Confluence Journal of Pure and Applied Sciences, Volume 5 Issue 1, March, 2026 Pp 28-41

ORIGINAL RESEARCH

Molluscicidal Activity of *Vernonia amygdalina* and *Azadirachta indica* Leaf Extracts against *Bulinus globosus*

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ABSTRACT

Bulinus globosus is a freshwater snail that serves as an intermediate host for *Schistosoma* species, the causative agents of the neglected tropical disease schistosomiasis. Trematode parasites of the genus *Schistosoma* rely on such snail hosts for transmission, making snail control a critical component of disease management. Consequently, one of the most important aspects of integrated schistosomiasis control strategies is the reduction of snail populations. This study evaluated the molluscicidal activity of methanolic leaf extracts of *Vernonia amygdalina* (bitter leaf) and *Azadirachta indica* (neem), applied individually and in combination, against adult *Bulinus globosus*. Qualitative phytochemical screening was conducted using standard procedures. Molluscicidal bioassays were performed at concentrations ranging from 0 to 128 mg/L, using ten snails per concentration in triplicate, and mortality was recorded at 1, 4, 6, 12, 24, and 48 h. Both plant extracts exhibited concentration- and time-dependent molluscicidal effects, with no mortality observed in control groups. At 48 h, the LC50 values were 56.76 mg/L for *V. amygdalina*, 51.09 mg/L for *A. indica*, and 51.03 mg/L for the combined extract, while LC90 values ranged from 108.39 to 123.23 mg/L. Statistical analysis revealed significant differences ($p < 0.05$) in snail mortality across concentrations and exposure periods. The findings indicate that *V. amygdalina* and *A. indica* possess promising molluscicidal properties and may serve as potential plant-based alternatives to synthetic molluscicides in schistosomiasis control programmes. However, the study was limited to adult *Bulinus globosus*, did not assess effects on non-target aquatic organisms, and utilized crude extracts without characterization of active compounds, which may affect environmental safety assessment and reproducibility.

Keywords: Molluscicidal activity; *Vernonia amygdalina*; *Azadirachta indica*; *Bulinus globosus*; Schistosomiasis

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INTRODUCTION

Bulinus globosus is a freshwater snail that serves as an intermediate host for *Schistosoma* species, the causative agents of schistosomiasis (Kokaliaris et al., 2022). Transmission occurs when infected snails release cercariae into freshwater bodies, which penetrate human skin during contact. The persistence of this disease is therefore closely linked to the presence of suitable snail hosts, particularly in endemic regions such as sub-Saharan Africa (Danlami et al., 2025). It is estimated that at least 253.7 million people required preventive treatment for schistosomiasis in 2024, with the highest burden occurring in African countries (World Health Organization [WHO], 2026). Given the central role of Bulinus globosus in disease transmission, controlling its population through molluscicidal interventions is essential. In Nigeria, schistosomiasis is widely distributed, especially in rural and peri-urban communities where activities such as fishing, bathing, irrigation, and laundry increase human–water contact (Oyetunde et al., 2020). Current control strategies rely largely on mass drug administration with praziquantel. Although praziquantel is effective in reducing morbidity, it does not prevent reinfection, and emerging concerns regarding reduced drug sensitivity have been reported (Eastham et al., 2024). Environmental modification, water resource development, and population movements further complicate disease control efforts (Buchwald et al., 2021).

To address these challenges, the World Health Organization recommends an integrated approach to schistosomiasis control, including snail population management (Kokaliaris et al., (2022). Chemical molluscicides such as niclosamide have been widely used; however, their application is limited by high cost, toxicity to non-target organisms, and environmental persistence (Mtemeli et al., 2021). These limitations have prompted increased interest in plant-derived molluscicides as environmentally friendly and cost-effective alternatives (Sokolow et al., 2018).

Azadirachta indica (neem; family Meliaceae) and *Vernonia amygdalina* (bitter leaf; family Asteraceae) are medicinal plants commonly found in Nigeria, typically growing in tropical and subtropical environments such as savannahs, forest margins, and cultivated farmlands, and are known to contain diverse bioactive compounds, including alkaloids, flavonoids, tannins, and saponins. These phytochemicals have been associated with antimicrobial, insecticidal, and antiparasitic activities (Erhabor & Erhabor, 2024; Basha et al., 2024). Plant extracts have been reported in numerous studies to have the potential to control schistosome-transmitting snails (Mtemeli et al., 2021). The present study therefore evaluated the molluscicidal activity of methanolic leaf extracts of *V. amygdalina* and *A. indica*, individually and in combination, against adult *Bulinus globosus* under laboratory conditions.

