of the snails. Three replicates were used, each of 10 snails, for each concentration. The exposure period of snails to the studied concentration was 24 h. then they were removed from the experimental container and left in clean containers for a recovery period (24 h.). The percentages of observed mortalities were recorded and the median lethal concentration was calculated by Probit analysis.

Biochemical measurements

Tissue preparation

Snails were crushed between two slides, and their soft tissues were withdrawn from the shell using forceps, weighed and then homogenized in ice cold. One gram tissue of each group/10 ml phosphate buffer saline (PBS) was homogenized using a glass Dounce homogenizer. The homogenates were centrifuged at 8000 rpm for 15 min at 5 °C in cooling centrifuge (Sigma laborzentrifugen, model 2-16PK, made in Germany), then the deposits were discarded, and the supernatants were stored at − 80 °C until used.

Investigation of the antioxidant activity

Antioxidant enzymatic activities were detected in the supernatant of the tissue homogenate for each group using Biodiagnostic kits (Biodiagnostic Dokki, Giza, Egypt) for the determination of catalase (CAT) (Damerval et al. 1986). In addition, reduced glutathione (GSH) was done according to the method of (Beutler 1963). Also, glutathione S-transferase (GST) was determined according to Habig et al. (1974).

Histopathological examination

Digestive gland was isolated, washed with saline and fixed in 10% buffered formalin for histopathological investigations. Samples were dehydrated in ascending grades of alcohol, cleared with xylene and embedded in paraffin and 5 µm sections were prepared and stained with hematoxylin and eosin (H& E). The stained sections were examined and photographed using light microscope (LEICA DM 750) supplied with a LEICA ICC 50 HD Camera.

Genetic study

DNA extraction and RAPD-PCR analysis

Samples of the *E. vermiculata* snails were dissected and their soft parts were preserved in 100% ethyl alcohol at −20 °C until used. Total genomic DNA was extracted from frozen ethanol-preserved mantle using Qiagen Dneasy tissue kit (Valencia, CA, USA) according to the manufacturer's manual. Six primers were used in the present work,

No.	Name	Seq.
P1	C1	TTCGAGCCAG
P2	P13	GGAGTGCCTC
P3	N8	ACCTCAGCTC
P4	B12	CCTTGACGCA
P5	H5	AGTCGTCCCC
P6	P8	GGAGCCCAG

Only 5 primers worked out (C1, P13, B12, H5 and N8). Amplifications were performed by modifying the protocol reported by Williams et al. (1990). Twenty-five µl mixture contained 1 µl of template DNA, 12.5 µl Dream Taq Green PCR Master Mix (2X), 2 µl primer and water nuclease-free to 25 µl (Thermal). Each amplification reaction was performed using a single primer and repeated twice to verify band auto similarity. Amplifications were performed in T-personal thermal cycler (Techne, TC-3000G), programmed for 42 cycles of 92 °C for 1 min, 35 °C for 1 min and 72 °C for 1 min. An initial denaturation step (5 min, 92 °C) and a final extension holding (10 min, 72 °C) were included in the first and last cycles, respectively. Ten µl of the reaction products were resolved by 2% agarose gel electrophoresis at 85 V in 1 × TAE (Tris–acetate-EDTA) buffer. The gel was stained with ethidium bromide and photographed by a gel documentation camera. For the comparison of the amplified products, population-specific fragments were detected using Gene Ruler 100 bp DNA Ladder from Thermal scientific.

Estimation of genomic template stability

The polymorphic pattern generated by RAPD-PCR profiles by using the selected primers allowed the calculation of Genomic Template Stability (GTS, %) as follows:

$$GTS = (1 - a/n) * 100$$

where *a* is the average number of polymorphic bands and *n* the number of total bands in the non-treated cells. Polymorphisms in RAPD profiles included disappearance of a band and appearance of a new one with respect to the control profile. To compare the sensitivity of genomic template stability, changes in these values were calculated as a percentage of their control.

Statistical analysis

Toxicity results were analyzed by Probit analysis (Finney 1971) using SPSS v. 17.0 for Windows (SPSS Inc. 2008). The data were expressed as mean ± S.D and the comparison between 2 means was done using student's t-test (Snedecor and Cochran 1991).

