MEDWIN PUBLISHERS
Committed to Create Value for Researchers

International Journal of Zoology and Animal Biology
ISSN: 2639-216X

Testing the Molluscicidal Effect of Some Endozoic and Endophytic Fungal Species against *Biomphalaria Alexandrina* Snails

Abdel-Wareth MTA*

Department of Environmental Research and Medical Malacology, Theodor Bilharz Research Institute, Giza, Egypt

***Corresponding author:** Marwa TA Abdel-Wareth, Department of Environmental Research and Medical Malacology, Theodor Bilharz Research Institute, Giza, Egypt, Email: marwatamim2001@hotmail.com

Research Article

Volume 4 Issue 4

Received Date: August 03, 2021

Published Date: August 19, 2021

DOI: 10.23880/izab-16000317

Abstract

Controlling snails which are the intermediate hosts of schistosomiasis is one of the strategies used to reduce disease incidence. Researchers are always seeking molluscicides of biological origin to avoid using chemical molluscicides which are toxic to the environment. The aim of this study is to investigate the molluscicidal activity of fungal species isolated from the habitat of *Biomphalaria alexandrina* snails. Fungal species were isolated from the inner tissues of *B. alexandrina* snails, and from the tissues of *Eichhornia crassipes* which is an aquatic plant associated with these snails. The results indicated that the ethyl acetate extracts of endozoic fungi and endophytic ones showed LC_{90} values higher than 600 ppm and 400 ppm, respectively.

Keywords: *Biomphalaria Alexandrina* Snails; *Eichhornia crassipes*; Endozoic Fungi; Endophytes

Introduction

Biomphalaria alexandrina snails are the intermediate hosts of schistosomiasis which is caused by *Schistosoma mansoni*. Controlling this snail is one of the methods used to prevent the spread of schistosomiasis [1]. Researchers all over the countries that suffer from the dissemination of the disease have investigated the molluscicidal effect of different biological agents, as an urgent step to substitute chemical molluscicides with all their drawbacks and negative effects on the aquatic environment [2]. Plants, algae, bacteria and fungi were tested as powders or extracts against *B. alexandrina* snails. Some of them showed promising results such as *Casimiroa edulis* and *Cestrum diurnum* plants [3], *Spirulina platensis* alga [4], *Paenibacillus* bacterial strain [5], besides *Paecilomyces lilacinus* and *Beauveria bassiana* fungal extracts [6]. In this study I tried seeking molluscicidal agents from unexpected habitats. Despite the fact that these habitats can be considered as *B. alexandrina* habitat, there was a 50/50 probability that the fungi isolated from the selected habitats might be effective. The first group of fungi were isolated from the inner tissues of *B. alexandrina* which are known as endozoic fungi, while the second group of fungi were isolated from the tissues of *Eichhornia crassipes* plant which are known as endophytic fungi. All tested fungi were extracted with ethyl acetate to ensure getting their secondary metabolites which were shown to have multifaceted activities [6].

Materials and Methods

Endozoic Fungi

Isolation of fungi from *Biomphalaria alexandrina* snails: *Biomphalaria alexandrina* snails were dried using filter paper. Then their surfaces were sterilized with cotton pieces

soaked with alcohol. Under aseptic conditions, I crushed the snails between two sterile glass slides, and shell parts were removed. Then, the snails were dissected using sterile tools to get the soft parts. The soft part of each snail was homogenized under aseptic conditions in 2 ml of sterile water and centrifuged at 40000 rpm for 15 min. Then, 0.5 ml of the supernatant was spread onto the surface of solidified Czapek agar medium of the following composition; 30 g sucrose, 2 g sodium nitrate, 1 g dipotassium phosphate, 0.5 g magnesium sulphate, 0.5 g potassium chloride, 0.01 g ferrous sulphate, 15 g agar and 1 L of distilled water. Three replicates were cultured, and then incubated at 28°C for 10 days [6].

Extraction of fungal metabolites: Scale-up fermentation was done using three 1 L Erlenmeyer flasks for each fungus, each containing 100 g rice and 100 ml distilled water, sterilized at 121°C for 20 min. Each flask was inoculated with spore suspension from 10 days old cultures. To ensure complete growth of fungal species and production of secondary metabolites, the flasks were incubated at 30°C for 15 days. The medium was extracted several times with ethyl acetate till exhaustion [7].

Endophytic Fungi

Isolation of fungal species from *Eichhornia crassipes* plant: *Eichhornia crassipes* plant was collected from watercourses in Egypt. The plant was washed thoroughly with tap water, and then surface sterilized with 70% ethanol for 1 min, 4% sodium hypochlorite for 3 min, and again with 70% ethanol for 1 min, finally, they were rinsed twice with sterile distilled water. The plant parts were then dried under sterile conditions, cut into smaller pieces with a sterilized scalpel [8], and then placed onto malt extract agar plates amended with chloramphenicol to suppress bacterial growth. All cultivated plates were incubated at 28°C for one week. When the growth of endophytic fungi was observed, it was purified on malt extract agar plates.

