BF-1790
[1-7]

RESEARCH ARTICLE Indian Journal of Animal Research

Potential of Animal Bile as Bio-molluscicide against Land Snail *Monacha cartusiana*

Mutee Murshed[1], Amin Nagi Al Ansi[2], Osama B. Mohamed[1]

10.18805/IJAR.BF-1790

ABSTRACT

Background: One of the pests that can be detrimental to crops in agroecosystems is the land snail, also known as *Monacha cartusiana*. Molluscicides that are chemical are the first line of defense against land snails; however, because of their toxicity to both land and aquatic life, there has been an increase in interest in the discovery of biological molluscicides that are acceptable and eco-friendly. These molluscicides are a natural and environmentally safe alternative to synthetic poisons.

Methods: The purpose of this study is to examine the efficacy of animal bile liquid against *Monacha cartusiana in vitro*. Molluscicides were tested using a dipping approach on snails isolated from agricultural land. PBS was utilized as a control. Furthermore, four different concentrations of extract (12.5, 25, 50 and 100%) were evaluated across different periods, and changes in snail viability were recorded (5, 10, 20 and 40 minutes). A chemical analysis was carried out using GC-MS to determine the presence of numerous expected active chemical components in animal bile fluids.

Result: The results clear that the bile had significant efficacy in killing snails at 100%, 61%, 41% and 20% after 20 hours. The findings suggest that bile contains potent chemicals and is a promising strategy for molluscicide. More research is needed to identify the specific active compounds found in animal bile, as well as their modes of action and application *in vivo*.

Key words: Bile liquid, Biocontrol, Eco-friendly, *Monacha cartusiana*.

INTRODUCTION

Molluscs are the second-largest invertebrate category after arthropods in the animal kingdom and they make a substantial contribution to the overall biodiversity of the planet (Rosenberg 2014). Vegetables and ornamental plants are typically severely damaged by terrestrial snails, which are typically found in gardens and grassy areas (Capinera and White 2011). Both animals and humans are unable to consume these plants because of the unpleasant odour and taste that are left behind by the mucous secretions that snails leave behind on plants throughout their movements (Abd El-Atti *et al.,* 2019). It has been determined by Iglesia *et al.,* (2003) that snails and slugs are among the most dangerous agricultural pests, as they cause significant economic damage to field crops, vegetables and horticultural plants. Land snails are responsible for the transmission of bacteria, viruses and fungi to plants by the act of scratching plant parts when they are feeding (Raut and Barker, 2002). This affects plants in both a direct and indirect manner. According to Castle *et al.* (2017), the most common approach to controlling land snails comes in the form of chemical molluscicides. According to Khalil (2016), methomyl is the most widely used molluscicide because of its capacity to kill molluscs and its ease of application. This particular molluscicide, on the other hand, is toxic to beneficial invertebrates and poses a threat to humans, other animals, and the environment as a whole (Moustafa *et al.,* 2016). According to Geasa *et al.,* (2013), natural molluscicides are considered to be the most effective alternative to chemical molluscicides. This is because natural molluscicides are less expensive and pose fewer dangers to the general environment. Natural enemies, which include predators, diseases and parasites, have the potential to be a successful technique for the biological control of snails even over the long term. A few years ago, biological treatment with microbial agents against various land snails and slugs gained a lot of interest (Kramarz *et al.,* 2007; Genena and Mostafa 2008; Shahawy 2018).

Several pharmacologically active chemicals have been derived from traditional medicinal plants, according to Yousif *et al.* (2007). The World Health Organization (WHO) estimates that between 65 and 80 percent of the population in developing countries relies on traditional medicine to cover their primary healthcare needs (Kumar *et al.,* 2021). For this reason, a significant amount of work has been put into locating plant items that are safe for the environment, do not contain any poisonous substances and are selectively active for the integrated management of snails. Several of these plant

[1]Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh-11451, Kingdom of Saudi Arabia.
[2] Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, 1145, Kingdom of Saudi Arabia.