Results

The present results confirmed the molluscicidal activity of chitosan against adult *E. vermiculata* snails after 24 h. of exposure, followed by another 24 h. for recovery (Table 1). The data revealed that chitosan induced toxicity to *E. vermiculata* snails at (LC_{50} 222.4 mg/l).

Table 1 Molluscicidal activity of chitosan against adult *Eobania vermiculata* snails (24 h. exposure)

Concentration (mg/l)					
Mortality of *E. vermiculata*	LC_{10} (mg/l)	LC_{25} (mg/l)	LC_{50} (mg/l)	LC_{90} (mg/l)	Slope
	54.8	134.2	222.4	390.01	1.3

Biochemical analysis

Exposure of *E. vermiculata* snails to LC_{50} chitosan for 24 h./day (one day) exhibited a slight increase in CAT levels, while a significant decrease after 3, followed by a slight decrease after 7 days. Also, GSH activity significantly decreased after 1 and 3 days, while exhibited a slight decrease after 7 days. GST was significantly increased after 1and 3 days and slightly decreased after 7 days as compared with the control group (Table 2).

Histopathological study

Digestive gland of control *E. vermiculata* showed healthy and intact organ with no histopathological findings compared to the treated one. Typical features of digestive gland observed with a bilobed mass. Each lobe consists of many digestive tubules separated by intertubular connective tissues containing hemocytes and hemolymph spaces. The epithelium of each digestive tubule is lined with three different types of cells laying on a basement membrane; digestive cells, calcium cells and excretory cells, where all these cells are arranged around a narrow lumen (Fig. 1a). The digestive cells represent the major cell type component of the digestive gland with simple columnar epithelium while excretory cells are distinguished by the presence of single large vacuole containing excretory granules. The calcium cells are fewer in number than the two previous types and have a pyramidal shape. Sections of digestive gland isolated from treated chitosan snails after 24 h. showed clear signs of histopathological alterations. The stress induced by chitosan exposure led to irregular shape of cells, became flattened; dilatation of the intertubular spaces, destruction of tubules with increase in lumen size, necrosis of digestive cells with rise in vacuoles number and increase in calcium cells number (Fig. 1b). Considerably greater damage was observed with increasing time of exposure. Treated snails exposed to chitosan for 3 days revealed destructive effect in the digestive tissues (Fig. 1c), where there is a deformation of cells. Excretory and calcium cells were increased in number. Also, the presence of vacuoles, rupture and degeneration of digestive, excretory and calcium cells were more evident. While a moderate morphological change observed in the digestive tubules followed exposure for 7 days, the cells try to make a recovery, sections showed normal digestive, excretory and calcium cells. Besides the presence of vacuoles in some digestive cells and the lumen inside the tubule is increased (Fig. 1d).

Table 2 Effect of LC_{50} exposure of chitosan on the oxidative stress biomarkers of *Eobania vermiculata* snails

Groups	Control	1 Day	3 Days	7 days
CAT	8.45 ± 0.17	8.58 ± 0.07	6.48 ± 0.08*	8.3 ± 0.16
GSH	3.07 ± 0.14	1.89 ± 0.26*	1.54 ± 0.19**	3.05 ± 0.31
GST	7.85 ± 0.11	8.015 ± 0.18	8.78 ± 0.27*	7.68 ± 0.14

*Significant at $p < 0.05$

Genetic studies

Random amplified polymorphic DNA was employed to evaluate the chitosan toxicity effect on the genomic level of land snail *E. vermiculata*. RAPD profiles was performed on DNA extracted from control and treated snails. Out of 6 primers tested, 5 gave clear and reproducible bands (Fig. 2). The sizes of DNA fragments were between 100 and 1000 bp and polymorphic bands included appearance of new bands and disappearance of normal one is detected. High polymorphic profiles showed for primers C1, N8 and B12 (100%) as shown in Table 3. RAPD fingerprints indicated virtual differences between treated and control snails represented by change in the DNA band patterns. The greatest number of PCR fragments was found with primer P13 (8 bands), while less number were with primer B12 (4 bands). All experimental groups, 1, 3 and 7 days, gave variable bands, compared to the control one, reflected by the changes in appearance of new bands, disappearance of normal one. The apparent of new bands increased as duration time of exposure to chitosan decreased, treated snails exposed for 24 h. displayed 12 bands, followed by 11 and 7 bands for 3 and 7 days of exposure, respectively, while the number of lost bands were 6, 4 and 6 for the experimental groups, 1, 3 and 7 days, respectively, compared with the control one.