Extraction of Fungal Metabolites: Fresh cultures of fungi grown on malt extract agar medium for 7 days were inoculated into malt extract broth and incubated at 28°C for 3 weeks. Fungal filtrates were then extracted with ethyl acetate. The extraction procedure was performed in triplicates. The organic fractions were combined and concentrated to dryness under reduced vacuum to obtain crude fungal extracts [9].

Identification of Fungal Isolates

Using standard media, taxonomic identification based on morphology characteristics down to species level was carried out according to identification keys: *Penicillium* (on Czapek yeast extract agar) [10]; *Aspergillus* (on Czapek agar) [11,12]; dematiaceous hyphomycetes (potato carrot agar) [13,14]; miscellaneous fungi (on malt extract agar, potato dextrose agar and Czapek yeast extract agar) [15-17] and ascomycetes [18].

Molluscicidal Test

Each fungal extract was tested against *Biomphalaria alexandrina* snails. A series of concentrations was prepared using dechlorinated tap water at 22±2°C to determine the most effective fungal extract. Three replicates were used; each of ten snails (8-10 mm in diameter) for each one. The exposure period was 24 h at room temperature. Another group of snails was maintained under the same experimental conditions as a control group [19]. At the end of the exposure period, these snails were removed from each tested concentration, washed thoroughly with dechlorinated tap water and transferred to another container for a recovery period of 24 h. Then, the number of dead snails in each concentration was recorded.

Results and Discussion

We found that the tested fungal extracts were not effective (Tables 1&2) as their LC_{90} values exceeded the standard set by WHO for extracts with molluscicidal activity which are supposed to have LC_{90} of 20 ppm [20]. Also when we compared our results with the previous studies, we found that LC_{90} values of the already applied molluscicidal compounds such as niclosamide and copper sulphate are very low as they equal 0.33 and 0.97 ppm, respectively [21]. The ineffectiveness of the tested extracts can be explained in the light of their sources; the endozoic fungi which were investigated in the present study were isolated from the inner tissues of *Biomphalaria alexandrina* snails which means that they live in commensalism with this molluscan species, so they did not show harmful or killing effect when they were extracted and tested against it as a mollusciciding agent. Also, the endophytic fungi which were isolated from water hyacinth (*Eichhornia crassipes*) were also non effective, because water hyacinth is one of the aquatic plants which are associated with *B. alexandrina* snails [22], where snails in general are using this plant as shelter, and prefer a habitat with aquatic vegetation [23], to get higher amounts of dissolved oxygen, that help snails to tolerate unfavorable conditions [24]. Our results are in accordance with Saad et al. [25] who reported that the filtrates of *Aspergillus niger* and *Aspergillus flavus* isolated from watercourses in Egypt were not effective against *B. alexandrina* snails as they recorded very high LC_{90} values.

Table 1: Molluscicidal effect of ethyl acetate extract of endozoic fungi isolated from *Biomphalaria alexandrina* snails tissues on *Biomphalaria alexandrina* snails after 24 h.

Ethyl acetate extract of fungi	LC_{90} (ppm)
Mucor hiemalis	>600
Penicillium islandicum	>600
Cladosporium cladosporioides	>600
Aspergillus flavus	>600
Aspergillus niger	>600
Penicillium variabile	>600
Paecilomyces variotii	>600
Penicillium chrysogenum	>600
Trichoderma harzianum	>600
Niclosamide	0.33*
Copper sulphate	0.97*

* Ismail, et al. [21].

Table 2: Molluscicidal effect of ethyl acetate extract of endophytic fungi isolated from *Eichhornia crassipes* on *Biomphalaria alexandrina* snails after 24 h.

Ethyl acetate extract of fungi	LC_{90} (ppm)
Aspergillus niger	>400
Aspergillus flavus var. *flavus*	>400
Aspergillus sydowi	>400
Niclosamide	0.33*
Copper sulphate	0.97*

* Ismail, et al. [21].

Conclusion

The fungi which are isolated from the tissues of *Biomphalaria alexandrina* snails (endozoic) and those which are isolated from the tissues of *Eichhornia crassipes* (endophytic) have the same properties of their hosts, as they showed no molluscicidal effect on *B. alexandrina* snails.

Acknowledgment

I am grateful to each of Prof. Mohamed Abdel-Aziz (National Research Centre), Prof. Ahmed Abdel-Azeem (Suez Canal University), and Dr. Eman Abdullah (Cairo University) who helped me in this work.

References

1. WHO (2008) African Network for drug/diagnostics discovery and innovation (ANDI) 'Creating a sustainable platform for R&D innovation in Africa'. 1st meeting, Abuja, Nigeria.