Corresponding Author: Mutee Murshed, Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh-11451, Kingdom of Saudi Arabia. Email: mmurshed@ksu.edu.sa

How to cite this article: Murshed, M., Ansi, Al. A.N. and Mohamed, O.B. (2024). Potential of Animal Bile as Bio-molluscicide against Land Snail *Monacha cartusiana.* Indian Journal of Animal Research. DOI: 10.18805/IJAR.BF-1790.

Submitted: 27-03-2024 **Accepted:** 04-05-2024 **Online:** 07-06-2024

products have demonstrated very encouraging results. Recently, for instance, we have conducted research that shows the potential for Trichoderma harzianum to act as a biocontrol agent against the land snail (Ahmed *et al.,* 2023). Recent studies have demonstrated that certain natural compounds, like as extracts of Pulsatilla chinensis (Bunge) Regel, can kill *O. hupensis*. These extracts have been shown to have molluscicidal activity. According to Chen *et al.,* (2012), they pose a lower risk of being detrimental to aquatic creatures that are not the intended targets of the molluscicide niclosamide. Additionally, Agave attenuata Salm-Dyck is poisonous to the snail Bulinus africanus, which is the target species, but it has fewer negative effects on fish and animals, or none at all (Brackenbury and Appleton 1997).

Traditional Chinese medicine (TCM) has utilized gallbladder liquid and various bile components derived from a wide variety of animals for several centuries (Wang and Carey, 2014). According to the biochemical compounds and pharmacological activities of bile, such as specific bile salts, the bile pigment bilirubin and its glucuronides, secondary bile ingredients such as Vitamins A, D, E, K and melatonin synthetic ingredients, animal bile, such as cow, sheep and others promising candidates for bear bile's similar and alternative therapeutic purposes (Li *et al.,* 2016). Animal bile has been shown to have anti-inflammatory, antioxidant, antipyretic, anodyne, anticonvulsive, antiallergic, anti-congestive, antidiabetic and antispasmodic characteristics (Wang and Carey 2014; Zehua 2015). Additionally, it has been shown to dissolve gallstones, improve liver function and suppress the reproduction of bacteria and viruses. Research conducted in the field of modern therapeutics has revealed that bear bile possesses a wide variety of pharmacological qualities. These properties include hepatoprotection, antibacterial, antiviral, anti-inflammatory, anti-gallstones, hypolipidemic and other actions (Kou *et al.,* 2014; Zhao *et al.,* 2015). According to Wang and Carey (2014), traditional Chinese medicine made use of goat bile as a medicinal agent since it was believed to be effective in the treatment of optic atrophy, acute hemorrhagic conjunctivitis and several infectious skin illnesses. Chicken bile has been used to control coccidiosis, as it has shown a destructive effect on the oocysts and their morphology of *Eimeria papillate* (Murshed *et al.,* 2023). The present study was the first to evaluate the potential activities of sheep bile against *M. carutisiana*.

MATERIALS AND METHODS

Animal material

Bile liquid was acquired from the intact gallbladder of lambs from a slaughterhouse in Riyadh (Saudi Arabia). Gallbladders were isolated from 8 free-disease lambs. Gallbladders were sterilized with 80% methanol before eradicating the gallbladder, then Suctioning bile fluid with a syringe, transferred and collected to clean tubes and kept at 4°C until they were utilized.

Snails collection

The experimental design was carried out at the Zoology Department, Laboratory of Parasitology, Faculty of Science, King Saud University, for almost 2-3 months in September-November 2023. *M. cartusiana* adult snails were collected from the Al Ammariyah district of Riyadh, Kingdom of Saudi Arabia. Snails were transported in muslin bags, placed in plastic containers with sterilized, moist sandy loam soil in a 1:1 (V:V) ratio and given fresh lettuce (*Lactuca sativa*) leaves for 2 weeks to adapt to laboratory conditions.

