CATRINA (2023), 28 (1):-53-60

Evaluating the Effectiveness of *Acremonium* sp. Protease as a Natural Molluscicide Agent: A Toxicological and Histological Investigation on Land Snails

Eman M. Abd-ElAzeem*, Asmaa M. A. El-Sayd, Fatma I. ElAkhrasy
Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

ABSTRACT

Our goal nowadays is to reduce the use of chemical pesticides and replace them with biocides, aiming to mitigate environmental pollution and minimize the impact of climate change. In this study, we successfully demonstrated the toxic effects of *Acremonium* sp., a bioagent that produces the protease enzyme, in comparison to the conventional pesticide methomyl, on the land snails *Monacha cartusiana* and *Theba pisana*. We also examined the corresponding histological responses of these snails under controlled laboratory conditions. The mortality percentages increased with increasing the concentration of protease derived from *Acremonium* sp. and the duration of exposure. For the highest concentrations (20%) of *Acremonium* sp. and (2%) of methomyl, the mortality rates after 96 hrs were 53.33%, 26.67%, 93.33%, and 86.67% for *M. cartusiana* and *T. pisana*, respectively, using the poisonous baits technique. Conversely, the dipping technique yielded mortality rates of 100%, 46.67%, 100%, and 100%, respectively. The dipping technique proved to be more effective than the poisonous baits technique, with *M. cartusiana* displaying greater sensitivity compared to *T. pisana*. Histological examinations of snails exposed to methomyl revealed significant alterations in the digestive glands, resulting in the loss of their normal architecture. This damage subsequently impaired feeding and movement activities, potentially leading to snail mortality. Microbial agents demonstrated promising results as molluscicides, providing a cost-effective and superior alternative to chemical-based molluscicides for managing snail pests in Egyptian agriculture. Notably, *Acremonium* sp. induced various histopathological disorders in the treated snails, distinguishing it from methomyl in terms of its impact on snail health.
Keywords: *Acremonium* sp.; Histological analysis; Land snails; Methomyl; *Monacha cartusiana*; *Theba pisana*.

INTRODUCTION

Mollusca, the second largest group of invertebrates after Arthropoda, encompass approximately 85,000 living species of molluscs (Rosenberg, 2014). In Egypt, land snails pose a significant threat to agricultural productivity across various provinces. They infest and cause extensive damage to economically important crops such as orchard trees, vegetables, field crops, and ornamental plants (Desoky, 2018). The consumption and destruction of leaves, blooms, flowers, fruits, trunks, limbs, and bark by land-dwelling snails present a constant menace to host plants (Ismail *et al*., 2003). Moreover, the unpleasant odor produced by snails while in motion deters consumption by humans and animals, further impacting the marketability of crops contaminated with snail mucus (Sallam *et al*., 2009; Baker and Hawke, 1990; Ittah and Zisman, 1992). Given these challenges, effective control measures for snails are becoming increasingly crucial. The use of alternative molluscicides that are safe, environmentally friendly, and cost-effective is imperative, as they are less toxic to non-target organisms and do not contribute to environmental contamination (Gabr *et al*., 2006). In recent years, the application of microbial agents, specifically bacteria and fungi, for the biological control of land snails has emerged as a promising alternative to conventional pesticide-based approaches. This approach gained significant attention as it offered a different method to manage land snail populations without relying on chemical solutions (Genena and Mostafa, 2008). One significant element of biological control approaches was pathogenic microorganisms (Moussa *et al*., 2014). According to Wenzel Rodrigues *et al.* (2016), it is not very hazardous to the environment or ecosystem, there is little chance that target pests will develop a resistance to it, and it is also inexpensive to reproduce and register.

Fungal biopesticides can serve as a substitute method for managing terrestrial gastropods. (Hendawy *et al*., 2015). Metabolites derived from microorganisms, used as molluscicide agents for controlling land snails, offer a safe, effective, affordable, and easily accessible alternative. Furthermore, they contribute to reducing environmental pollution caused by conventional pesticides (El-Sayd, 2017). The fungus *Acremonium* sp. possesses the capability to generate enzymes that can degrade the cuticles of pests, including protease and lipase. As a result, it can play a crucial role in pest control, offering a safe method that helps in reducing environmental pollution. (Abd-ElAzeem *et al*., 2019).

The digestive gland in molluscs serves multiple functions, including extracellular digestion of food, absorption of essential nutrients, storage of lipids, glycogen, and minerals, as well as detoxification of harmful substances (Boer and Kits, 2005; EL-Alakhrasy, 2017). El-Sayd (2017) demonstrated that oral admini-stration of *Aspergillus flavus* resulted in significant structural alterations in the digestive gland of *M. cartusiana* snails, deviating from its normal appearance. Consequently, the treated snails exhibited reduced feeding,

* *Corresponding author: emo1azem@yahoo.com*

loss of appetite, and sluggish movement. Therefore, the primary objective of this research is to conduct a comparative analysis of the toxicological and histological effects of methomyl and *Acremonium* sp. on the digestive gland tissues of *M. cartusiana* and *T. pisana* under controlled laboratory conditions.