Genomic templates stability

Genomic templates stability (GTS, %) is a percentage value that reflects PCR amplification profile changes generated by chitosan, in relation to profiles obtained from the control group. Changes in the RAPD patterns are expressed as decreases in GTS, the percentage of genome stability was calculated from the analysis of RAPD profiles using five primers (Table 4). GTS values were decreased with the increase time of exposure. Exposure of chitosan for 1 day produced high value of GTS (63.6%), followed by decline in genomic stability (36.4, 18.2% GTS) for 3 and 7 days of exposure, respectively. These results confirmed the effect of DNA damage caused by chitosan.

Discussion

Control of snails on different crops is heavily dependent on the use of pesticides that limit the effect of these pests below damaging level (Genena and Mostafa 2008). Chitosan, a natural product produced from crustacean shells (Muthu et al. 2021), serves a variety of purposes in the domains of biomedical and

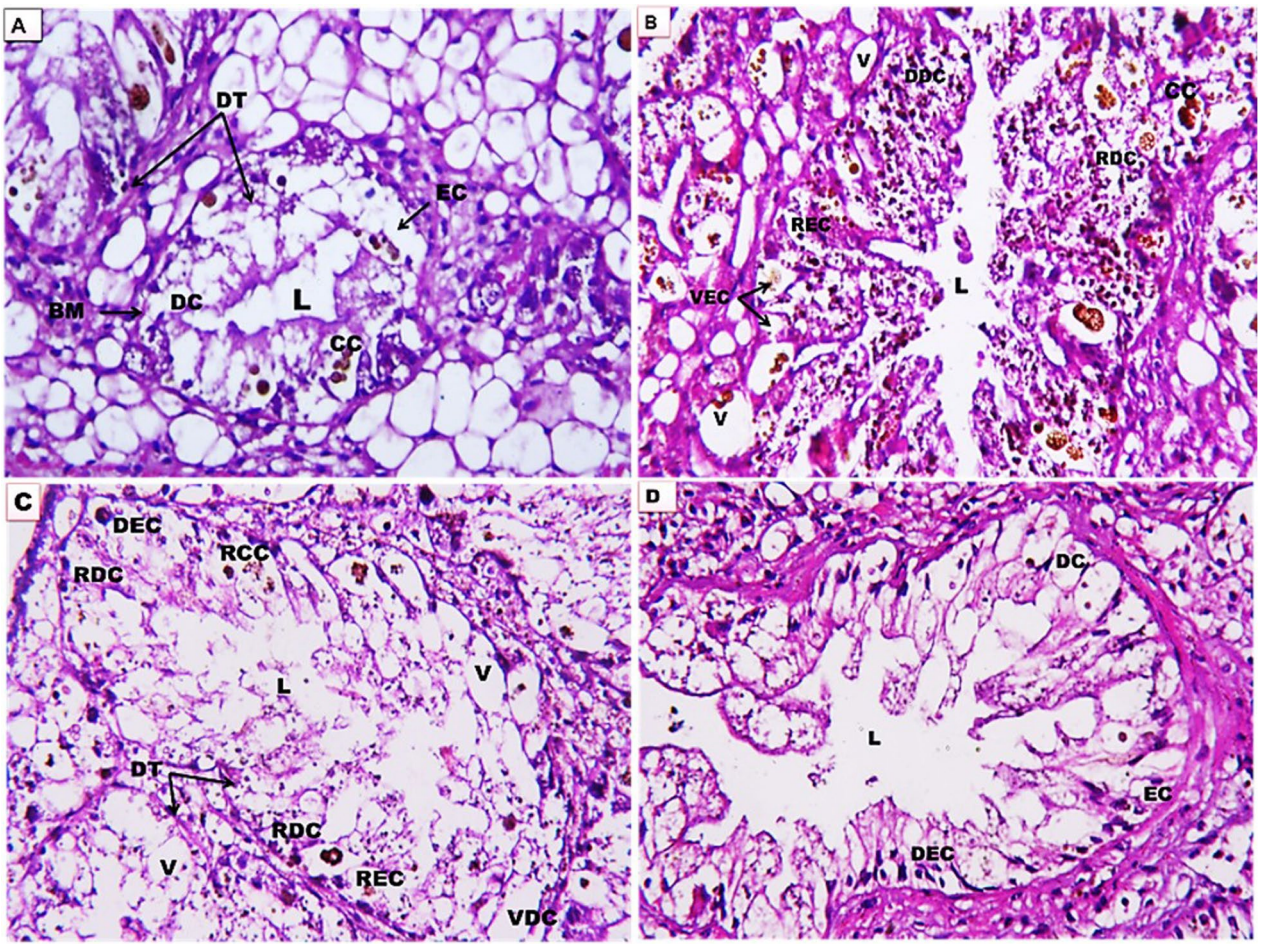

Fig. 1 Digestive gland of *Eobania vermiculata* snail. **a** Digestive gland of untreated *E. vermiculata* snail (control group) display regular structure with normal digestive tubule (DT) lined with digestive cells (DC), calcium cells (CC) and excretory cells (EC) resting on a basement membrane (BM) and are arranged around a narrow lumen (L). **b** Digestive gland of treated *E. vermiculata* snail after 1 day of chitosan LC_{50} exposure exhibited rupture of digestive cells (RDC) and excretory cells (REC), vacuolated digestive cells (VDC), vacuolated excretory cells (VEC), degeneration of digestive cells (DDC) and the lumen (L) inside tubule increased in size. **c** Digestive gland of *E. vermiculata* snail exposed to LC_{50} of chitosan for 3 days