MATERIALS AND METHODS

Study Area

The study was carried out at Minna, Niger State, North-Central Nigeria (9°35′N, 6°33′E). The region has a tropical climate with wet seasons (April–October) and dry seasons (November–March). In addition to seasonal patterns, the area supports freshwater habitats suitable for snail breeding due to the presence of slow-moving or stagnant water bodies, moderate temperatures, near-neutral pH, and abundant aquatic vegetation. These environmental conditions collectively promote the proliferation of freshwater snails, particularly *Bulinus globosus*, which serve as intermediate hosts in the transmission of schistosomiasis.

Plant Collection and Processing

Fresh leaves of *Vernonia amygdalina* and *Azadirachta indica* were collected from the premises of the Federal University of Technology, Minna. To get rid of any dirt, the leaves were rinsed with distilled water and then left to air-dry at room temperature (25–30 °C) for three to four weeks. Dried leaves were ground into fine powder using an electric blender.

Preparation of Methanolic Extracts

500g of powdered leaf material from each plant were subjected to Soxhlet extraction using methanol as solvent for five hours. A rotary evaporator was used to concentrate the extracts under reduced pressure at 40 °C, and they were then kept in airtight containers at 4 °C until needed.

Snail Collection and Maintenance

Adult *Bulinus globosus* snails were collected from freshwater bodies in Bosso Local Government Area using a scoop net between 07:00 and 09:00 hours. Snails were transported to the laboratory in containers containing the source water, which was maintained under ambient laboratory conditions, specifically at room temperature (25–30 °C) and near-neutral pH. The stocking density was maintained at ten snails per litre and identified using standard WHO identification keys (WHO, 2020). Cercarial shedding was assessed using the light-induced method, and infected snails were excluded from the study. Snails were fed blanched lettuce (*Lactuca sativa*) every other day, and water was renewed twice weekly.

Molluscicidal Bioassay

Molluscicidal assays were conducted following standard procedures described by Otarigho (2012). One gram of the crude methanolic extract was dissolved in 500 milliliters of distilled water to create a stock solution. Serial dilution was used to prepare working concentrations of 0, 16, 32, 64, and 128 mg/L. The selected concentration range was based on preliminary range-finding tests and guided by previously reported molluscicidal studies (Danlami et al., 2025; Erhabor and Erhabor, 2024), which indicated that plant-derived crude extracts typically exhibit bioactivity within this concentration range. The gradient was designed to span from sub-lethal to potentially lethal doses to enable accurate determination of concentration-dependent effects and facilitate estimation of lethal concentrations (e.g., LC_{50} and LC_{90}). Ten adult snails were exposed to each concentration in 500 mL of test solution, with each treatment performed in triplicate. Control groups contained distilled water only. Mortality was recorded at 1, 4, 6, 12, 24, and 48 hours. Snails were considered dead if they showed no movement and failed to retract upon probing.

Phytochemical Screening

Alkaloids, flavonoids, glycosides, saponins, tannins, phenols, and steroids were found in the extracts using qualitative phytochemical screening utilizing conventional techniques.

Statistical Analysis

The mean ± standard deviation was used to express the mortality statistics. The LC50 and LC 90 values at 24 and 48 hours were estimated using probit analysis. Using SPSS version 23.0, one-way analysis of variance was carried out independently for every exposure duration. Duncan's

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multiple range test was then used to determine mean separation. The threshold for statistical significance was set at $p < 0.05$.

RESULTS

Qualitative Phytochemical Composition

Qualitative screening revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, phenols, and steroids in both *A. indica* and *V. amygdalina* leaf extracts (Table 1)

Table 1: Qualitative Phytochemical Constituents of *Azadirachta indica* and *Vernonia amygdalina* Leaf Extracts

Phytochemical constituents	Azadirachta indica	Vernonia amygdalina
Alkaloids	+	+
Flavonoids	+	+
Glycosides	+	+
Saponins	+	+
Tannins	+	+
Phenols	+	+
Steroids	+	+

Footnote:(+) present; (−) absent.