MATERIALS AND METHODS

Tested snails

Adults of the glassy clover snail, *M. cartusiana* and the small garden snail, *T. pisana*, around the same age and size, collected at dawn or early evening from infested fields crops at Banadf village, Meniet El-Kamh district, Sharkia Governorate, Egypt. Snails were introduced into muslin bags and directly transferred to the laboratory and kept in a glass container (30×30×50 cm) containing moist clay soil and covered with muslin cloth for preventing snails escaping at (20 ± 3ºC) and (80 ± 5% R.H.) through the experimental period as described by (Ghamry *et al*., 1993). Snails were fed daily on fresh leaves of lettuce for 14 days to acclimatization. Dead and unhealthy snails were removed and only healthy ones were used in the experiments (Godan, 1983).

Toxicological studies

Tested material

Methomyl: (Agrinate 24% SL. 1liter/ feddan), Carbamate is recommended molluscicide. Chemical name: S-methyl N (methylcarbamoyloxy) thioacetimidate

Tested organism

Acremonium sp. EZ1 isolated from a dead spiny bollworm (Abd-ElAzeem *et al*., 2019) was used through this investigation. Its identification was confirmed using 18s rRNA and its accession number MN25101.

Acremonium sp. propagation

Spore suspensions were obtained by washing the 7 days old slant of tested fungal isolates (Dulmage *et al*., 1971; MohdSalleh and Lewis 1983) then inoculated a 100 ml of Czapeck's Dox's agar medium (Oxoid, 1982) composed of (g/l), 20 sucrose, 2.0 $NaNO_3$, 1.0 KH_2PO_4, 0.5 $MgSO_4.7H_2O$, 0.5 KCl, 20.0 agar-agar and dissolved in 1L tap water, pH 5.0 in a 250 ml Erlenmeyer flask with each suspension. The inoculated broth was incubated at 30^{o}C for 7 days (each ml contains a concentration of 5x10^7 spores of *Acremonium* sp.).

Protease production evaluation

For protease production, *The Acremonium sp.* isolate was grown in 250ml Erlenmeyer flask containing 100 ml of modified Czapek's broth medium. The flask was sterilized and inoculated with 1ml of the inoculum of fungal spores and then incubated at 28±2ºC for 7 days. After the incubation period, the contents of the flasks were centrifuged and the supernatant (cell-free filtrate, CFF) was used as crude enzyme (Sahab *et al*. 2019).

To determine the activity of the protease enzyme in the tested isolate, Dox media method was used as recommended by Ammar *et al*. (1991). In this method, we replaced $NaNO_3$ with 0.2% gelatin. The prepared medium was poured into individual Petri-dishes. After allowing the plates to solidify, wells on each agar plate were made. Then, 0.1 ml (100 µl) of the cell-free filtrate (CFF) obtained earlier was added to each well using the well diffusion technique. The plates were then incubated at a temperature of 28 ±2°C for 24 hours. Following incubation, the clear zone appeared surrounding each well was measured. The diameter of this zone indicates the extent of protease enzyme hydrolysis on media containing protein, as described by Reda *et al*. (2013) and Abd-ElAzeem *et al*. (2019).

Acremonium protease bioassay on land snails

The solutions preparation

To prepare the required concentrations for the *Acremonium* protease bioassay on land snails, 20 ml of *Acremonium* sp. culture was added to 80 ml of sterile water to obtain a 20% concentration. This 20% solution was then further diluted to achieve concentrations of 10% and 5%. In a similar manner, three concentrations of methomyl (0.5%, 1%, and 2%) were prepared. using the same method.

Poisonous Bait Technique

For the poisonous bait technique, the appropriate amount of each compound was incorporated into bran bait. To conduct the experiment, three plastic boxes with a capacity of 3/4 kg were utilized for each concentration. In each box, five grams of the respective bait mixture was evenly spread. As a control treatment, bran bait without any added compounds was used.

Dipping technique

In the dipping technique, fresh lettuce leaves of equivalent size were immersed in a solution containing crude protease from *Acremonium* sp. and methomyl for a duration of 30 seconds. Afterward, the treated lettuce leaves were left to dry before being offered to the snails. As for the control treatment, fresh lettuce leaves were dipped in distilled water without any added compounds.

Mortality Assessment Technique

In the preceding two techniques, ten adult snails were introduced into plastic boxes. The boxes were then covered with muslin cloth and securely fastened with rubber bands to prevent snail escape. To assess mortality, stainless steel needles, following the methodology outlined by El-Okda (1981), were employed. Snails showing no contraction were recorded as deceased, and the deceased snails were subsequently removed from the boxes.

Mortality percentages were calculated after 24, 48, 72 and 96 hrs. and corrected by Abbott's formula (1925). as follows:

Mortality percentages=

$$1-\frac{n\ in\ T\ after\ treatment}{n\ in\ Co\ after\ treatment}\ X100$$

Where, T is the number of alive snails in the treated box; Co, is the number of alive snails in control.

Histological Studies

For the histological studies, two groups of tested land snails, each consisting of 5 snails, were utilized. Similar pieces of fresh lettuce leaves were dipped in a 2% methomyl solution and a 20% *Acremonium* sp. solution for duration of 30 seconds. After 48 hrs, the snails were allowed to relax in warm water. Once the snails released the biomass from their shells, the shell was separated from the remaining biomass. The digestive gland was then isolated from the other organs and fixed in 10% formalin, following the protocol outlined by Runham and Hunter (1970).