revealed deformation of cells where the lumen (L) is increased, many vacuoles (V) found and presence of vacuolated digestive cells (VDC). Many cells were ruptured and degenerated (RDC, RCC, REC and DEC). **d** Digestive gland of *E. vermiculata* snail after 7 days of chitosan LC_{50} exposure showed moderate morphological changes, with normal digestive, excretory and calcium cells and some vacuoles, H&E × 10

pharmaceutical products, food preservation, microbial control and biological controls (Vinsova and Vavrikova 2008). The present study revealed the molluscicidal activity of chitosan against *E. vermiculata* snails after 24 h. of exposure at LC_{50} 222.4 mg/l. On the same line, Khidr (2018) confirmed the molluscicidal activity of chitosan and nano-chitosan on the land snails *E. vermiculata* and *Monacha obstructa*. Similarly, Kandil et al. (2020) found that chitosan had a twofold higher molluscicidal efficacy than emamectin benzoate against *E. vermiculata* snails, with an LC_{50} of 68.8 g/l. Liu et al. (2008) hypothesized that chitosan's molluscicidal action was related to its method of lowering cholesterol absorption in the small intestine and increasing bile acid excretion.

Oxidative stress biomarkers could be helpful in the environmental toxicological studies (Bhagat et al. 2017). Digestive glands in gastropods are known to be one of the important sites of multiple oxidative reactions and maximal free radical generation (Ali et al. 2012). The increase or decrease in antioxidant activity in organisms results to an oxidative stress (Ojha et al. 2011), leading to damage in DNA and lipids (Schieber and Chandel 2014).

Stress markers are reliable indicators of the physiological state of animals (Nazarizadeh and Asri-Rezaie 2016). In the present study, levels of oxidative stress markers of