Chemical analysis using (GC-MS)

Utilizing a Trace Ultra Gas Chromatographer in conjunction with a DSQII Mass Spectrometer (Thermo Scientific), an analysis of the volatile components of bile was carried out. To chromatographically separate the components according to the method of Adams (1995), the TR-5 MS capillary column was utilized. This column had a length of 30 m, a diameter of 0.25 mm and a length of 0.25 m. The substances were discovered by using pertinent data that was kept in databases from literature and equipment (Adams Book 07, Nist 98 and Xcalibur). A variety of n-alkanes, ranging from C8 to C24, were utilized in the calculation of the Relative Retention Index. Compounds' relative percentages have been obtained by electronic means, using data on the percentage of the region.

TPC, TFC and TTC measurement

The phenolic and flavonoid, content of bile were determined according to the method by Ordonez *et al.,* 2006. The blue color absorbance was measured at 765 nm with a UV-visible spectrophotometer. Based on the curve calibration equation:

$$(y=0.005 - x - 0.0088)$$

$$(y=0.0011x + 0.0928)$$

The bile TPC and TFC, content was estimated as gallic acid equivalent and quercetin (mg/g DW), where (y) is absorbance and (x) is concentration.

The total tannin content was determined by utilizing this approach for the bile. An overall volume of 0.1 mL of the extract samples was put into an Eppendorf tube with a capacity of 2 mL, which already included 1.5 mL of Milli-Q water and 0.1 mL of the Folin-Ciocalteu phenol reagent. This mixture was incubated for 8 minutes. After that, 0.3 mL of a sodium carbonate solution containing 35 % was added to the mix. After that, the ingredients were thoroughly combined and then placed in a dark, ambient temperature area for twenty minutes. The measured value for the wavelength was 700 nm. The following equation, was utilized to determine the total tannin content that was present in the leaf extract. The total tannin content that was calculated was given in units of mg/g dry weight (DW).

$$(Y= 0.0013x + 0.0052)$$
$$\text{with } (R2=9937)$$

Cytotoxicity evaluation (MTT assay)

Cell culture

The Hep-G2/2.2.15 Human Hepatoblastoma Cell Line was gained of (Sigma-Aldrich Chemie GmbH,Taufkirchen - Germany). The cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) that was supplemented with 10% fetal bovine serum (FBS) in an atmosphere that was humid and contained 5% CO_2 at 37°C.

Cell viability assay

The detection of cell viability and cell growth was performed using MTT Assay (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium Bromide, cat#475989-1GM, Sigma-Aldrich, Germany). Briefly, aliquots of 120 µl of the suspended cells (5×10^4 mL^{-1}) were given to 60 µL of a serial dilution of the bile in a 96-well plate. After 3 days of incubations, 20 µl of MTT-solution was given to each well (Mosmann 1983) and the cells were further cultivated for an additional two hours. Formazan crystals were dissolved in isopropanol. The intensity of the resulting color was measured at 595 nm as described previously.

Cell Viability (%)=

$$\text{Mean absorbance} \frac{\text{(treated cells)}}{\text{(untreated cells)}} \times 100$$

Experimental design

The snails were kept at room temperature (23-28°C) and 75% relative humidity. Snails were divided into 5 groups, each of five snails. The first group was treated with sterilized water and served as a control group. The second to fifth groups were treated with the concentration (12.5, 25, 50 and 100%) of bile liquid.

Statistical analysis

An analysis of variance (ANOVA) with a single-way was performed on the data using SPSS (version 18) (SPSS Inc., Chicago, IL, USA). A two-tailed paired t-test and Duncan's multiple ranges were utilized to investigate the statistical differences that existed between the means of the various groups.