To prepare the glands for histological studies, the tissues were dehydrated using an ascending series of ethyl alcohol. Subsequently, they were cleared in xylene for 2 minutes and then immersed in three changes: the first change consisted of xylene and wax in a 1:1 ratio, while the second and third changes involved wax for 1/2 h each. Embedding in paraffin and blocking were performed under vacuum conditions. Serial transverse sections with a thickness of 6 µm were mounted on clean slides without using any adhesive material. For general histological studies of the tested organs, Ehleish's hematoxylin and eosin staining method, as described by Drury and Wallington (1980), was employed. The sections were then mounted and covered with a glass cover. Histological sections were captured using a photo-automated camera.

Statistical analysis

The obtained results were subjected to statistical analysis using the CoStat computer program, developed by Cohort Software (CoStat program, 2005).

RESULTS

Toxicological studies

Poisonous baits technique

The results presented in Table (1) provide an overview of the mortality percentages observed in the land snails, *M. cartusiana* and *T. pisana*, following exposure to various concentrations of *Acremonium* sp. and methomyl for different time periods. Results demonstrate the molluscicidal activity of methomyl within 24 hrs post-treatment at all three concentrations tested. For *M. cartusiana*, the mortality percentages were recorded as 13.33%, 33.33%, and 66.67% for the concentrations of 0.5%, 1.0%, and 2.0%, respectively. In the case of *T. pisana*, the mortality percentages were 6.67%, 26.67%, and 53.33% for the same concentrations. Additionally, after 24 hours of exposure to *Acremonium* sp., the mortality percentages for *M. cartusiana* were 6.67% and 13.33% when subjected to 10% and 20% concentrations, respectively. As the exposure time increased, the mortality percentages for *M. cartusiana* reached 53.33% and 93.33% after 96 hrs post-treatment for the highest concentration of *Acremonium* sp. and methomyl, respectively. Similarly, for *T. pisana*, the mortality percentages increased to 26.67% and 86.67% after 96 hrs post-treatment with the highest concentration of *Acremonium* sp. and methomyl, respectively. These results demonstrate the varying effects of *Acremonium* sp. and methomyl on the mortality rates of *M. cartusiana* and *T. pisana*, revealing a significance clear trend of increasing mortality percentages with higher concentrations and longer exposure durations.

Dipping technique

Data presented in Table (2) revealed that (20%) *Acremonium* sp. and (2%) methomyl had a high effect against *M. cartusiana* snails after 24h. post treatment where reach 73.33 and 80.00 %, respectively. The effect of *Acremonium* sp. on *T. pisana* snails was the lowest one where gave 6.67% after 24hrs. post treatment for 20% percentage. The mortality percentages increased by increasing the concentration and exposure period until reaching 100 and 100% for *M. cartusiana* snails and 46.67and 100% for *T. pisana* snails after 96h. post treatment for (20%) *Acremonium* sp. and (2%) methomyl. Data reported that a high significance difference between the concentration in tested snail by time elapsing.

Histological Studies

The control digestive gland

In both of the tested land snails, a bilobed tubuloacinar gland was observed in the dorsal region on both sides of the stomach. This gland, known as the digestive gland, is comprised of spherical digestive tubules that are separated by loose connective tissue. Additionally, each tubule is surrounded by a circular muscle layer. Upon closer examination of the epithelium within the digestive gland tubules, various distinct cell types were identified. These include digestive cells, calcium cells, and excretory cells. For a visual representation, please refer to Figure (1A and 1B).

The treated digestive gland

After treatment by methomyl assessed *T. pisana* the basement membrane had lysis and dissection occur in various places in the digestive gland tissue where the connective tissue was degenerated and the lumen dilation leading to fusion together (Fig. 2C). However, when *M. cartusiana* is treated with methomyl it results in the appearance of vacuole in the gland tissue, where the lumen is dilated and widened compared with the reference untreated snails. The connective tissue separated from the gland tubules dissected and degeneration appeared in different gland cells (Fig. 2 D).

The result in the case of using *Acremonium* sp. with *M. cartusiana* has the same effect in *T. pisana* as the connective tissue dissected and the basement membrane lysis because of the proteolytic effect of the protease enzyme. Also, the vacuolization happened with the appearance of degeneration. The lumen loses its regular shape and fuses together Figure (3 E and F).

The *Acremonium* sp. treatment on *M. cartusiana* the lumen fusion also occurs with dissected connective tissue and the basement membrane degenerate. More vacuoles appear all over the gland tissue.

Table (1): Comparative analysis of mortality percentages in *M. cartusiana* and *T. pisana* snails exposed to *Acremonium* sp. and methomyl by poisonous baits technique at different concentrations and time intervals.

Comparison category	Conc. used (%)	Mortality percentages at different periods (hrs)							
		24		48		72		96	
		Tested land snail							
		M. cartusiana	*T. pisana*	*M. cartusiana*	*T. pisana*	*M. cartusiana*	*T. pisana*	*M. cartusiana*	*T. pisana*
Control	**0.0**	00.00^{d}	00.00^{c}	00.00^{e}	00.00^{d}	00.00^{d}	00.00^{e}	00.00^{g}	00.00^{e}
	5.0	0.00^{d}	0.00^{c}	0.00^{e}	0.00^{d}	6.67^{d}	0.00^{e}	13.33^{f}	0.00^{e}
***Acremonium* sp.**	**10.0**	6.67^{cd}	0.00^{c}	13.33^{c}	0.00^{d}	26.67^{c}	13.33^{d}	26.67^{e}	13.33^{d}
	20.0	13.33^{c}	0.00^{c}	33.33^{c}	6.67^{cd}	53.33^{b}	26.67^{c}	53.33^{c}	26.67^{c}
	0.5	13.33^{c}	6.67^{c}	26.67^{c}	13.33^{c}	33.33^{c}	26.67^{c}	40.00^{d}	33.33^{c}
Methomyl	**1.0**	33.33^{b}	26.67^{b}	46.67^{b}	40.00^{b}	53.33^{b}	46.67^{b}	66.67^{b}	60.00^{b}
	2.0	66.67^{a}	53.33^{a}	73.33^{a}	66.67^{a}	80.00^{a}	73.33^{a}	93.33^{a}	86.67^{a}
***p* value**		0.0001^{***}	0.0001^{***}	0.0001^{***}	0.0001^{***}	0.0001^{***}	0.0001^{***}	0.0001^{***}	0.0001^{***}
L.S.D.$_{0.05}$		9.86	7.64	9.86	8.82	10.8	9.86	10.81	9.87

