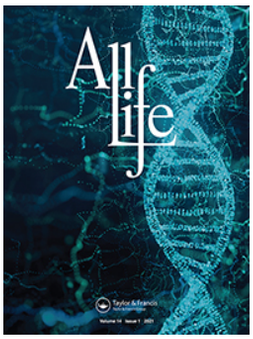

All Life

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tfls21

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To cite this article: Floryn L. Mtemeli, Irene Walter, Tawanda Tinago & Ryman Shoko (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, DOI: 10.1080/26895293.2021.1901788

To link to this article: https://doi.org/10.1080/26895293.2021.1901788

Published online: 29 Mar 2021.

ALL LIFE
2021, VOL. 14, NO. 1, 244–255
https://doi.org/10.1080/26895293.2021.1901788

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EPIDEMIOLOGY, GENETICS & GENOMIC

An assessment of the molluscicidal potential of *Cucurbita maxima* seed extracts on *Biomphalaria pfeifferi and Bulinus globosus* snails

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ABSTRACT

Reports of snails' resistance to niclosamide appeared recently and finding new molluscicides becomes necessary. We investigated the molluscicidal effects of *Cucurbita maxima* seed extracts on *Biomphalaria pfeifferi*, and *Bulinus globosus* snails under laboratory conditions. For *B. pfeifferi*, we tested seed extracts on juvenile and adult snails while only adult *B. globosus* were available for testing. Snails were exposed to water and crude ethanol extracts for 72 h and significant concentration-dependent mortality rates were observed. The number of *B. pfeifferi* juveniles collected was not enough for a comprehensive investigation against both solvents. We, therefore, tested them against water extracts only. A lethal concentration of 0.02 mg/mL killed 50% of the snails (LC50) for both water and ethanol extracts on adult *B. pfeifferi* snails. Our results suggest that pumpkin seed extracts have a significant molluscicidal effect on *B. pfeifferi* and *B. globosus* snails. The LC50 values for all the extracts in *B. pfeifferi* and *B. globosus* snails are within the threshold set for potential molluscicides by the World Health Organisation. We propose that *C.maxima* seed extracts be considered as potential molluscicidal agents in Schistosomiasis transmission control.

ARTICLE HISTORY
Received 16 October 2020
Accepted 7 March 2021

KEYWORDS
Schistosomiasis; *Biomphalaria*; *Bulinus*; molluscicidal activities; LC50

Introduction

Schistosomiasis (also known as Bilharzia) is a helmi nthic infection caused by a digenean trematode of the genus *Schistosoma* (Katsurada 1904). The disease leads to an estimated 200 000 deaths *per* year globally and at least 229 million people required treatment for the disease in 2018 alone (World Health Organisation (WHO) 2020). Like all digenetic trematodes, the intermediate host for schistosomes is the snails. In Zimbabwe, the snail vectors are *Bulinus globosus* for *Schistosoma haematobium* and *Biomphalaria pfeifferi* for *Schistosoma mansoni* (Chimbari 2012). Although Schistosomiasis is one of the most persistent Neglected Tropical Disease (NTD), treatment and disease control are based on the use of a single drug, praziquantel (PZQ), otherwise called biltricide (Sokolow et al. 2013).

Controlling or preventing morbidity in subjects using PZQ has not been entirely successful in restricting transmission in high-risk areas, as there have been reports of PZQ schistosomal resistance (Ismail et al. 1999; Qi and Cui 2012; Doenhoff et al. 2014; Augusto et al. 2017). WHO has set a roadmap that includes the elimination of Schistosomiasis as a public health problem by the year 2030 (WHO 2020) and efforts to achieve this goal using Mass Drug Administration (MDA) alone have proved inefficient. To address the inadequacy of current Schistosomiasis control endeavors and move towards its elimination, there is an urgent need to improve existing intervention measures (Molyneux et al. 2017; Krauth et al. 2019; Engels and Zhou 2020). As a responsive measure, in the light of its call to reach the elimination milestone, WHO discusses Schistosomiasis management through the ecological control of the intermediate host population of *Schistosoma* snails from the *Biomphalaria* and *Bulinus* genus (WHO 2014; Augusto et al. 2017). In 2017, WHO published an operational manual for the field use of molluscicides in Schistosomiasis control (WHO 2017) and supports member states in the implementation of snail control activities (WHO 2020). It is, therefore, largely agreed that the regulation of the snail

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population is an essential part of the control of Schistosomiasis (Bossier et al. 2019).

