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Evaluation the effectiveness of *Chrysomya marginalis* maggots extract in controlling land snail *Theba pisana*

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ABSTRACT

Maggots of some blowflies (Diptera: calliphoridae) are typically used in a wide range of medical and forensic applications. One of these flies is *Chrysomya marginalis*. This fly has not received attention in research in Egypt. Accordingly, we start to examine the effect of *Chrysomya marginalis* maggots extract on the land snails. Nevertheless, the effect of this extract on the environment is still under research. Thus, the present investigation was performed to determine the median lethal concentration of *Chrysomya marginalis* maggots extract (96-h LC_{50}) and to evaluate the effectiveness of maggots extract in controlling land snails *Theba pisana* by measuring levels of biochemical, oxidative and antioxidant parameters and the analysis at the molecular level. The results showed that LC_{50} of maggots extract for *T. pisana* for 96 hrs was 235.5 g/l. 1/2, 1/4 and 1/10 LC_{50} (117.75, 58.875 and 23.55 g/l) values were chosen for sublethal studies for 28 days. The results indicated that aspartate amino transferase (AST) and alanine transferase (ALT) levels were significantly higher, while total protein (TP), lactate dehydrogenase (LDH), hexokinase (HK) and pyruvate kinase (PK) levels were significantly lower in maggots extract-exposed snail compared to the control. Lipid peroxidation (LPO) and nitric oxide (NO) levels were significantly higher, while catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) levels were significantly lower in maggots extract-exposed snail as opposed to the control group. A significant down-regulation was noticed in gene expression levels of Cu-Zn SOD in the exposed group. On the other hand, exposure to tested extract caused significantly up-regulated gene expression levels of HSP 70 compared with the control group. In conclusion, *Chrysomya marginalis* maggots extract caused alteration in biochemical, and antioxidant activities and gene expressed changes.

Introduction

Land snails are regarded as significant pests that seriously harm all plant parts while attacking a variety of crops. These gastropods prey on plants during various phases of growth, thereby decreasing their yields Khidr *et al*. (2011). In order to determine the best molluscicide for managing these pests, numerous studies were conducted evaluating a variety of utilizing pesticides and biocides to combat land snails in both laboratory and field (Farag, 2012; Ismail *et al*., 2015). *Theba pisana* (*T. pisana*), a white garden snail belonging to the Helicidae family of gastropods, is an invasive terrestrial snail that has spread throughout numerous Mediterranean countries (Neubert, 1998; Herbert, 2010). Because of its small size, ability to live for extended periods of time, and rapid reproduction rates which can produce up to 3,000 snails on a tree in less than five years *T. pisana* is especially dangerous (Cowie, 2009; Deisler *et al*., 2015).

The first line of defence against land snails is chemical molluscicides, but because these products are toxic to both aquatic and terrestrial life, there is a growing interest in finding acceptable biologically ecofriendly molluscicides. The molluscicidal activity of a fungal isolate (*Trichoderma harzianum*) against the land snail, *Monacha cartusiana*, was tested (Ahmed *et al*., 2023) as a natural and environmentally safe substitute for synthetic chemicals.

Biological insecticides, which include bacteria, fungi, viruses, and non-target insects, are real living things or the toxins they produce (Geasa *et al*., 2013). *Metarhizium anisopliae* is a naturally existing entomopathogenic fungus serves as the basis for the biological insecticide biomagic. One of the best fungi to combat the two land snails, *Eobania vermiculata* and *M. cartusiana*, are fungus isolates of *M. anisopliae* (Gabalah, 2013). The native *M. anisopliae* fungal isolates at Kafr Elsheikh Governorate were compared to methomyl insecticide in terms of their ability to suppress Monacha spp. In the biological mechanisms of land snails, transaminase enzymes, acetylcholine esterases, total proteins, and total lipids are important (Abd-El-Aal, 2004). In the past few decades, biological control of land snails through the use of pesticides derived from bacteria and fungi has garnered significant attention globally (Genena and Mostafa, 2008). *Beauveria bassiana* (Balsamo), one of the entomopathogenic fungi used in control methods, has been viewed as a promising substitute for chemicals (Chandler *et al*., 2001). A commercial fungal insecticide called Biozed (2.5×10^4 spores/ml WP) was isolated from *Trichoderma album* (Verma *et al*., 2007). Conversely, El-Sabbagh *et al*. (2013) reported that *Bacillus thuringiensis* is the source of the bacterial result of Biogard (6.5% WP), which has insecticidal effects.