Data with different letters, per column for each time exposure, are significantly different; ***, high significant differences.

Table (2): Comparative analysis of mortality percentages in *M. cartusiana* and *T. pisana* snails exposed to *Acremonium* sp. and methomyl, by dipping techniques, at different concentrations and time intervals

Comparison category	Conc.used (%)	Mortality percentages at different periods (hrs)							
		24		48		72		96	
		Tested land snail							
		M. cartusiana	*T. pisana*	*M. cartusiana*	*T. pisana*	*M. cartusiana*	*T. pisana*	*M. cartusiana*	*T. pisana*
Control	**0.0**	00.00^{e}	00.00^{d}	00.00^{e}	00.00^{d}	00.00^{e}	00.00^{f}	00.00^{e}	00.00^{f}
	5.0	20.00^{d}	0.00^{d}	20.00^{d}	0.00^{d}	26.67^{d}	13.33^{e}	26.67^{d}	13.33^{e}
***Acremonium* sp.**	**10.0**	33.33^{c}	0.00^{d}	40.00^{c}	6.67^{d}	46.67^{c}	26.67^{d}	53.33^{c}	26.67^{d}
	20.0	73.33^{a}	6.67^{d}	86.67^{a}	20.00^{c}	100^{a}	40.00^{c}	100^{a}	46.67^{c}
	0.5	26.67^{cd}	26.67^{c}	33.33^{c}	26.67^{c}	40.00^{c}	33.33^{cd}	53.33^{c}	46.67^{c}
Methomyl	**1.0**	46.67^{b}	40.00^{b}	66.67^{b}	53.33^{b}	73.33^{b}	66.67^{b}	86.67^{b}	80.00^{b}
	2.0	80.00^{a}	73.33^{a}	93.33^{a}	86.67^{a}	100^{a}	93.33^{a}	100^{a}	100^{a}
***p* value**		0.0001^{***}	0.0001^{***}	0.0001^{***}	0.0001^{***}	0.0001^{***}	0.0001^{***}	0.0001^{***}	0.0001^{***}
L.S.D.$_{0.05}$		10.81	8.82	11.96	9.87	8.81	10.79	8.8	9.86

Data with different letters, per column for each time exposure, are significantly different; ***, high significant differences

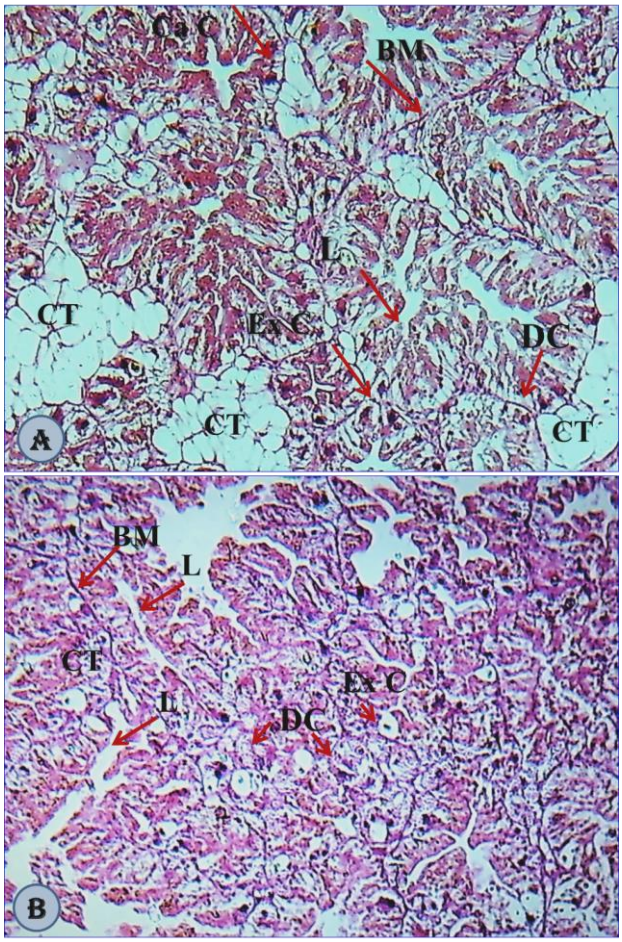

Figure (1): Photomicrographs of digestive gland cross-sections in control snails, A, *T. pisana* and B, *M. cartusiana*: Comparative analysis of connective tissue (CT), basement membrane (BM), lumen (L), calcium cells (Ca C); digestive cells DC); and excretory cells (Ex C). 100X Magnification (stained with HX and E).