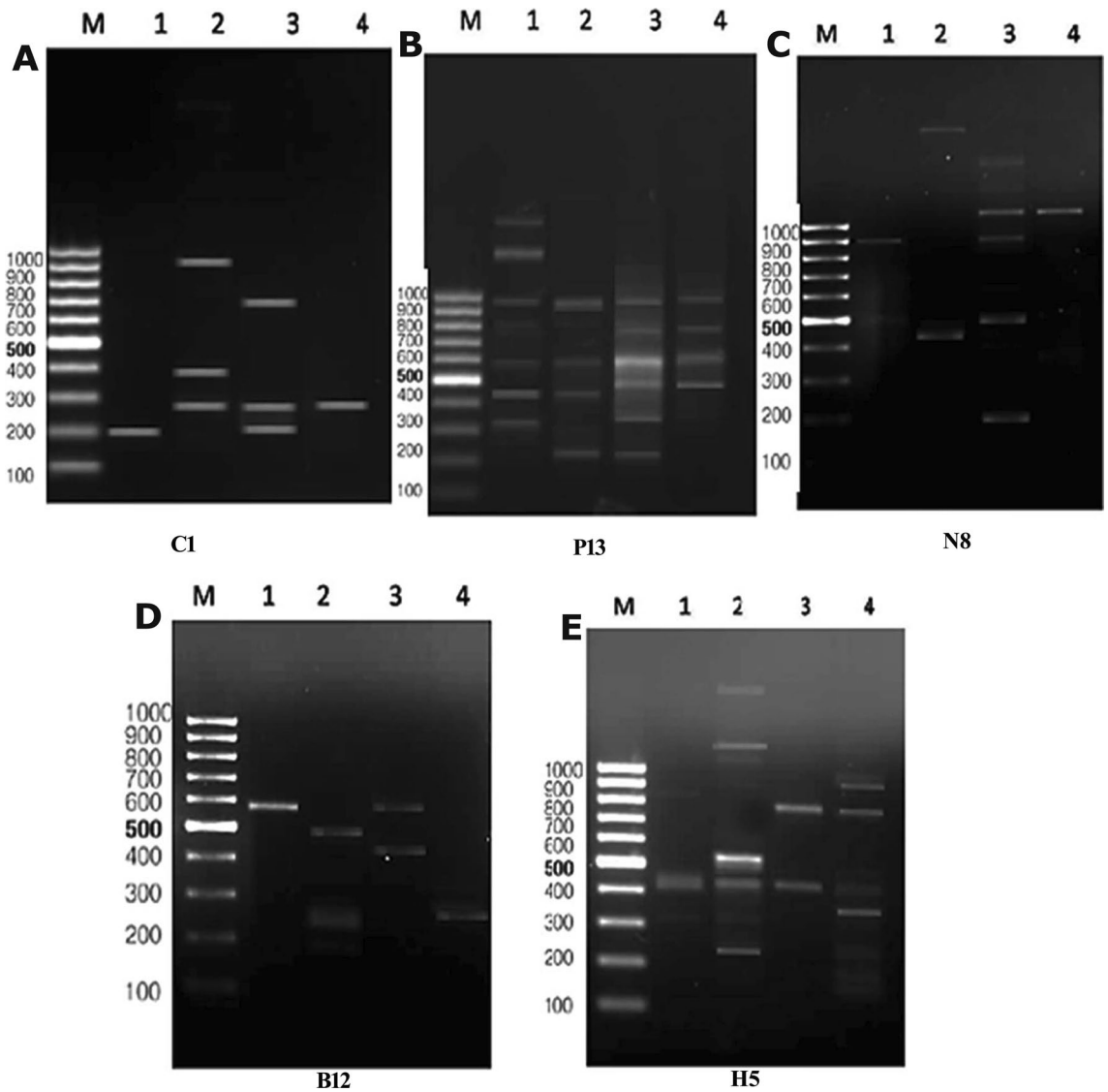

Fig. 2 Random amplified polymorphic DNA profiles of genomic DNA from *Eobania vermiculata* under different duration of chitosan exposure using five primers. **a** Profile generated by primer C1, **b** profile generated by primer P13, **c** profile generated by primer N8, **d** profile generated by primer B12, **e** profile generated by primer H5, Lanes **m** 100 bp plus DNA ladder; **1:** Control, **2:** 1 day **3:** 3 days **4:** 7 days

Table 3 RAPD primers used to evaluate genotoxicity in *Eobania vermiculata* snails after different duration of exposure to chitosan

Primer	Primer sequence (5′→3′)	Length (bp)	Polymorphic bands	Monomorphic bands	% of polymorphism
C1	TTCGAGCCAG	10	5	0	100
P13	GGAGTGCCTC	10	5	3	62.5
N8	ACCTCAGCTC	10	7	0	100
B12	CCTTGACGCA	10	4	0	100
H5	AGTCGTCCCC	10	7	1	87.5

Table 4 Summarizes the changes detected in the RAPD profile of each sample, total bands in control, varied band by primers set and % GTS in *Eobania vermiculata* snails under different duration of exposure to chitosan