Various insect families, including the Diptera (Scomyzidae and Sarcophagidae) and the Coleoptera (Carabidae, Lampyridae, and Staphylinidae) families, were thought to possess the ability to biologically regulate aquatic, semi-aquatic, and terrestrial gastropods. The vinegar fly *Drosophila melanogaster* (Diptera: Drosophilidae) is a natural enemy of land snails, according to Mohamed and Ali (2013) report, which focused on the family Drosophilidae of the order Diptera and its potential use as a biocontrol agent of land snails. This role was unintentionally discovered when a land snail culture (*Eremina irrigularis*) became contaminated by this fly. Many arthropod pests, such as *Rhipicephalus microplus, Deois flavopicta, Mahanarva fimbriolata*, and *Cornitermes cumulans*, have been effectively managed with entomopathogenic agents (Pereira, 2008; Fernandes and Bittencourt, 2008; Loureiro, 2012). The present study aimed to control the snail *T. pisana* using maggots extract as a natural and alternative solution instead of using harmful chemical molluscicides.

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Materials and methods

Collection of Maggots

Third instar larvae (maggots) of *Chrysomya marginalis* used in this study were raised under controlled sterile conditions. Eggs were collected from adult cages previously reared at Entomology laboratory, Zoology Department, Faculty of Science, Zagazig University, Egypt. The collected eggs were washed three times by ethanol followed by distilled water and then transferred to a fresh beef meat till hatching. Hatched larvae were examined daily and upon forming the third instars, they were collected, washed successfully with ethanol and distilled water three times and then dried with filter papers.

Preparation of crude maggots extract

All Dried maggots were transferred to a sterile jar and then each 100 maggots (0.6g/maggot) were homogenized with 10 ml of phosphate buffer saline (PBS) solutions in sterile porcelain mortars. After complete homogenization, the homogenates were centrifuged at 12000 rpm for 20 min. at 4ºC. The supernatants (crude extracts) were filtrated using sterile micro syringe filters (0.22 µm pore of the membrane) and then transferred to new clean sterile tubes which were frozen at – 80ºC. After freezing, the extracts were kept in a deep freezer at -20ºC for further study.

Snail's collection and acclimatization

Adult *T. pisana* snails were collected by hand from gardens in Abees area, few kilometers south of Alexandria, Egypt, during May 2023. The gathered snails (13±0.5 mm shell length, and 1.2±0.14 g weight) were transferred to the laboratory and kept in plastic boxes (40x30x30 cm, with 10 cm height of moistened soil and covered with a nylon-mesh covering). At 27–30°C and 63–65% humidity, the snails under test were kept in their enclosure. For a week, they were fed only fresh lettuce leaves every day.

Baits preparation and experimental design

Bait technique was made in accordance with Ebenso (2004) method. The baits were made by mixing 5% molasses, 80% wheat-bran, little of water and tested concentrations of *Chrysomya marginalis* maggots extracts (100, 200, 400 and 600 g/l). Five g of the bait sample was put on plastic sheet on the surface of the soil in each box. Ten snails of adult *T. pisana* were divided into three replicates and exposed to the tested concentrations of the maggot extracts. Parallel to this, thirty snails in three boxes were used as control group. When an animal was probed with a dissecting needle and did not respond to tactile stimuli, it was deemed dead (WHO, 1963). Death was counted every 24 h and dead snails were removed. Mortality percentages at each precise day were calculated. LC_{50} values for maggot extract were calculated by using probit analysis based on Finney (1971).