DISCUSSION

Toxicological studies resulted in Tables (1 and 2) proved that *Acremonium* sp. had a proteolytic activity and caused a high mortality effect on both tested snails. The same results investigated by (Nada and Abdel-Azeem, 2005) recorded the mortality effect of protease produced from entomopathogenic fungi, *Paecilomyces violacea* and *Paecilomyces variotii* against *Pectinophora gossypiella*, while Jain *et al*. (2012) recorded the damaged effect of protease produced from *Acremonium* sp on the insect cuticle. Also, Reda *et al*. (2013) studied pathogenicity of protease and lipase enzymes produced from *Streptomyces vinaceusdrappus* against the *P. gossypiella*.

In a study conducted by El-Akhrasy (2017), the chemical compound Neomyl was investigated for its effectiveness against *M. cartusiana* snails. The study found that Neomyl exhibited the highest efficacy in controlling adult snails compared to Amino and Vertimic. Furthermore, Bayoumi (2018) examined the impact of various plant parts, biocides, and chemical compounds as toxic baits against *M. cartusiana* under laboratory conditions. Among the tested compounds, Neomyl demonstrated the highest effectiveness, resulting in 100% mortality after 7 days at five different concentrations. Following Neomyl, Gastrotox showed significant efficacy. On the other hand, Urea exhibited the least effectiveness, with mortality rates of 26.6% and 46.6% for the highest concentrations of 3 and 4%, respectively. These findings highlight the potential of Neomyl and Gastrotox as effective compounds for controlling *M. cartusiana* snails, while suggesting limited effectiveness of Urea.

The histological study showed three cell types observed in the epithelium of the digestive gland tubules. These cells are: digestive cells, calcium cells and excretory cells are in harmony with several studies that have described the morphology and histology of the hepatopancreas of the land snail *Monacha* sp. as (Parvate and Thayil, 2017); *Eobania vermiculata* (Hemmaid *et al*., 2017). Abd El-Atti *et al*. (2020) revealed that the digestive gland of untreated *M. cartusiana* consists mainly of three cell types that observed previously (digestive, excretory and calcium cells).

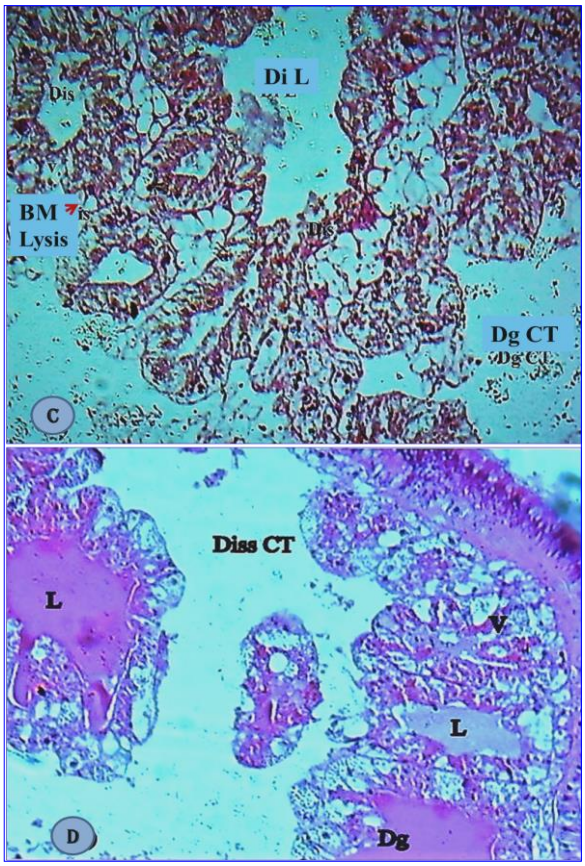

Figure (2): The photomicrograph of the digestive gland cross-section in *T. pisana* (C) and *M. cartusiana* (D) treated with methomyl. C, showed degeneration of connective tissue (Dg CT); basement membrane lysis (BM lysis); dilatation of the lumen (Di L); dissection (Diss.), and the presence of vacuoles (V). X100, magnification (stained with HX and E). Similarly, in *M. cartusiana* (D), treated with methomyl, the image displayed degeneration (Dg), a visible lumen (L), dissection of connective tissue (Diss. CT), and the presence of vacuoles (V). X100, magnification (stained with HX and E).