Primer number	Primer	Control	1 day		3 days		7 days	
			p	d	p	D	p	d
1	C1	1	3	1	2	0	1	1
2	P13	7	1	3	3	3	1	3
3	N8	1	2	1	4	0	1	1
4	B12	1	2	1	1	1	1	1
5	H5	1	4	0	1	0	3	0
	Total bands	11	12	6	11	4	7	6
	A		18		15		13	
	a\n		1.63		1.36		1.18	
	1-(a/n)		0.63		0.36		0.18	
	% GTS		63.6		36.4		18.2	

p is appearance band in treated sample, d is disappearance band in treated sample, a is polymorphic profile (a = d + p)

E. vermiculata snails were altered. Catalase is the initial line of defense in antioxidant systems due to their significant function against oxidative stress. In this study, the significant increase in the activity of CAT observed in the digestive gland of *E. vermiculata* snails exposed to LC_{50} of chitosan for 24 h. Similarly, Radwan et al. (2010) found that CAT activity was significantly increased in the digestive gland of *Theba pisana* snails after 48 h. of exposure to 80% LC_{50} of Zn. This rise might be attributed to increased oxygen free radical generation, which could boost antioxidant activities to protect cells from harm (Torres et al. 2002). While the level of CAT showed a significant decrease after 3 days and a slight decrease after 7 days, compared to the control group. Slight decrease in CAT activity might be due to the activation of the natural antioxidant defense system by the xenobiotics (El-Shenawy et al. 2012). Moreover, glutathione (GSH) is a non-enzymatic antioxidant that protects cells from oxidative damage (Dickinson and Forman 2002). The present study revealed significantly ($p < 0.05$) decreased in GSH content after 1 and 3 days, while a slight decrease after 7 days of exposure to LC_{50} chitosan. These findings were in-line with those of El-Shenawy et al. (2012) who discovered that GSH levels in the digestive glands of *E. vermiculata* snails taken from WadiWaj garden and Qurwa farm were reduced by 47.4 and 53.4%, respectively, as compared to El-Shareef farm. This decrease might be attributed to the intensification of turnover between reduced and oxidized glutathione after exposing to toxic material (Farid et al. 2009).

Additionally, GST are multifunctional enzymes that play essential role in the defense system against oxidative damage and peroxidative products of DNA or lipids (Van der Oost et al. 2003). As a result, it provided sensitive biochemical indicators for environmental monitoring of exposure and toxicity (Bhagat et al. 2017). Our findings demonstrated a substantial increase in GST levels after 1, 3 days, while marginally lower after 7 days in comparison to the control group. This coincides with El-Shenawy et al. (2012) who stated that the activity of GST was significantly increased in the digestive gland tissue of *E. vermiculata* snails collected from Wadi Waj garden and Qurwa farm by 1.1- and 1.6-fold, respectively. Also, Radwan et al. (2010) reported that GST activity was significantly increased in the digestive gland of *T. pisana* snails after 48 h. of exposure to 80% LC_{50} of Pb. The increase in GST activity might be due to the xenobiotics activating the endogenous antioxidant defense mechanism (Elia et al. 2007).

The digestive gland, also known as hepatopancreas, which is the main organ of gastropod, since it is responsible for the production of digestive enzymes, nutrient absorption, food endocytosis, food storage, and excretion (Dussart 2003). Radwan et al. (2008) found that exposure to organic and inorganic chemicals causes cellular changes in the digestive gland. There are limited investigations on the biochemical and histological alterations in terrestrial gastropod digestive gland tissues caused by natural molluscicides (Hamed et al. 2007). The present investigation showed differences in the cell type composition between the control and treated snails, where the digestive cells decreased in number with increase in excretory and calcium cells. Likewise, Zaldibar et al. (2007) found that around 750 (17%) digestive cells of *Arionater* slugs were lost, following Cd + kerosene treatment whereas after 7 days of detoxification about 1500 new digestive cells appeared. Similar changes were identified in Sharaf and El-Atti (2015), following exposure to both Methiocarb and Chlorpyrifos, such as significant tubular rupture, vaculation, pyknotic nuclei and necrosis of

digestive tubules of *Helicella vestalis* digestive gland. On the same line, Zaldibar et al. (2007) identified general alterations in the digestive gland architecture of the terrestrial gastropods exposed to pollutants; basal membranes are more thickened, digestive cells are vacuolated and reduced in numbers, calcium cells become hypertrophied, calcium and excretory cells become relatively abundant, blood cells and migrating connective tissue cells accumulate around digestive tubules. These changes in *E. vermiculata* digestive gland following exposure to sub-lethal chitosan concentrations might be attributable to a general stress response that varied depending on time intervals, species, pollutant, concentration, exposure method and environmental variables (Marigómez et al. 1996).