Biochemical biomarkers

After being exposed to snails for 28 days, 0.5g of the control group's digestive gland and 1/2, 1/4, and 1/10 LC_{50} of maggot extract were removed and homogenized in 10 mL of ice-cold 0.05M phosphate buffer saline. The supernatants were stored at -80 °C after the homogenates were centrifuged at 6000 rpm and 4 °C. Total proteins (TP) were estimated according to the Biuret method (Gornall *et al*., 1949). Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were estimated according to Breuer (1996) method. Lactate dehydrogenase (LDH) was measured according to Cabaud and Worblewski (1958). Hexokinase (HK) was assessed according to Uyeda and Racker (1965). Pyruvate kinase (PK) was estimated according to McManus and James (1975).

Oxidative and antioxidant biomarkers

Digestive gland homogenates were used to determine lipid peroxidation (LPO) according to Ohkawa *et al.* (1979) method. Nitric oxide (NO) was measured as stated by Green *et al.* (1982). Catalase (CAT) was assayed as stated by Aebi (1984). Superoxide dismutase (SOD) was estimated according to Nishikimi *et al*. (1972). Glutathione (GSH) was assessed according to Ellman (1959).

Molecular analysis

RNA extraction

Using the QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH), 200 µl of the sample were added to 600 µl of RLT buffer containing 10 µl β-mercaptoethanol per 1 ml, and the mixture was then incubated for 10 minutes at room temperature. The cleared lysate was mixed with one volume of 70% ethanol, and the Purification of Total RNA protocol of the QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH) was followed.

Oligonucleotide Primers

The primers utilized, which are mentioned in Table 1, were provided by Metabion (Germany).

SYBR green rt-PCR

The primers were used in a 20-µl reaction that included 3 µl of water, 5 µl of RNA template, 10 µl of the 2x HERA SYBR® Green RT-qPCR Master Mix (Willowfort, UK), 1 µl of RT Enzyme Mix (20X), and 0.5 µl of each primer at a concentration of 20 pmol. A step one real-time PCR machine was used to carry out the reaction.

Analysis of the SYBR green rt-PCR

The step one software produced amplification curves and ct values. The CT of each sample was compared with that of the positive control group using the ratio 2-DDct, in accordance with the "ΔΔCt" method described by Yuan *et al.* (2006), in order to estimate the variation of gene

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions for SYBR green rt-PCR.

Target gene	Primers sequences	Reverse transcription	Primary denaturation	Amplification (40 cycles)			Reference
				Secondary denaturation	Annealing (Optics on)	Extension	
B- actin (F)	5′ TCACCATTGGCAACGAGCGAT 3′						Liu *et al*. (2018)
B- actin (R)	5′ TCTCGTGAATACCAGCCGACT 3′						
HSP70 (F)	5′ CGAAACGATACGACTACCTAGCAGA 3′	65˚C 60 min.	95˚C 15 min.	95˚C 15 sec.	60˚C 1 min.	72˚C 30 sec.	Liu *et al*. (2018)
HSP70 (R)	5′ AGGAAGGTAAGAACCGAGCAAT 3′						
Cu-Zn SOD (F)	5′ TAGTTGCCAATGCTGACGGT 3′						Turki *et al*. (2022)
Cu-Zn SOD (R)	5′ GGACCACAACACTACGACCA 3′						

expression on the RNA of the different samples.

Statistical analysis

Statistical analysis was done using SPSS version 20 (SPSS, Richmond, VA, USA). To compare significant differences between treatments at $P < 0.05$, all data were calculated as mean ± SE and analyzed using One-way ANOVA, followed by Duncan's multiple range tests as a post-hoc test.

Results

Toxicity test

Table 2 reveals that the highest mortality rate occurred at *Chrysomya marginalis* maggots extract concentration of 600 g/l; however, the lowest mortality percentage occurred at *Chrysomya marginalis* maggots extract concentration of 100 g/l for *T. pisana*. It was found that, the mortality percentages increased as the concentrations of *Chrysomya marginalis* maggots extract and the time of exposure increased. Table 3 reveals that, LC_{50} of *Chrysomya marginalis* maggots extract for *T. pisana* was 235.5 g/ l. On the other hand, 1/2, 1/4 and 1/10 LC_{50} of *Chrysomya marginalis* maggots extract was 117.75, 58.875 and 23.55 g/ l, respectively for the tested snail.

Table 2. Effect of various concentrations of *Chrysomya marginalis* maggots extract on mortality percentages of *T. pisana* at different exposure periods.