RESULTS AND DISCUSSION

GC/MS analysis was performed on the volatile contents of bile liquid, it was shown that bile contains 12 ingredients

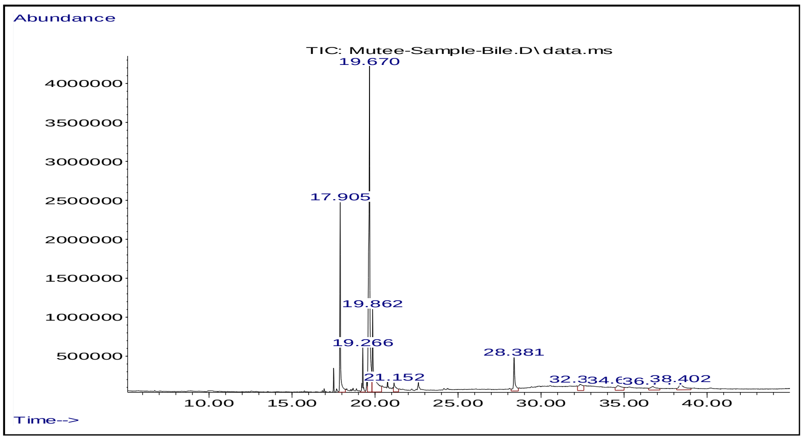

Fig 1: The chemical ingredients of sheep bile liquid by GC-MS.

Table 1: The Chemical makeup of bile was determined using GC-MS.

tR (min)	Proposed compound	MW	Formula	Peak area%
17.50	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$	1.80
17.90	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	19.44
19.27	9-Octadecenoic acid (Z)-, methyl ester	296	$C_{19}H_{36}O_2$	3.53
19.51	Heptadecanoic acid, 16-methyl-, methyl ester	298	$C_{19}H_{38}O_2$	1.48
19.61	Linoelaidic acid	280	$C_{18}H_{32}O_2$	13.03
19.67	Oleic Acid	282	$C_{18}H_{34}O_2$	42.14
19.86	Octadecanoic acid	284	$C_{18}H_{36}O_2$	7.77
20.75	Arachidonic acid methyl ester	318	$C_{21}H_{34}O_2$	0.79
22.62	Glycidyl oleate	338	$C_{21}H_{38}O_3$	1.33
28.38	Cholesterol	386	$C_{27}H_{46}O$	5.45
32.34	Deoxycholic acid	392	$C_{24}H_{40}O_4$	0.52
38.40	Methyl cholate	422	$C_{25}H_{42}O_5$	2.73

(Table 1), with oleic acid being the most prominent component (Fig 1). These compounds belong to the classes of monoterpenes, furanes, isocyanate metabolites, alkanes and alcohols, which are comparable to the compounds that were reported by Murshed *et al.* (2022). Because of the presence of multiple bioactive components, it is not possible to attribute the biocontrol actions of bile to a single bioactive constituent; rather, it is more likely to be attributed to the synergism among the various bioactive constituents.

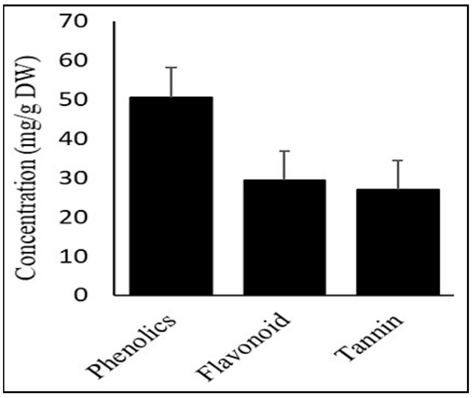

Fig 2: Total of phenols, flavonoids and tannin in methanolic of bile.

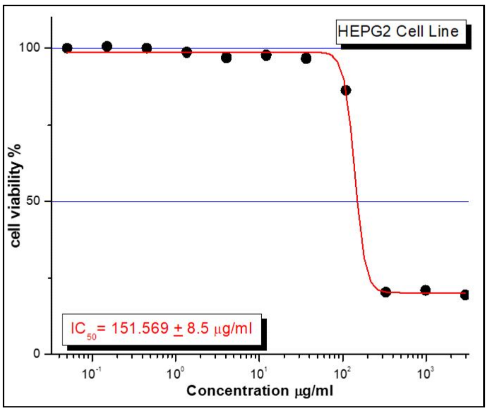

Fig 3: The effect of different doses of bile on the cytotoxicity and subsequent survival of the HT29 cell line. HT-29: Human Colorectal Adenocarcinoma Cell Line.