Molluscicidal Activity

Both plant extracts produced concentration and time-dependent mortality in *B. globosus,* with no mortality observed in control groups. For the individual extracts, mortality increased markedly at higher concentrations, with complete mortality recorded at 128 mg/L after 48 hours of exposure. The combined extract also produced increased mortality with prolonged exposure,

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although higher concentrations were initially required to achieve comparable effects (Tables 2–3).

Table 2: Mean Mortality of *Bulinus globosus* Exposed to Methanolic Leaf Extracts of *Vernonia amygdalina*, *Azadirachta indica*, and Their Combination.

Treatment (mg/L)	Extract Type	1 h	4 h	6 h	12 h	24 h	48 h
Control	*V. amygdalina*	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	A. indica	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	Combined	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
16	*V. amygdalina*	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
	A. indica	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	2.50±0.50[b]
	Combined	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	1.00±0.00[b]	3.00±0.00[b]
32	*V. amygdalina*	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	1.00±0.00[a]	3.00±0.00[b]	5.00±0.00[b]
	A. indica	0.00±0.00[a]	0.00±0.00[a]	0.50±0.50[a]	1.00±0.00[b]	2.00±0.00[b]	3.00±0.00[c]
	Combined	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	1.00±0.00[b]	2.00±0.00[c]	4.50±0.50[c]

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Treatment (mg/L)	**Extract Type**	**1 h**	**4 h**	**6 h**	**12 h**	**24 h**	**48 h**
64	***V. amygdalina***	**0.00±0.00[a]**	**0.00±0.00[a]**	**0.00±0.00[a]**	**1.00±0.00[a]**	**3.50±0.50[b]**	**6.00±0.00[c]**
	A. indica	**0.00±0.00[a]**	**0.50±0.50[a]**	**2.00±0.00[b]**	**2.00±0.00[c]**	**4.00±0.00[c]**	**7.00±0.00[d]**
	Combined	**1.00±0.00[a]**	**2.00±0.00[b]**	**2.00±0.00[b]**	**2.50±1.00[c]**	**4.00±0.00[d]**	**5.00±0.00[c]**
128	***V. amygdalina***	**0.00±0.00[a]**	**0.00±0.00[a]**	**0.00±0.00[a]**	**3.00±0.00[b]**	**6.50±0.50[c]**	**10.00±0.00[d]**
	A. indica	**0.00±0.00[a]**	**5.00±0.00[b]**	**7.00±0.00[c]**	**10.00±0.00[d]**	**10.00±0.00[d]**	**10.00±0.00[e]**
	Combined	**1.00±0.00[a]**	**2.00±0.00[b]**	**2.00±0.00[b]**	**3.00±0.00[c]**	**5.50±1.00[d]**	**9.50±0.50[d]**

Values with different superscripts within the same column are significantly different at $p < 0.05$.