Conc. (g/l) \ Time	Percentage of mortality (%)			
	24 h	48 h	72 h	96 h
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
100	20.00±0.00	26.67±3.30	26.67±3.30	30.00±0.00
200	36.67±3.30	36.67±3.30	40.00±0.00	40.00±0.00
400	46.67±3.30	50.00±0.00	53.33±3.3	56.76±6.70
600	63.33±3.30	76.67±3.3	93.33±3.3	100.00±0.00

Data are represented as means of Mean ±SE.

Effect of 1/2, 1/4 and 1/10 LC_{50} of Chrysomya marginalis maggots extract on biochemical parameters of T. pisana for 28 days

After 28 days of exposure, AST, and ALT levels in *T. pisana* exposed to 1/2, 1/4 and 1/10 LC_{50} of *Chrysomya marginalis* maggots extract were significantly increased ($P < 0.05$) compared with the control group. Whereas, levels of TP, LDH, HK and PK were significantly decreased ($P < 0.05$) compared with the control group (Table 4).

Effect of 1/2, 1/4 and 1/10 LC_{50} of Chrysomya marginalis maggots extract on Oxidative and antioxidant biomarkers of T. pisana for 28 days

After 28 days of exposure, LPO and NO levels in *T. pisana* exposed to 1/2, 1/4 and 1/10 LC_{50} of *Chrysomya marginalis* maggots extract were significantly increased ($P<0.05$) compared with the control groups.

Table 3. Lethal toxicity values and sublethal concentrations of *Chrysomya marginalis* maggots extract against *T. pisana* under laboratory conditions.

Tested concentration (g/l)	Exposure periods	*Chrysomya marginalis* maggots Extract concentrations
LC_{50}	96 h	235.5
1/2 LC_{50}	28 days	117.75
1/4 LC_{50}	28 days	58.88
1/10 LC_{50}	28 days	23.55

Whereas, levels of CAT, SOD and GSH were significantly decreased ($P < 0.05$) compared with the control group (Table 5).

Effect of sublethal concentrations (1/2, 1/4 and 1/10 LC_{50}) of Chrysomya marginalis maggots extract on Cu-Zn SOD and HSP70 mRNA expression by real time-PCR for 28 days.

Data in Fig. 1 showed that a significant down-regulation ($P<0.05$) was noticed in gene expression levels of Cu-Zn SOD in the exposed group to 1/2 and 1/4 LC_{50} of *Chrysomya marginalis* maggots extract compared with the control group. On the other hand, exposure to 1/2 and 1/4 LC_{50} of *Chrysomya marginalis* maggots extract caused significantly up-regulated ($P<0.05$) gene expression levels of HSP 70 in contrast to the group under control.

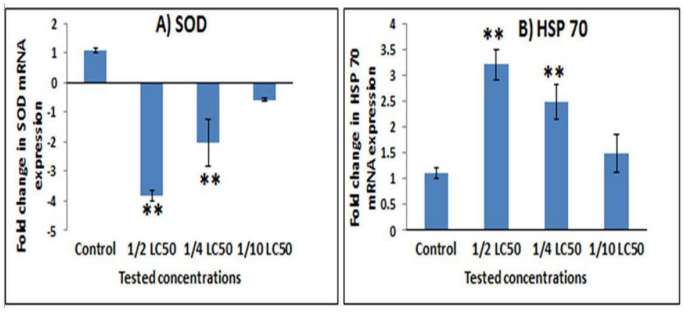

Fig. 1. Effect of sublethal concentrations (1/2, 1/4 and 1/10 LC_{50}) of *Chrysomya marginalis* maggots extract for 28 days on Cu-Zn SOD and HSP70 mRNA. Results are expressed as means ± SE (n=3) and standardized to β-actin and represented as fold change (log2 scale), compared to mRNA levels in control. **: significant change at $P<0.05$ compared to control group

Discussion

Molluscicidal agents of biological origin is required to avoid the disadvantages of using chemical molluscicides (Amina *et al.*, 2022). Observing the definition offered by Eilenberg *et al.* (2001), we have adopted the following definition of biological control, which has undergone numerous definitions in recent years: "The application of living things to reduce a pest organism's population density or impact, making it less prevalent or harmful than it otherwise would be." *T. pisana* snails were used as the target pest in this study in order to evaluate the harmful effects of the extract from *Chrysomya marginalis* maggots.