Fig 2 revealed the findings of the phenolics TPC, flavonoids TFC and tannin TTC measurements taken of the bile liquid. The total potency concentrations of the bile were determined to be 50.65079365±0.809 mg GAE/g DW, 29.39351852±0.386mg QE/g DW, and 27.41669074±1.066 mg TAE/g DW, respectively (Fig 2).

The findings regarding the cytotoxicity of the bile liquid are presented in Fig 3, which shows the results of testing it at varying concentrations. The test included exposing Hep-G2 (hepatoblastoma cell line) cells to diverse concentrations of bile, namely 1, 12.5, 25, 50, 100, 200, 400 and 800 µg/mL. The survival ratio of the cells at these concentrations was found to be 99.76, 84.00, 73.19, 62.27, 53.11, 35.29, 18.81 and 5.19%, respectively. The IC50 value for Hep-G2 was calculated to be 151.569±8.5 µg/mL. As can be explained in Fig 3, the percentage of cells that managed to survive was reduced as the concentration of the bile was raised (Fig 3).

Molluscs are the second-largest invertebrate group in the animal kingdom, following arthropods (Rosenberg 2014). Due to snail resistance, great effort is being put into creating new, safe and effective treatments for *M. cartusian*. After being submerged in concentrations, the snails generally displayed agitated behavior, moving in circles before slowing down and eventually dying. In the contact assay, bile at various doses demonstrated significant molluscicide activity against adult *M. cartusian* (Fig 4). Since it is believed to be ideal for pest management tactics and helps in avoiding the health and environmental concerns of chemical pesticides, the genus bile is now attracting a lot of research (Kumar *et al.,* 2019). This is because it is supposed to be suitable for these strategies. To combat a variety of plant infections and insect pests, bile is widely utilized as a biocontrol agent (Napitupulu *et al.,* 2019). The development of several secondary metabolites that are volatile, non-volatile, antifeedant and repellant, such as pyrones, sesquiterpenes and peptaibols, was found to be connected with its potential for utilization (Reino *et al.,* 2008). In light of this, the purpose of this research was to determine the chemical components of bile and to assess the efficacy of bile as a molluscicide against *M. cartusiana* in a laboratory setting. The bile is efficacious in

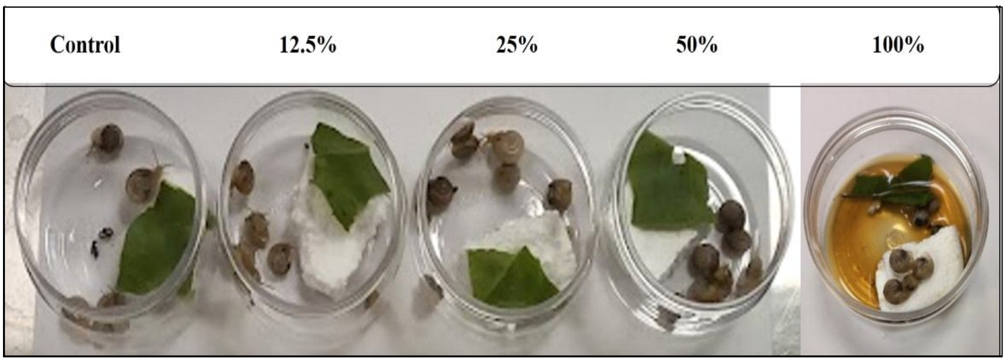

Fig 4: An illustrative image of the effect of the bile on S. snails at a concentration of 100%

Table 2: *In vitro mollusca* lethal effect of bile against *M. cartusian* snail.