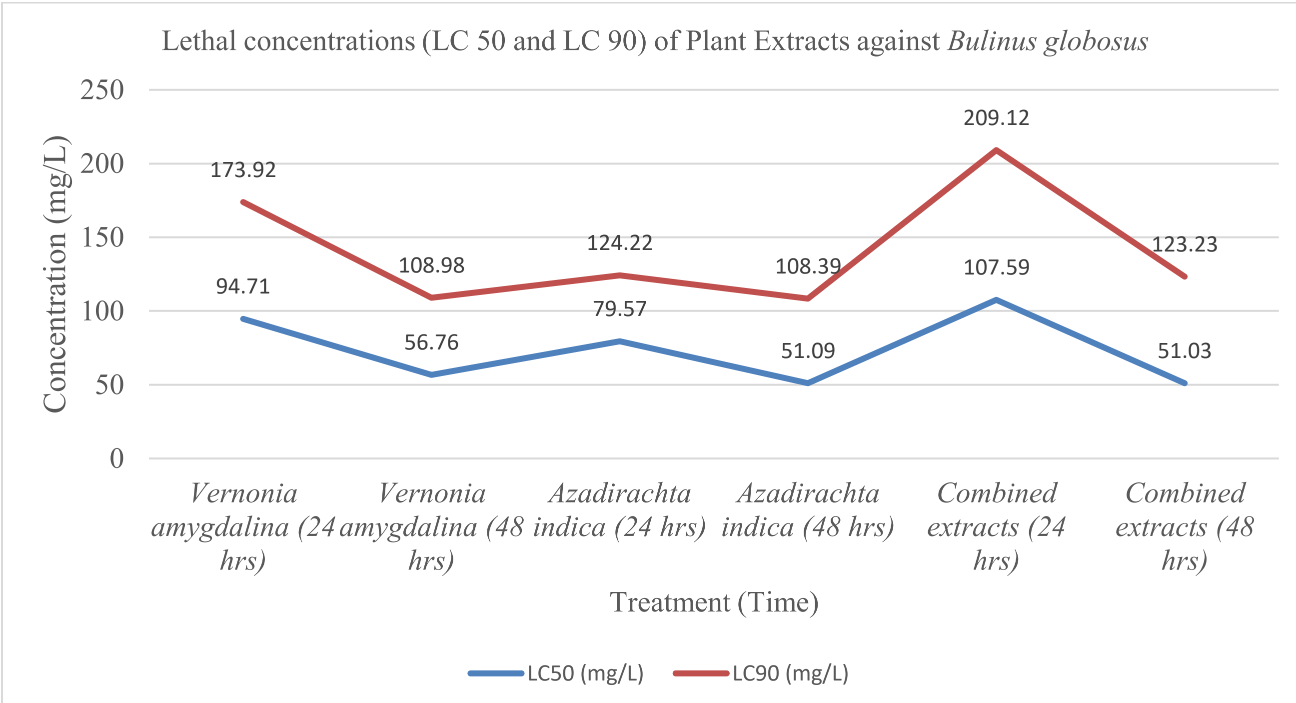

Figure 1: Lethal concentrations (LC 50 and LC 90) of methanolic leaf extracts against *Bulinus globosus*

DISCUSSION

The present study demonstrates that methanolic leaf extracts of *Vernonia amygdalina* and *Azadirachta indica* possess significant molluscicidal activity against adult *Bulinus globosus*, characterised by clear concentration- and time-dependent effects. These findings align with the growing body of evidence supporting the use of plant-derived products as viable alternatives to synthetic molluscicides in schistosomiasis control programmes.

The observed molluscicidal activity is likely attributable to the diverse phytochemical constituents detected in both extracts. Compounds such as saponins and tannins are known to disrupt cell membrane integrity, leading to increased permeability and eventual cell lysis, while alkaloids and flavonoids may interfere with neuromuscular coordination and metabolic pathways in molluscs. The presence of these bioactive compounds provides a plausible mechanistic basis for the mortality patterns observed in this study and is consistent with reports from previous investigations involving plant-based molluscicides (Mandefro et al., 2017; Mtemeli et al., 2021).

Comparatively, *A. indica* exhibited slightly lower LC50 values than *V. amygdalina* at 48 h, suggesting a higher molluscicidal potency under the experimental conditions. This observation corroborates earlier studies that have highlighted the strong bioactivity of neem-derived compounds against snail vectors (Danlami et al., 2025; Acheampong et al., 2020). Nevertheless, the molluscicidal performance of *V. amygdalina* was also notable, highlighting its potential utility as a locally available and affordable control option.

The combined extract demonstrated enhanced molluscicidal effectiveness over prolonged exposure, although the results suggest an additive rather than a conclusively synergistic interaction. While combined plant extracts may broaden the spectrum of bioactive compounds acting on target organisms, definitive conclusions regarding synergism require more rigorous analytical approaches, such as isobologram or combination index analyses. Future studies employing such methods would be valuable in elucidating interaction dynamics between plant-derived molluscicides.

From a public health perspective, the use of plant-based molluscicides offers several advantages, including reduced environmental persistence, lower toxicity to non-target organisms, and improved community acceptability when compared with synthetic chemicals (Das, 2022). Incorporating such botanicals into integrated schistosomiasis control strategies could complement existing measures such as mass drug administration and environmental management, thereby contributing to more sustainable disease control.