According to Asran (2001); Abd El Rahman and Al Akra (2012); Mwonga *et al.* (2015); Farag (2017); Prabhakaran *et al.* (2017) and Abd El-Atti *et al.* (2020), and numerous toxicological studies have been conducted in both

Table 4. Biochemical parameters of *T. pisana* exposed to 1/2, 1/4 and 1/10 LC_{50} of *Chrysomya marginalis* maggots extract for 28 days.

Tested conc.	TP (mg/g)	AST (U/g)	ALT (U/g)	LDH (U/mg)	HK (U/mg)	PK (U/mg)
Control	23.5±1.3^{b}	28.2±2.4^{a}	102.2±11.7^{a}	64.5±3.0^{C}	24.3±0.9^{b}	0.91±0.1^{b}
1/2 LC_{50}	13.4±0.8^{a}	65.8±3.75^{c}	167.1±10.8^{b}	18.02±0.22^{a}	13.45±2.3^{a}	0.045±0.04^{a}
1/4 LC_{50}	17.2±1.4^{a}	59.5±4.5^{bc}	134.2±5.7^{b}	40.7±3.8^{b}	17.1±0.25^{a}	0.29±0.02^{a}
1/10 LC_{50}	19.1±0.9^{ab}	42.2±3.5^{ab}	110.6±10.1^{a}	53.2±1.95^{bc}	19.7±1.1^{ab}	0.42±0.08^{ab}
F-value	13.9	19.2	8.8	58.13	11.7	8.35
P-value	0.01	0.01	0.03	0.00	0.02	0.03

Values are represented as means of three samples ± SE ; Means with dissimilar alphabetical superscripts at each column are significantly different at $P < 0.05$. TP: Total protein; AST: Aspartate amino transferase; ALT: Alanine amino transferase; LDH: Lactate dehydrogenase; HK: Hexokinase; PK: Pyruvate kinase.

Table 5. Oxidative and antioxidant biomarkers of *T. pisana* exposed to 1/2, 1/4 and 1/10 LC_{50} of *Chrysomya marginalis* maggots extract for 28 days.

Tested conc.	LPO (µmol/g)	NO (µmol/g)	CAT (U/g)	SOD (U/g)	GSH (µmol/g)
Control	42.5±2.99^{a}	39.2±4.05^{a}	12.3±0.4^{b}	58.2±6.9^{b}	52.7±3.15^{b}
1/2 LC_{50}	146.9±3.67^{c}	87.45±6.2^{b}	7.1±0.7^{a}	27.8±2.8^{a}	24.4±0.91^{a}
1/4 LC_{50}	133.5±4.5^{c}	54.1±3.9^{b}	8.2±0.05^{a}	36.5±2.5^{ab}	30.8±2.4^{a}
1/10 LC_{50}	71.52±5.7^{b}	41.5±2.5^{a}	10.02±0.9^{ab}	47.5±3.1^{ab}	49.5±1.5^{b}
F-value	131.5	25.8	6.8	10.2	40.6
P-value	0.00	0.00	0.04	0.02	0.00

Values are represented as means of three samples ± SE -Means with dissimilar alphabetical superscripts at each column are significantly different at $P < 0.05$. LPO: Lipid peroxidation; NO: Nitric oxide; CAT: Catalase; SOD: superoxide dismutase; GSH: Glutathione.

laboratory and field settings to assess the molluscicidal efficacy of chemical pesticides, plant extracts, and biopesticides against harmful land snails. Because of their environmentally friendly qualities, biopesticides are being created and used against a wider range of agricultural pests. The findings showed that as the concentrations of *Chrysomya marginalis* maggot's extract and the exposure duration increased, the mortality percentages increased. The acute toxicity data from the current study clearly demonstrated that the extract from maggots is lethal to *T. pisana* snails. This is agreed with the results of Ali *et al.* (2017), who discovered mortality rates in the land snails *Eobania vermiculata* and *Succinea putris* four weeks after *Trichoderma album* treatment. Comparably, Abd El-Atti *et al*. (2020) found that at high concentrations, the fungus biozed exhibits molluscicidal activity against *Monacha cartusiana*, resulting in 66.6% mortality.