	The average number of snail dead at 40 minutes after exposure			
Dose rate	5 min	10 min	20 min	40 min
Control	0.0±0.0 c	0.0±0.0 c	0±0.0 c	0±0.0 c
12.5%	0.0±0.0 c	1.1±0.5 b	2±3.8b	3.3±2.7 b
25%	0.0±0.0 c	2.3±2.8 ab	5±12.5b	10.3±4.1ab
50%	2.2±1.1b	4.7±8.3b	13.3±14.4b	17.7±6.2ab
100%	4.9±1.1b	8.5±4.7a	13.3±16.6a	25±1a

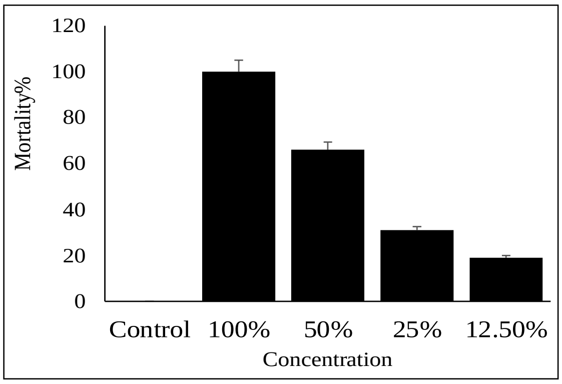

Fig 5: Mortality % of *M. cartusian* assayed with bile at various exposure periods to concentrations (12.5, 25, 50 and 100 mg/mL), compared with the reference drug.

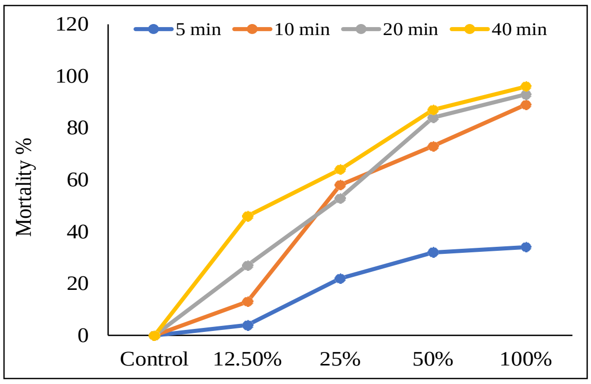

Fig 6: Mortality % of *M. cartusian* assayed with bile at various exposure periods (5, 10, 20 and 40 min). compared with control.

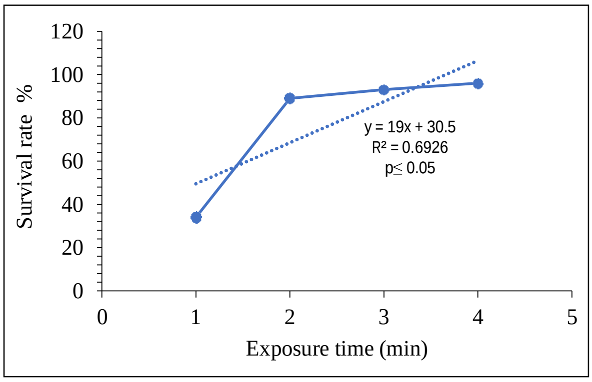

Fig 7: Mean mortality % of *M. cartusian* adults at 100% concentration for various exposure times

preventing it due to its inhibitory capability and influence on the sporulation of coccidian oocysts of *E. stiedae* (Murshed *et al.,* 2022)

The results showed that the bile molluscicidal effect against the *M. cartusian* snail was concentration-dependent. It was discovered that as bile concentration increased, the survival rate of *M. cartusian* snails significantly decreased (Fig 5). The highest mortality (97%) was attained at the dose of 100% of bile.