Molluscicide programmes have been employed in the control of Schistosomiasis for years (WHO 1961; McCullough 1980). Niclosamide is recommended by the WHO as the only chemical molluscicide to be used for snail control (WHO 1998) despite concerns of resistance of *Oncomelania* snails to the molluscicide (Dai Li and Wang 2014, 2018). The toxicity of niclosamide to non-target organisms such as fish is also of great concern. This is because the majority of the population that live in Schistosomiasis endemic areas rely on fish as a source of protein and income (WHO, 2020). The high cost of niclosamide also precludes its use in the endemic areas as they are usually characterised by low-income and high levels of poverty. Studies that will facilitate the development of new, affordable molluscicides with potentially low toxicity to non-target organisms are crucial (Sokolow et al. 2018). WHO, and other researchers, therefore, recommend further studies on plant molluscicides as these tend to be cost-effective and environmentally friendly (Coelho and Cadeira 2016; Augusto et al. 2017). The growing interest in plant-based molluscicides is also influenced by the fact that they degrade very rapidly when released into the environment, despite them being highly toxic to the snails (WHO 1983).

There has been a progression of several reports on the efficacy and molluscicidal activity of extracts from many researchers including Lemma et al. (1972) who discovered the molluscicidal activity of butanol extracts of *Phytolacca dodecandra* on *Biomphalaria* snails and Rug and Ruppel (2000) who studied the molluscicidal activity of *Jatropha curcas* on *Biomphalaria glabrata*, *Bulinus natalensis & Bulinus. truncatus* snails. Ojewole (2004) also found 14 of plants indigenous to South Africa to possess molluscicidal activity on *B. pfeifferi* and *Bulinus africanus*. Elsherbini et al. (2009) studied the lethal molluscicidal capability of some *Solanum* species on *Biomphalaria alexandrina* snails and their vulnerability to infection with *Schistosoma*. Victor (2015) studied the molluscicidal effects of *Ocimum americanum*, *Brideli amicrantha* and *Chenopodium ambrosoides*; Amalammar *et al.* (2016) found the molluscicidal effects of *Callistemon citrinus*, *Punica granatum* and pumpkin on *B. alexandrina* and Augusto et al. (2017) studied the double impact of *Euphorbia milii latex* on *S. mansoni* cercariae and their vectors, *Biomphalaria* snails.

Pumpkins (*Cucurbita maxima*) are known not only for their edible fruit but also for their several health benefits and thus have been used for a long time in traditional medicine in many countries such as Turkey and China (Young Kim et al. 2012). Pumpkin seeds have been used in different parts of the world as a traditional medicine for the treatments of gastrointestinal parasites such as anthelmintic (Ayaz et al. 2015), urinary dysfunctions, hyperplasia of the prostate, dysuria, cardiovascular disease, enuresis, and lowering blood glucose (Medjakovic et al. 2016; Lestari and Meiyanto 2018). Among the studies that have been done on pumpkin seeds, their anthelmintic potential has proved to be a success on *S. mansoni* (Beshay et al. 2018). However, there is a dearth of literature on the molluscicidal effects of pumpkin seeds on the vector snails. A successful trial of pumpkin seeds as a molluscicide would mean a double impact on both the vectors and the cercarial stage of the *S. mansoni* parasite in freshwater. Molluscicidal plant extracts may offer affordable, locally produced, biodegradable, and effectual control means in the rural parts of low-income countries where Schistosomiasis is prevalent (Hamed 2010).

In this study, we show that *C. maxima* seed water and ethanol crude extracts have molluscicidal effects against *B. globosus* and *B. pfeifferi*. To our knowledge, our work represents the first investigation to assess the molluscicidal activity of *C. maxima* extracts on the planorbid snails of the *B. pfeifferi* and *B. globosus* species. The work thus opens an opportunity for further research on the development of cost-effective alternatives for the control of Schistosomiasis based on natural compounds.

Materials and methods

Study site

The bio-assays and the seed extraction process were carried out in the Chinhoyi University of Technology biology and chemistry laboratories, respectively.

Collection of pumpkin seeds and snail vectors

Organic pumpkins were bought from a local supermarket in Chinhoyi, Zimbabwe. The pumpkins were thoroughly washed and cut to separate the seeds from the fruit.

B. globosus and *B. pfeifferi snails* were sampled using a sweep net in October 2018 at Madzorera dam in Murombedzi, Zimbabwe. Both snail species were identified using morphological keys according to Krauss (1848) and Morelet (1866). For *B. pfeifferi*, both adults and juveniles were obtained. However, only adults were obtained for *B. globosus*. The snails were kept in open plastic bottles and covered with moist cotton wool to keep them alive before reaching the laboratory.