CONCLUSION

The findings of this study demonstrate that methanolic leaf extracts of *Vernonia amygdalina* and *Azadirachta indica* exhibit significant, concentration and time-dependent molluscicidal activity against *Bulinus globosus*, an important intermediate host of schistosomiasis. The relatively low LC 50 and LC 90 values obtained further highlight their effectiveness, both individually and in combination. These results suggest that the studied plants possess potent bioactive compounds capable of disrupting snail survival, thereby offering a viable means of reducing disease transmission. Given their availability, biodegradability, and potential cost-effectiveness, these plants' extracts represent promising eco-friendly alternatives to synthetic molluscicides.

RECOMMENDATIONS

Further studies should investigate the effects of the extracts on different developmental stages of *Bulinus globosus* (eggs, juveniles, and adults) to determine stage-specific susceptibility and optimize control strategies. There would also be a need to examine the effects of the extracts on different developmental stages of *Bulinus globosus* (eggs, juveniles, and adults) to determine stage-specific susceptibility and optimize control strategies. The study did not perform toxicity tests on non-target organisms. It is therefore recommended that further studies should conduct comprehensive toxicity assessments on non-target aquatic organisms to establish environmental safety profiles. and isolate active bioactive compounds responsible for molluscicidal activity, characterized, and quantified to enhance reproducibility, dosage precision, and potential formulation into standardized products.

AUTHORS' CONTRIBUTION

MIO and AKA conceptualized the study. MIO, AKA, YH, and HM designed the study. MIO, AKA, YH and HM participated in fieldwork and data collection. AKA, YH and HM performed the data analysis; MIO and AKA interpreted the data. MIO prepared the first draft of the manuscript, reviewed by AKA, YH and HM. All authors contributed to the development of the final manuscript and approved its submission.

CONFLICT OF INTEREST

None

STATEMENT OF INFORMED CONSENT

There are no human subjects in this article and informed consent is not applicable.

FUNDING

The study did not receive any external funding.

REFERENCES

Acheampong, D. O., Owusu-Adzorah, N., Armah, F. A., Aninagyei, E., Asiamah, E. A., Thomford, A. K. & Anyan, W. K. (2020). Ethnopharmacological evaluation of schistosomicidal and cercaricidal activities of some selected medicinal plants from Ghana. *Tropical Medicine and Health*, 48: 19. https://doi.org/10.1186/s41182-020-00205-y

Augusto, R., Tetreau, G., ChanMarie-Laure, P., Balieu, W., Claudia,M., Santos, P. & Grunau, C. (2017). Double impact: natural molluscicide for schistosomiasis vector control also impedesthe development of *Schistosoma mansoni* cercariae into adultparasites. *PLoS Negllected Tropical Diseases,* 11**:** 1 - 19. https://doi.org/:10.1371/journal.pntd. 0005789.

Basha, H., Debella, A., Endale, M., Debebe, E., Mathewos, M., Biftu, T. & Mamo, H. (2024). Molluscicidal activity ofextracts and fractions from *Hagenia abyssinica*, *Rosa abyssinica*, and *Cucumis ficifolius* Against *Biomphalaria* and *Bulinus* Snails. *Journal of Parasitology Research,* 25: 1–9. https://doi.org/:10.1155/japr/7968654. PMID: 39624255; PMCID: PMC11611416.

Buchwald, A. G., Grover, E., Van Dyke, J., Kechris, K., Lu, D., Liu, Y., Zhong, B. & Carlton, E. J. (2021). Human Mobility Associated with Risk of Schistosoma japonicum Infection in Sichuan, China. *American Journal of Epidemiology*, 190(7): 1243–1252. https://doi.org/10.1093/aje/kwaa292.

Coelho, P. & Cadeira, R. (2016). Critical analysis of molluscicide application in schistosomiasis control programs in Brazil. *Infectious Diseases of Poverty*. 5 (1): 57. https://doi.org/:10.1186/s4024901601536.