The data revealed substantial differences between the mortality means of 100% concentration after 40 minutes, 50% concentration of bile after 20 minutes and the remaining mortality means (Table 2). There were also significant changes between the control and 100% concentrations after 40 minutes (Fig 6). The maximum mortality occurred after 40 minutes at 50 and 100% doses. The lowest mortality was seen after 5 minutes at all doses (Table 2). Significant differences ($P<0.05$) were seen in *M. cartusian*. Adult death rates by bile concentration (12.5, 25, 50 and 100%) and control (Fig 7). These findings demonstrated that bile has activity against snails.

CONCLUSION

Bile is a very promising candidate that could be used to control land snails, or exploited as a starting point to develop new, effective and environmentally friendly molluscicides. Bile showed molluscicide activity almost comparable to that of the recommended chemical molluscicides and in contrast, bile is less harmful to the environment and health. The exact mode of action should be investigated.

ACKNOWLEDGEMENT

We thank the Project of Supporting Researchers No. RSP2024R94, at King Saud University, Saudi Arabia.

Conflict of interest

The authors declare that there are no conflicts of interest.

REFERENCES

Abd El-Atti, M.S., Khalil, A.M., Elsheakh, A.A. and Elgohary, W.S. (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.

Adams, R.P. (2017). Identification of essential oil components by gas chromatography/mass spectrometry. 5 online ed. Gruver, TX USA: Texensis Publishing.

Ahmed, H.Y., Al-Quraishy, S., Hassan, A.O., Abdel-Baki, A.A.S. and Abdel-Tawab, H. (2023). Biocontrol potential of trichoderma harzianum against the land snail *Monacha cartusiana*: lab and field trails.

Brackenbury T.D., Appleton C.C. (1997) A comprehensive evaluation of agave attenuata, a candidate plant molluscicide in South Africa. Acta Trop 68: 201-213.

Capinera, J.L. and White, J. (2011). Terrestrial snails affecting plants in Florida. Publication number *EENY-497. Gainesville:* Department of Entomology. University of Florida.

Castle, G.D., Mills, G.A., Gravell, A., Jones, L., Townsend, I., Cameron, D.G. and Fones, G.R. (2017). Review of the molluscicide metaldehyde in the environment. Environmental Science: Water Research and Technology. 3(3): 415-428.

Chen Y.Q., Xu Q.M., Liu Y.L. *et al.* (2012) Laboratory evaluation of the molluscicidal activity of pulsatilla chinensis (Bunge) Regel saponins against the snail Oncomelania hupensis. Biomed Environ Sci. 25: 224-229.

Geasa, N.S., Heiba, F.N., Mortada, M.M. and Mosbah, I.S. (2013). Molluscicidial activity of certain biological insecticides against land snails *Monacha cartusiana* and Succinea oblonga in laboratory and field conditions. Egypt J Zool 60: 179-188.

Genena, M.A. and Mostafa, F.A. (2008). Impact of eight bacterial Isolates of bacillus thuringiensis against the two land snails, monacha cantiana and eobania vermiculata (GASTROPODA: Helicidae). Journal of Plant Protection and Pathology. 33(4): 2853-2861.

Iglesias, J., Castillejo, J. and Castro, R. (2003). The effects of repeated applications of the molluscicide metaldehyde and the biocontrol nematode Phasmarhabditis hermaphrodita on molluscs, earthworms, nematodes, acarids and collembolans: A two year study in north west Spain. Pest Management Science: Formerly Pesticide Science. 59(11): 1217-1224.

Khalil, A.M. (2016). Impact of methomyl lannate on physiological parameters of the land snail Eobania vermiculata. The journal of basic and applied zoology. 74: 1-7.

Kou, G., Zhang, C., Wang, Z., Xu, Q. and Wang, B. (2014). Study progress on pharmacology of bear bile powder. Chinese Journal of Drug Evaluation. 31: 142-145.

Kramarz, P.E., De Vaufleury, A. and Carey, M. (2007). Studying the effect of exposure of the snail Helix aspersa to the purified Bt toxin, Cry1Ab. Applied Soil Ecology. 37(1-2): 169-172.