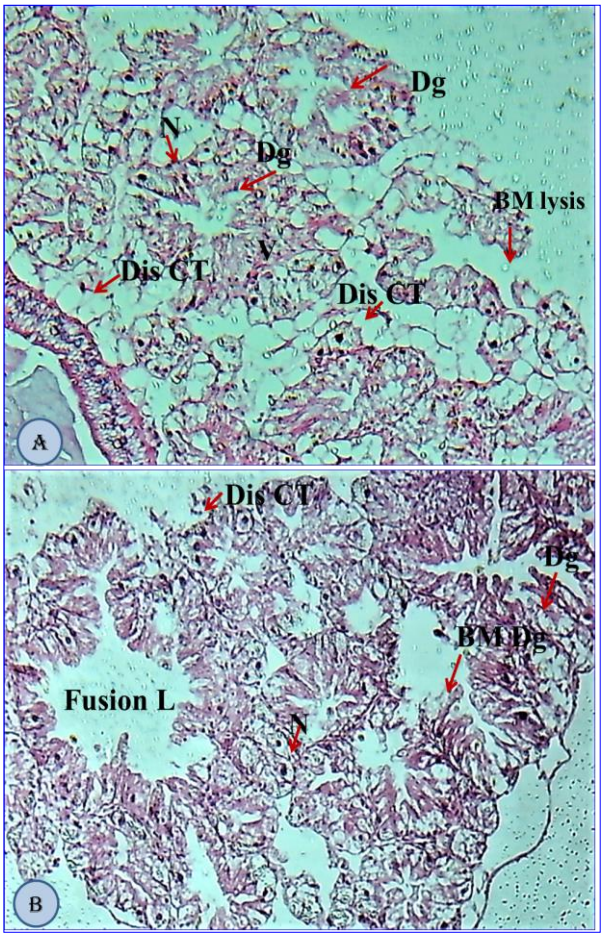

Figure (3): Photomicrographs of digestive gland cross-sections in treated snails, A, *T. pisana* and B, *M. cartusiana:* Comparative analysis showing dissociated connective tissue (Dis CT), basement membrane degenerated (BM lysis), vacuole (V); nucleus (N); digestive cells DC). 100X Magnification (stained with HX and E).

A histological section of the control digestive gland for land snail M. cartusiana was consists of digestive tubules lined with three different simple epithelium cells resting on a thin basement membrane. These cells were seen and differentiated into digestive, excretory, and calcium cells (Gaber *et al*., 2022).

Kandil *et al.* (2014) and El Akhrasy (2017) studied the histopathological effect of LC_{50} of methomyl as partial as well as complete disappearance, necrosis and atrophy of mucus glandular tissue of *E. vermiculata* and *M. cartusiana*. It also caused focal necrosis, especially with degeneration. The treated digestive gland showed rupture in the digestive envelope of tubules that disrupted columnar digestive cells and appeared without its content (Ali and said 2019; Peña *et al.*, 2017). Hemmaid *et al.* (2017) illustrated that the application of 1/2 LC_{50} of Neomyl resulted in cytoplasmic degeneration in the digestive cells of the land snail *Eobania vermiculata* and LC_{50} of Neomyl showed that the digestive cells were widely degenerated. Abdel-Rahman (2020) cleared that the tissue of the hepatopancreas of *Monacha* sp. exhibited marked histological alterations, Neomyl showed cell's necrosis, degeneration of the digestive tubules and Neomyl precipitation. The alterations in the digestive gland are shown as remarkable degeneration and rupture for digestive and execratory cells and, vacuolization and hemolytic infiltration (Gaber *et al.*, 2022).

As a result of the proteolytic effect of the protease enzyme that The *Acremonium* sp. produced. The digestive tracts of African giant land snail *Archachatina marginate* are provided with multiples of enzymes like cellulase, trypsin, lipase, α-glucosidase and protease (Adedire *et al.*, 1999). The presence of protease and lipase in the gut regions suggests that the experimental snails consumed fatty and protic food substances along with their normal carbohydrate diets. The presence of protease and lipase in the gut regions explained as the experimental snails consumed fatty and proteinoids food substances and their normal carbohydrate diets.

CONCLUSION

In conclusion, this study aimed to compare the effectiveness of *Acremonium* sp. and different concentrations of methomyl as toxic baits for controlling *M. cartusiana* and *T. pisana* snails under laboratory conditions. The results revealed that *Acremonium* sp. showed varying levels of toxicity, with higher concentrations leading to increased mortality rates in both snail species. Methomyl exhibited dose-dependent toxicity, with higher concentrations resulting in higher mortality percentages. The study also included photomicrographs of the digestive gland cross-sections, highlighting the structural components of the snails' digestive systems. Overall, these findings suggest the potential of *Acremonium* sp. and methomyl as effective toxic baits for snail control, with implications for pest management strategies. These findings highlight the potential of *Acremonium* sp. in comparable to methomyl as effective toxic baits for snail control. The study contributes to the existing knowledge on the use of these substances for managing snail populations, providing valuable information for further research and potential practical applications in pest management strategies. However, replace chemical pesticide by biopesticide aiming to mitigate environmental pollution and minimize the impact of climate change is nowadays the target for the ecological impacts in natural conditions.

REFERENCES

ABBOTT, W.S. 1925. A method of computing the effectiveness of insecticides. J Econ Entomol 18:265-267.

ABD EL-ATTI, M.S., A.M. KHALIL, ELSHEAKH, A.A. AND W.S. ELGOHARY. 2020. Biological control of *Monacha cartusiana* "glassy clover land snails" by microbial biopesticides Biozed and Biogard, using bait technique. Biocatalysis and Agricultural Biotechnology 25:101572. doi: 10.1016/j.bcab.2020.101572.

ABD-ELAZEEM, E.M., A.Z.E. WARDA. AND M.S. HEND. 2019. Biological activities of spores and metabolites of some fungal isolates on certain aspects of the spiny bollworms *Earias insulana* (Boisd.) (Lepidoptera: Noctuidae). Egyptian J of Biological Pest Control 29(90):1-7. https://doi.org/10.1186/s-41938-019-0192-y

ADEDIRE, C.O., E.A. IMEVBORE, E.O. EYIDE AND W.I. AYODELE. 1999. Aspects of digestive physiology and the complementary roles of the microbial enzymes in the intestinal tract of the giant land snail, *Archachatina marginate* J Technosci 3: 6-13.