2. Oliveria Filho EC, Paumgartten FJ (2000) Toxicity of *Euphorbia milii* latex and Niclosamide to snails and nontarget aquatic species. Ecotoxicol Environ Safety 46(3): 342-350.

3. Saad AA, Aly RH, Ragab FMA, Abdel Wareth MTA (2012) Effect of *Cestrum diurnum* and *Casimiroa edulis* plants on the digestive gland and its related enzymes in the freshwater snail *Biomphalaria alexandrina*. Toxicol Environ Health Sci 4(2): 109-114.

4. Mostafa SM, Gawish A (2009) Towards to control *Biomphalaria alexandrina* snails and the free living larval stages of *Schistosoma mansoni* using the microalga *Spirulina platensis*. Aus J Basic Appl Sci 3(4): 4112- 4119.

5. Duval D, Galinier R, Mouahid G, Toulza E, Allienne JF, Portela J, et al. (2015) A novel bacterial pathogen of *Biomphalaria glabrata*: a potential weapon for schistosomiasis control?. PLoS neg trop dis 9(2): e0003489.

6. Abdel-Wareth MTA, El Hagrassi AM, Abdel Aziz MS, Nasr SM, Ghareeb MA (2019) Biological activities of endozoic fungi isolated from *Biomphalaria alexandrina* snails maintained in different environmental conditions. Int J Environ Stud 76 (5): 780-799.

7. Abdel Aziz MS, Ghareeb MA, Saad AM, Refahy LA, Hamed AA (2018) Chromatographic isolation and structural elucidation of secondary metabolites from the soil-inhabiting fungus *Aspergillus fumigatus* 3T-EGY. Acta Chromatograph 30: 243-249.

8. Nath A, Joshi SR (2015) Ultrastructural effect on mastitis pathogens by extract of endophytic fungi associated with ethnoveterinary plant, *Hibiscus sabdariffa* L. J Micro Ultra 3: 38-43.

9. Pavithra N, Sathish L, Ananda K (2012) Antimicrobial and enzyme activity of endophytic fungi isolated from Tulsi. J Pharm Biomed Sci 16(12): 1-6.

10. Pitt JI (1980) The Genus *Penicillium* and its Teleomorphic States *Eupenicillium* and *Talaromyces*. JSTOR 73(3): 582-584.

11. Raper KB, Fennell DI (1965) The Genus *Aspergillus* (Baltimore: Williams & Wilkins).

12. Klich MA (2002) Identification of Common *Aspergillus* Species. pp: 116.

13. Ellis MB (1971) Dematiaceous Hyphomycetes.

Commonwealth Mycological Institute, Kew, pp: 608.

14. Ellis MB (1976) More Dematiaceous Hyphomycetes, Commonwealth Mycological Institute, Kew, pp: 507.

15. Von Arx JA (1981) The Genera of Fungi Sporulating in Pure Culture. Journal of Botanical taxonomy and geobotany 93(12): 151-152.

16. Watanabe T (2002) Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species. In: 3rd (Edn.), pp: 426.

17. Guarro J, Gene J, Stchigel AM, Figueras MJ (2012) Atlas of Soil Ascomycetes. 10: 486.

18. Domsch KH, Gams W, Anderson TH (2007) Compendium of Soil Fungi (Eching: IHW-Verlag). In: 2nd (Edn.).

19. WHO (1965) Monograph of molluscicide screening and evaluation. Bulletin of World Health Organization. Series 50: 124-138.

20. WHO (1983) Special Progrmme for Research and Training in Tropical Diseases: Report of the Scientific Working Group on Plant Molluscicides. Geneva, 4: 833.

21. Ismail NM, Sedek MN, El Said KM, Marai AMA (2019) Effect of water bodies lining on the efficacy of molluscicides against *Biomphalaria alexandrina* snails with emphasis to their integrated control measures. Egypt J Aquat Biol Fish 23(5): 267-283.

22. El Khayat HM, Eissa FI, Mostafa MA, Flefel HE (2013) Distribution of schistosome intermediate hosts in relation to aquatic plants and physico-chemical characteristics in different watercourses among Kafr El-Sheikh centers, Egypt. Nat Sci J 11(12): 146-155.

23. Van Schayck IRCP (1985) Laboratory studies on the relation between aquatic vegetation and the presence of two Bilharzia-bearing snail species. J Aquat Plant Manag 23: 87-91.

24. El Khayat HMM, Mostafa BB, Mahmoud KMA, El Said KM, Ismail NMM (2009) The association between fresh water snails, macrophytes and water quality in different water courses in Egypt. New Egypt J Med 40: 381-392.

25. Saad AA, Khalil MT, Ragab FMA, Mekawey AAI, Abdel Wareth MTA (2016) Separation of a compound effective against *Biomphalaria alexandrina* snails from the filtrate of *Penicillium janthinellum*. Int J Env Stud 73(1): 1-17.