Kumar M., Rawat S., Nagar B., *et al.* (2021) Implementation of the use of ethnomedicinal plants for curing diseases in the Indian himalayas and its role in sustainability of livelihoods and socioeconomic development. Int J Environ Res Public Health. 18:1509.

Kumar R, K., Kumari, K.C., Hembram, L., Kandha, B.K., Bindhani (2019). Expression of an endo á-1, 3-Glucanase gene from Trichoderma harzianum in rice induces resistance against sheath blight. J. Plant Biochem Biotechnol. 28: 84-90.

Li, S., Tan, H.Y., Wang, N., Hong, M., Li, L., Cheung, F. and Feng, Y. (2016). Substitutes for bear bile for the treatment of liver diseases: Research progress and future perspective. Evidence-Based Complementary and Alternative Medicine, 2016.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 65(1-2): 55-63.

Moustafa, G.G., Shaaban, F.E., Hadeed, A.A. and Elhady, W.M. (2016). Immunotoxicological, biochemical and histopathological studies on roundup and stomp herbicides in Nile catfish (Clarias gariepinus). Veterinary world. 9(6): 638.

Murshed M., Al-Quraishy S. and Qasem M.A (2022). Evaluation of the anticoccidial activity of sheep bile against eimeria stiedae oocysts and sporozoites of rabbits: An *In Vitro* Study. Veterinary Sciences. 9(12): 658.

Murshed, M., Al-Quraishy, S. and Qasem, M.A. (2023). The anticoccidial effects of the poultry bile against Eimeria papillata oocysts of mice: *In vitro* study. Indian Journal of Animal Research. 57(1): 74-81.

Napitupulu, T.P., Ilyas, M., Kanti, A. and Sudiana, I.M. (2019). *In vitro* evaluation of trichoderma harzianum strains for the control of fusarium oxysporum F. sp. cubense. Plant Pathology and Quarantine. 9(1): 152-159.

Ordonez, A.A.L., Gomez, J.D. and Vattuone, M.A. (2006). Antioxidant activities of sechium edule (Jacq.) Swartz extract. Food chemistry. 97(3): 452-458.

Raut, S.K. and Barker, G.M. (2002) Achatina fulica bondich and other achatinidae as pests in tropical agriculture. In: Barker G.M., Ed., Molluscs as Crop Pests, CABI Publishing, Hamilton. 55-114. http://dx.doi.org/10.1079/9780851993201.0055.

Reino, J.L., Guerrero, R.F., Hernandez-Galan, R. and Collado, I.G. (2008). Secondary metabolites from species of the biocontrol agent trichoderma. Phytochemistry Reviews. 7: 89-123.

Rosenberg, G. (2014). A new critical estimate of named species-level diversity of the recent Mollusca. Amer Malacol Bull 32: 308-322

Shahawy, W.A. (2018). Biochemical effects of mullicicides against the land snails, helically vestalis and the ba piscina using sublethal doses. Journal of Plant Protection and Pathology. 9(4): 261-264.

Wang, D.Q. and Carey, M.C. (2014). Therapeutic uses of animal biles in traditional Chinese medicine: An ethnophar mmacological, biophysical chemical and medicinal Review. World Journal of Gastroenterology: WJG, 20(29): 9952.

Yousif, F., Hifnawy M.S., Soliman, G., *el at.,* (2007) Large-scale *in vitro*. Screening of egyptian native and cultivated plants for schistosomicidal activity. Pharm Biol. 45:501-510.

Zehua, L. (2015). Political and Intellectual Authority: The Concept of the “Sage-Monarch” and Its Modern Fate. Ideol. Power Power Ideol. Early China. 273-300.

Zhao, J.Y., Lin, W., Zhuang, Q.C., Zhong, X.Y., Peng, J. and Hong, Z.F. (2015). Bear bile powder inhibits angiogenesis *in vivo* and *in vitro*. Chinese Journal of Integrative Medicine. 21: 369-375.