For *T. pisana*, the lowest death percentage was observed at a concentration of 100g/l for the *Chrysomya marginalis* maggots extract, while the maximum mortality rate was observed at 600 g/l. *T. pisana*'s LC_{50} for the extract of Chrysomya marginali's maggots was 235.5 g/l. Conversely, for *T. pisana*, the 1/2, 1/4, and 1/10 LC_{50} of the *Chrysomya marginalis* maggot extract were 117.75, 58.875 and 23.55 g/l, respectively. This is consistent with Amina *et al.* (2022) findings that at LC_{50} of 222.4 mg/l, chitosan exhibited molluscicidal action against *E. vermiculata* snails after a 24-hour exposure. In a similar vein, Khidr (2019) confirmed that nano-chitosan and chitosan have molluscicidal effects on the land snails *Monacha obstructa* and *E. vermiculata*. Likewise, Kandil *et al.* (2020) discovered that, with an LC_{50} of 68.8 g/l against *E. vermiculata* snails.

Our observations demonstrated that ALT and AST activities rose considerably at sub-lethal concentrations of 1/2, 1/4, and 1/10 LC_{50} of maggot's extract when compared to the equivalent control at all experimental exposure periods (24, 48, and 72 hours). This is in line with research by Kammon *et al.* (2010), which demonstrated that damage to the digestive gland and insecticide-induced cell necrosis may be the reason of the rise in ALT and AST activity and the release of enzymes from the cells. On the other hand, our observations concur with El-Gohary and Genena (2011) that, when exposed to three different tested molluscicides, the activity of AST and ALT significantly increased in *M. cantiana* and E. vermiculta. However, our findings disagree with El-Gohary and Genena's assertion that, in *E. vermiculata*, ALT significantly decreased.

Also, the study's findings demonstrated that the snail exposed to three sublethal concentrations of maggot extract had a significantly lower level of total protein than the control group. According to El-Shenawy *et al*. (2012), *E. vermiculata* snails collected from two polluted areas had lower TP levels in their digestive glands than snails collected from unpolluted areas. The disparity between the rate of total protein synthesis and degradation in bodily tissues may be the cause of the drop in TP levels. El-Gohary and Genena (2011) concur with our findings that three distinct molluscicide treatments resulted in a decrease in the amount of TP in *M. cantiana*, but disagree with our findings when applying the same three molluscicides to *E. vermiculata*. There was a noticeable increase in TP. According to Shahawy (2018), land snails H. vestalis and *T. pisana*'s tissues had lower levels of TP following their exposure to the pesticides Agrinate and Bio magic. Additionally, El-Khayat *et al*. (2018) took into account our findings, confirming that *Biomphalaria alexandrina* snails exhibit higher TP in more polluted ecosystems than their counterparts in environments with high water quality.

Determining the activity of lipoprotein digestase (LDH), an enzyme found in all organs and tissues, is one method of evaluating the integrity of cell membranes Kending and Tarloff (2007). In the current investigation, *T. pisana* exposed to 1/2, 1/4, and 1/10 LC_{50} of *Chrysomya marginalis* maggots extract showed significantly lower LDH levels after 28 days of exposure when compared to the control group. Our findings contradict the theory that abamectin (ABM) at sub-lethal doses significantly increased the activity of LDH in the treated *T. pisana*, suggesting that ABM can alter the permeability of cell membranes and ultimately cause cell death (Amin and Hamza, 2005; Radwan and Gad, 2022). In line with Mohamed (2011) findings, which demonstrated a significant decline in LDH, hexokinase (HK), and pyru-vatekinase (PK) activity in the whole tissue extract of *Biomphalaria alexandrina* in response to pesticide treatment, the levels of TP, LDH, HK, and PK were significantly lower in the current study when compared to the control group.