RAPD-PCR technique appears to be a reliable procedure, this method involves amplification of random regions of genomic DNA using short arbitrary primers, with no prior knowledge of genomic deoxyribonucleic acid (DNA) required (Rocco et al. 2014). RAPD analysis was more sensitive than the comet and micronucleus assays, according to many studies, since it could identify DNA alterations at lower pollution concentrations (Zhou et al. 2011). In ecotoxicological investigations, RAPD test has been shown to be a useful method for assessing the impact of toxicants on organisms under ideal circumstances. The presence, absence and intensity of bands are linked to DNA damage and genotoxicant-induced mutations (Pal 2016). It is critical to assess genotoxin-induced DNA damage and mutation at the molecular level (Aslam et al. 2014).

In this study, RAPD analysis revealed substantial changes in RAPD patterns between the chitosan-treated and control groups. These differences occurred because of variation in the disappearance of existing bands, and the appearance of new ones. In a similar study, Bauranda et al. (2015) found that pesticides caused DNA damage in *Cantareus aspersus*, as evidenced by the development and removal of existing bands. In accordance with Abdel-Halim et al. (2019), who observed alterations in RAPD pattern on the freshwater snail *Lanistes carinatus* in Egypt as a result of industrial and urban waste water. Shen et al. (2013) also found that after diesel oil treatment, the DNA band alteration in RAPD profiles of Zhe Oyster, *Crassostrea plicatula* comprised the disappearance of normal DNA bands, the emergence of new DNA bands, and changes in DNA intensity when compared to non-treated oysters. According to Liu et al. (2005) genomic rearrangements, reduced point mutation, DNA damage in primer binding sites, and interaction of DNA polymerase in the test organism with damaged DNA were the primary causes of band disappearance. On the other hand, new bands might be the consequence of GTS due to DNA damage, as certain oligonucleotide priming sites may have been accessible to primers following structural alterations or changes in DNA sequence due to mutations, significant deletions and/or homologous recombination (Atienzar et al. 1999).

On the basis of novel biomarker assays for detection of DNA damage and mutations in living organisms, the RAPD bands were evaluated for genomic template stability (GTS) (Shahrtash et al. 2010). It is of interest to note that the genotoxic response observed early upon the studied snail, *E. vermiculata* after 24 h. of exposure with 63.6% GTS then declined to 36.4% after 3 days, followed by 18.2% after 7 days, suggesting that GTS decreases as chitosan exposure time increases. This finding of genomic stability matched to those of Shen et al. (2013) who found that the GTS value in the Zhe Oyster, *C. plicatula* exposed to diesel oil dropped as the time spent increased. In a similar vein, Kumar et al. (2015) found that GTS in *Cyprinus carpio* reduced as potassium dichromate concentrations and exposure duration increased.

In this way, a sustainable approach of biopesticide-based integrated pest management system for different crops should be undertaken, thus reducing pest management costs with minimal risk or hazard to humans and desirable components of their environment.

Conclusions

Chitosan has molluscicidal activity against the land snail *E. vermiculata*, making it the best candidate for biological application as a potentially simple, readily available, inexpensive and environmentally safe molluscicidal agent of natural origin for controlling snail pests in Egyptian agriculture.

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Authors' contributions

MF and SH have provided guidance during development of idea and AI, MM and MF prepared different figures required, MF and AI wrote and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

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Declarations

Ethics approval and consent to participate

All procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals and have been approved and authorized by Institutional Animal Care and Use Committee (IACUC) in Faculty of Science, Cairo University, Egypt.

Consent for publication
Not applicable.

Competing interests
The authors have indicated that they have no conflict of interest regarding the content of this article.

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