Das, B. (2022). Efficacy of some plant derived molluscicides for controlling pestiferous slug,Laevicaulis alte in Odisha. *Journal of Emerging Technologies and Innovative Research,* 9(7): 1-11

Danlami, A. U., Abolarinwa, S. O., Oladosu, M. A., Abah, M. A., Imade, B., Agbo, O. S., Agbolagade, F., Amuchechukwu, K. E., Olaitan, S. L., Farinde, T. D., Farinde, O. N., Ugwah, E. J., Oluwaseun, J. S. & Abah, S. O. (2025). Assessment of bioactive compounds and molluscicidal activity of neem (*Azadirachta indica*) and bitter leaf (*Vernonia amygdalina*) extracts against *Biomphalaria pfeifferi*, a schistosomiasis vector. *Plant Science Archives,* 10(4): 63-69. https://doi.org/10.51470/PSA.2025.10.4.63

Eastham, G., Fausnacht, D., Becker, M. H., Gillen, A. & Moore, W. (2024) Praziquantel resistance in schistosomes: a brief report. *Frontiers of Parasitology.* 3: 1471451. https://doi.org/10.3389/fpara.2024.1471451

Erhabor, A. P. & Erhabor, O. P. (2024). Comparative analysis of phytochemical composition of aqueous extracts from *Azadirachta indica* and *Vernonia amygdalina. Sciences of Phytochemistry*. 3(2): 91-97.

Kokaliaris, C., Garba, A., Matuska, M., Bronzan, R. N. & Colley, D. G., (2022). Effect of preventive chemotherapy with praziquantel on schistosomiasis among school-aged children in sub-Saharan Africa: a spatiotemporal modelling study. *Lancet Infectious Diseases.* 22(1): 136-149. https://doi.org/10.1016/S1473-3099(21)00090-6.

Macrotrends (2025). Minna Population. https://www.macrotrends.net/global-metrics/cities/23540/minna/population.

Mandefro, B., Mereta, S. T. & Tariku, Y. (2017). Molluscicidal effect of *Achyranthes aspera* L. (Amaranthaceae) aqueous extract on adult snails of *Biomphalaria pfeifferi* and *Lymnaea natalensis*. *Infectious Diseases of Poverty.* 6: 133. https://doi.org/10.1186/s40249-017-0349-4

Mtemeli, F. L., Walter, I., Tinago, T. & Shoko, R. (2021). An assessment of the molluscicidal potential of *Cucurbita maxima* seed extracts on *Biomphalaria pfeifferi and Bulinus globosus* snails. *All Life*. 14(1): 244–255. https://doi.org/10.1080/26895293.2021.1901788

National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Parasitic Diseases and Malaria (2024). Laboratory identification of parasites of public health concerns. https://www.cdc.gov/dpdx/index.html.

Otarigho, B. (2012). Efficacy of *Chromolaena odorata* leaf extracts on *Biomphalaria pfeifferi* eggs in control of Schistosomiasis. *Zoology and Ecology.* 22: 1-4. https://doi.org/10.1080/21658005.2012.745269.

Oyetunde, T. O., Wander, J. J., Rafaella, F. & Queiroz, G. (2020). Schistosomiasis in Nigeria: Gleaning from the past to improve current efforts towards control. *One Health*, 11: 2352-7714. https://doi.org/10.1016/j.onehlt.2020.100183.

Samah, I. G., Hanaa, M. A. & Shereen, M. M. (2024). Sensitivity of *Biomphalaria alexandrina* Snails and *Schistosoma mansoni* free larval stages to ethanol extract of *Jatropha gossypiifolia*. *Egyptian Journal of Aquatic Biology and Fisheries*. 28(1): 1255 – 1275. www.ejabf.journals.ekb.eg.

Şereflişan, H. & Duysak, Ö. (2021). Hibernation period in some land snail species (Gastropoda: Helicidae): Epiphragmal structure and hypometabolic behaviors. *Turkish Journal of Agriculture - Food Science and Technology.* 9: 166-171. https://doi.org/10.24925/turjaf.v9i1.166-171.3777.

Sokolow, S., Wood, C., Jones, I., Lafferty, K., Kuris, A., Hsieh, M. & DeLeo, G. (2018). To reduce the global burden of human schistosomiasis, Use 'Old fashioned' snail control. *Trends in Parasitology.* 34(1): 23–40. https://doi.org/10.1016/j.pt.2017.10.002.

Takougang, I., Meli, J., Wabo, Poné, J. & Angwafo, F. (2007). 3rd Community acceptability of the use of low-dose niclosamide (Bayluscide), as a molluscicide in the control of human schistosomiasis in Sahelian Cameroon. *Annals of Tropical Medicine and Parasitology,* 101: 479–486.

World Health Organization. (2026, January 19). *Schistosomiasis elimination review group*. https://www.who.int/news-room/articles-detail/schistosomiasis-elimination-review-group