AMMAR, M.S., S. LOUBOUDY. AND U.M. ABDUL-RAOUF. 1991. Purification and properties of mesophilic proteases produced by *Bacillus anthracis*; S-44 isolated from a temple in Aswan. A1-Azhar Bull Sci 2(1): 325-338.

BAKER, G.H. AND B.G. HAWKE. 1990. Life history and population dynamics of *Theba pisana* (mollusca: Helicidae), in a cereal pasture rotation. J of Applied Ecology 27:16-29.

BAYOUMI, S.M. 2018. Environmental studies on certain land snails under different conditions at Sharkia Governorate. M.Sc. Thesis, Institute of Environ-mental studies, and Research Ain Shams Univ183 pp.

BOER, H. H. AND K.S. KITS. 2005. Histochemical and ultrastructural study of the alimentary tract of the freshwater snail *Lymnaea stagnalis.* J of Morphology 205 (1):97-111.

COSTAT STATISTICAL SOFTWARE 2005. Microcomputer program analysis version, 6. 311. CoHort Software, Monterey, California

DESOKY, A.S.S. 2018. Identification of terrestrial gastro-pods species in Sohag Governorate, Egypt. Archives of Agriculture and Environmental Science, 3(1): 45-48, DOI: 10.26832/24566632.2018.030105.

DRURY, R.A.B. AND E.A. WALLINGTON. 1980. Carl-eton's Histological Techniques. 5th Edition, Oxford University Press, New York, 195.

DULMAGE, H.T., O.P. BOENING, C.S. REHNBORC AND G.D. HANSEN.1971. A proposed standardized bioassay for formulation of *Bacillus thuringiensis* based on the international unit. J Invertebr Pathol 18:240-245.

ELAKHRASY, F.I.H. 2017. Efficiency of certain chemicals and biocides on cells and tissues of some land snails infesting vegetable crops at Sharkia governorate. Ph D Thesis Fac Sci Zagazig Univ 221pp.

El-OKDA, M.M.K. 1981. Locomotion activity and infestation abundance of certain terrestrial mollusca in fruit orchards Alexandria province, A.R.E. Proc. 4th Arab pesticide conf Tanta University 11: 279-288.

El-SAYD, M.A.E. 2017. Studies on the biocontrol of certain land snails by some microbial isolates from different localities in Egypt. Ph D Thesis Fac Sci Zagazig Univ Egypt 186 pp.

GABER, O.A., A.E.A. ASRAN, H.M.K. ELFAY-OUMI, G. EL-SHAHAWY, F.K. KHIDER, H. ABDEL-TAWAB AND K.A. MAHMOUD. 2022. Influence of Methomyl (Copter 90%) on certain biochemical activities and histological structures of land snails *Monacha cartusiana.* Saudi J of Biological Sciences 29 (4): 2455-2462.

GABR, W.M., A.S. YOUSSEF AND F.K. KHIDR. 2006. Molluscicidal effect of certain compounds against two land snails *Eobania vermiculata* under laboratory and field conditions. Egypt J Agric Res 84(1): 43-50.

GENENA, M.A.M. AND F.A.M. MOSTAFA. 2008. Impact of eight bacterial isolates of *Bacillus thuringiensis* against the two land snails *Monacha cantiana* and *Eobania vermiculata* (Gastropoda: Helicidae). J Agric Sci Mansoura Univ 33: 2853-2861.

GHAMRY, E.M., H.I. EL-DEEB AND S.M. ABD EL-AAL. 1993. Efficacy of certain pesticides against some land snails under field conditions of Sharkia Governorate. Egypt J Appl Sci 8 (6): 764 -774.

GODAN, D. 1983. Pest slugs and snails, biology and control. Verlage Berlin 422 p.

HEMMAID, K.Z., S.A.A. ELMASRY. AND F.I. EL-AKHRSAY. 2017. Ultrastructural alterations in cells of the digestive gland of *Eobania vermiculata* (Muller) treated with three chemical compounds. Middle East J Appl Sci 7(3): 595-612.

HENDAWY, A.S., S.K. EL-FAKHANNY AND M.A. SAMY 2015. Laboratory and field evaluation of molluscicidal activity of native biological isolates compared to an insecticide against the land snails, *Monacha* spp. on lettuce and cabbage plantations. Egypt J Biol Pest Control 25(3): 675-678.

ISMAIL, A.A., S.A.A. EL-MASSRY, M.M. KHATTAB AND A. HASSAN. 2003. Daily activity and damage caused by *Eobania vermiculata* Müller (Gastropoda) in citrus orchards. Egyptian J of Agric Research 18: 1-6.

ITTAH, Y. AND U. ZISMAN. 1992. Evaluation of volatile allyl alcohol derivatives for control of snails on cut roses for export. Pest Science 35:183-186.

JAIN, P., V. AGGARWAL, A. SHARMA, RAM KUMAR AND R. M. PUNDIR. 2012. Isolation, production, and partial purification of protease from an endophytic *Acremonium* sp. J Agric Technol 8(6): 1979-1989.

MOHD-SALLEH, M.B. AND L.C. LEWI. 1983. Comparative effects of spore-crystal complexes and thermostable exotoxins of six subspecies of *Bacillus thuringiensis* against *Ostrinia nubilalis* (Lepidoptera: Pyralidae). J Invertebr Pathol 41:336-340.

MOUSSA, S., H. ABOUELMAATY, H.A. HAMADA AND E.A. HEMIEDA. 2014. Evaluation of *Bacillus thuringiensis* Cry 1 ca strain and *Metarhizium anisopliae* fungus against potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). Egypt J Biol Pest Control 24 (2): 515-521.