It is believed that oxidative stress results from an inequity in the ratio of biological oxidants to antioxidants. Nucleic acids, proteins, carbs, and lipids can all be harmed by oxidative stress. The abnormal production of ROS is typically a crucial sign of oxidative damage and can cause serious damage to the structure of cells (El-Demerdash, 2007). The current study found that treatment with the concentrations of maggots extract significantly reduced the levels of the tested antioxidant enzymes (CAT, SOD, and GSH). According to Radwan *et al.* (2010), the activation of an antioxidant a preventative measure the accumulation of reactive oxygen species (ROS) can explain the significant induction of CAT and SOD activities observed in this study. In contrast, Khene *et al.* (2017) discovered that the *Helix aspersa* snail's CAT activity significantly increased following its exposure to TiO_2 nanoparticles. Additionally, it was reported by Ugokwe *et al.* (2020) and Abd El-Atti *et al.* (2023) that the actions of SOD, GSH and CAT in the digestive gland of land snails had significantly increased.

The complex process known as lipid peroxidation (LPO) changes polyunsaturated fatty acids in biological membranes through a series of chain reactions to generate lipid hydroperoxides, which break down unsaturated fatty acid double bonds and disturb membrane lipid (Gutteridge, 1995). The gastrointestinal tract of *T. pisana* snails in the current study had noticeably higher levels of LPO.

An essential component of innate immune responses is Nitric oxide (NO). Haemocytes in molluscs produce NO (Bernice *et al*., 2006). The highly reactive chemical NO is produced by the cells of mammals, invertebrates, and plants. The current study revealed a highly significant increase in NO activity. Nevertheless, compared to their corresponding control (Fayez *et al*., 2016), there was a decrease in the activity of the NO in the hemolymph of *B. alexandrina* snails following acute and long-term treatment with Cu_2ONPs.

This snail is a bioindicator of toxicity species, but it is poorly understood at the molecular level. A well-known class of proteins known as HSPs can be activated by a variety of environmental stressors. Numerous organisms have been studied pertaining to the various families of HSPs. Sequences encoding for heat shock proteins (Hsp70, Hsp17.2, and Hsp19.8), superoxide dismutase (SOD Cu/Zn, Mn-SOD), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Turki, 2022). While the stress response revealed a weak response to hsp70 at the highest concentration tested in the digestive gland, oxidative stress induction was not observed. But the majority of organisms have the HSP gene hsp70, which all prokaryotes and eukaryotes share a high degree of conservation that have been studied to date (Huang *et al*., 2008). Several insects have been shown to exhibit up-regulation of hsp70 mRNA levels in response to cold and heat shock, density, starvation, and diapause (Sinclair *et al*., 2007). The current investigation found that, in comparison to the control group, exposure to 1/2 and 1/4 LC_{50} of *Chrysomya marginalis* maggots extract resulted in significantly higher levels of HSP 70 gene expression.

In current study, comparing the exposed group to the group under control, a significant down-regulation ($P<0.05$) in the gene expression levels of Cu-Zn SOD was observed at 1/2 and 1/4 LC_{50} of *Chrysomya marginalis* maggot's extract. This disagree with Rossbach *et al.* (2020) who reported that Silver nanoparticles Ag-NPs have been shown to initiate the transcriptional activity of sod-1 (also called Cu–Zn SOD) in *Caenorhabditis elegans*, and to upregulate Sod2 (also called Mn–SOD) in *Drosophila melanogaster*, which also has an impact on hemocytes. Nair *et al.* (2013) reported that Ag-NPs induce both SOD genes in the marine larvae of *Chironomus riparius*. Our results demonstrate that snails *T. pisana* treated with extract from *Chrysomya marginalis* maggots developed antioxidant defense system. The biochemical analysis of this common snail can yield results that are helpful in determining how best to use antioxidant responses as biomarkers to track the effects of maggot extract.

Conclusion

Chrysomya marginalis maggots extract inspire relatively high toxic effects against land snail *T. pisana*. The tested extract resulted in various biochemical, antioxidant and gene expression disorders within exposed snails. The tested extract demonstrated toxic effects on *T. pisana*. Using this natural product instead of dangerous chemical mollucicides is thought to be an alternate and successful method of controlling land snails.

Conflict of interest

The authors declare no competing interests.

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