NADA, M.A. AND E.M. ABD-EL-AZEEM. 2005. Effect of two types of entomopathogenic fungi, *Paec-*

ilomyces violacea and *Paecilomyces variotii* on biological aspects of pink bollworm *Pectinophora gossypiella* (Saunders). Egypt J Appl Sci 20 (12): 691-698.

OXOID, L. 1982. The Oxoid manual of culture media, ingredients and other laboratory services, 5th eds. Turnergraphic Ltd. England.

PARVATE, Y.A. AND L. THAYIL. 2017. Toxic effect of clove oil on the survival and histology of various tissues of pestiferous land snail *Lissachatina fulica*. J Exp Agric Sci 5(4):492-505.

PEÑA, S.C., G.N. POCSIDIO AND E.L. Co. 2017. Histological responses of golden apple snail (*Pomacea canaliculata*) to copper. Philippine J Sci 146(3) :315-321.

REDA, F.M., M.A. NADA AND ABD-ELAZEEM 2013. Biological activity of some actinomycetal and bacterial isolates on certain aspects of the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). Egypt J Biol Pest Control 23(2):297-303.

ROSENBERG, G. 2014. A new critical estimate of named species-level diversity of the recent mollusca. American Malacological Bulletin 32(2):308-322.

RUNHAM, N.W. AND P.J. HUNTER. 1970. Terrestrial slugs. Hutchison University Library, London 185 pp.

SAHAB A. F., N. M. SIDKEY, N. N. ABED AND A. MOUNIR. 2019. In-vitro Effect of Different Physiological and Biochemical Parameters on Two Fungal Isolates Involved Biodeterioration of Egyptian Ancient Old Documents, Egypt. J Microbiol 54: 1-12.

SALLAM, A.A.A., S.A. EL-MASSRY AND I.N. NASR, 2009. Chemical analysis of mucus from certain land snails under Egyptian conditions. Archives of Phytopathology and Plant Protection 42: 874-881.

WANZEL, R.I.M., M.R. FORIM, M.F.G.F. DA SILVA, J.B. FERNANDES AND A.B. FILHO. 2016. Effect of ultraviolet radiation on fungi *Beauveria bassiana* and *Metarhizium anisopliae*, pure and encapsulated and bio-insecticide action on *Diatraea Saccharalis*. Advances in Entomol 4(3): 151-162.

تقييم فاعلية انزيم البروتييز الناتج من *Acremonium* sp. كمبيد حيوي: دراسة سمية ونسيجية على القواقع الارضية

إيمان محمد عبد العظيم، أسماء محمد عبد المجيد السيد، فاطمة إبراهيم الأخرسي
معهد بحوث وقاية النباتات، مركز البحوث الزراعية، الدقي، جيزة، مصر

الملخــص العربي

هدفت هذه الدراسة التقليل من التلوث البيئي وتأثير التغيرات المناخية، ولذلك تم تقييم تأثيرات السمية لـ *Acremonium* sp وهو فطرله القدرة علي انتاج إنزيم البروتييز والذي يمكن استخدامه كمبيد حيوي للقواقع الارضية. و تم مقارنته بالمبيد الكيميائي التقليدي والمعروف باسم "الميثوميل".وتمت الدراسة على كلا من القواقع الأرضية *Monacha cartusiana* و *Theba pisana*. كما تم فحص انسجة هذه القواقع بعد المعالجة بالمبيد الحيوي و الكيميائي تحت ظروف معملية محكمة. اثبتت الدراسة ان ارتفاع نسب الموت يحدث بزيادة تركيز انزيم البروتيز والمنتج من *Acremonium* sp. وكذلك زيادة مدة التعرض له. واوضحت الدراسة ان لأعلى التركيزات 20%من *Acremonium* sp و2% من الميثوميل، ادت الي ارتفاع معدلات الموت بعد 96 ساعة كانت 53.33% و 26.67 % و 93.33% و 86.67% ل *T. pisana* و *M. cartusiana*. على التوالي، وذلك باستخدام تقنية الطعوم السامة. بالمقارنة، أظهرت تقنية الغمرمعدلات موت اعلي وسجلت النسب الاتية: 100%و 46.67 % و 100 % و100% على التوالي. كما أثبتت الدراسة احصائيا ان تقنية الغمرهي أكثر فعالية من تقنية الطعوم السامة، حيث أظهرت *M. cartusiana* حساسية أكبر مقارنة بـ *T.pisana*. . و أظهرت الفحوصات النسجية للقواقع التي تعرضت للميثوميل تغييرات ملحوظة في الغدد الهضمية، مما أدى إلى فقدان هياكلها الطبيعية. وقد أثر هذا الضرر على الأنشطة الغذائية والحركية، مما قد يؤدي هذا إلى موت القوقع. وأظهرت الدراسة ان العوامل الميكروبية لها نتائج واعدة كمبيدات للرخويات، مما يوفر بديلاً فعالاً، من حيث التكلفة و التاثير البيئي، من المبيدات الكيميائية لمكافحة القواقع في الزراعة المصرية. و جدير بالذكر أن *Acremonium sp*. أدى إلى اضطرابات نسجية متنوعة في القواقع التي تمت معالجتها، مما يميزه عن الميثوميل من حيث تأثيره على صحة القوقع.

.