Preparation of pumpkin seed ethanolic extracts

Pumpkin seeds were sun-dried for 72 h to a moisture content of 12.4%. Approximately 600 g of the seeds were milled into a fine powder using a mortar and pestle. The maceration technique was used to obtain the ethanolic crude extract. Thereafter, 900 ml of ethanol was added to 300 g of refined pumpkin seed powder and left in a dark cupboard for 7 days. At the end of this period, the mixture was filtered through a 0.1 mm Whatman filter paper grade using an EC vacuum pump (WP6122050) and then concentrated to dryness using a Buchi Rotavapor (R205) at 78°C to obtain pure crystals of the extract. The crystals were weighed and a total yield of 5 g was obtained. The crystals were dissolved in distilled water and the resulting solution of 100 mg/mL concentration was considered as the pure extract.

Preparation of pumpkin seeds water extracts

Approximately 600 ml of water was added to 300 g of fine pumpkin seed powder and left in a dark cupboard for 7 days. The mixture was filtered on 0.1 mm Whatman filter paper grade using an EC vapour pump (WP6122050) and the filtrate was concentrated to dryness using the Buchi Rotavapor (R205) to yield 8 g of crystals. The crystals were dissolved in 80 ml distilled water and the solution of 100 mg/ml concentration was considered as the pure extract.

Snail rearing

The snails were reared under laboratory conditions in plastic aquaria of 5 L holding capacity measuring 13 × 12 cm. The aquaria were provided with fresh water from the dams from which the snails were taken after every 2 days. No mud, sand, nor any other substratum was put in the aquaria. The laboratory in which they were kept was maintained at a room temperature of 25°C with natural fluctuations of +/−2°C for the length of the research. The snails were fed on oven-dried lettuce leaves (Chimbari and Shiff, 2008). The snails were allowed to acclimatise to laboratory conditions for 5 days.

Shedding of snails

To ensure that only healthy snails are used, cercariae were shed from the snails as described by El-sherbini et al. (2009), with modifications. Briefly, after being exposed to the dark for 8 h, snails were placed in 300 ml plastic bottles filled with non-chlorinated water and placed in direct sunlight for 8 h after which a drop of water from each of the bottles was then viewed under the light microscope to check for the presence of cercariae.

Molluscicidal activity assay

During the study, the snails were kept under normal diurnal lighting and room temperature. Snails were organised into 2 classes, based on their developmental stage and shell diameter. Snails with a shell diameter of less than 45 mm were considered to be juveniles while those with shell diameters above 45 mm were considered to be adults (Ciomperlik et al., 2013). Snails were deprived of food during the molluscicidal assays. Before the molluscicidal assays, preliminary molluscicidal assay tests were done to determine the minimum effective concentration. A range of 5 concentrations were randomly assayed; 20%; 40%; 60%; 80% and 100% of the ethanol and water extracts. A lethal effect in 2 h among all the concentrations was observed and, therefore, serial dilutions of the lowest concentration (20%) were used for the molluscicidal assays. A maximum of 6 serial dilutions of 20% of the pure water and ethanol extracts were made as *per* WHO guidelines (WHO 1983). The final concentrations of the water and ethanol extract serial dilutions were 20 mg/mL; 2 mg/mL; 0.2 mg/mL; 0.02 mg/mL; 0.002 and 0.0002 mg/mL to give 6 treatments for each solvent. A 0, 1 dilution of Thunder (Imidaclopride + Betacyfluthrine 100 + 45 g/L derivative) was prepared and used as positive control and plain dam water was used as a negative control. Thunder is currently used as a molluscicide in Zimbabwe. A second positive control of absolute ethanol was used to factor

Table 1. Showing design of the experiment.

Snail class	Treatment	Time of exposure		
Biomphalaria		24 hrs	48 hrs	72 hrs
adults	water extracts	R1 × 6concentrations R2 × 6concentrations R3 × 6concentrations	R1 × 6concentrations R2 × 6concentrations R3 × 6concentrations	R1 × 6concentrations R2 × 6 concentrations R3 × 6 concentrations
	ethanol extracts	R1 × 6concentrations R2 × 6concentrations R3 × 6concentrations	R1 × 6concentrations R2 × 6concentrations R3 × 6concentrations	R1 × 6concentrations R2 × 6concentrations R3 × 6concentrations
	positive control 1	R1 × 1concentration R2 × 1concentration R3 × 1concentration	R1 × 1concentration R2 × 1concentration R3 × 1concentration	R1 × 1 concentration R2 × 1 concentration R3 × 1 concentration
	positive control 2	R1 × 1concentration R2 × 1concentration R3 × 1concentration	R1 × 1concentration R2 × 1concentration R3 × 1concentration	R1 × 1 concentration R2 × 1 concentration R3 × 1 concentration
	negative control	R1 × 1concentration R2 × 1concentration R3 × 1concentration	R1 × 1concentration R2 × 1concentration R3 × 1concentration	R1 × 1 concentration R2 × 1 concentration R3 × 1 concentration
Biomphalaria juveniles	water extracts	R1 × 6concentrations R2 × 6concentrations R3 × 6concentrations	R1 × 6concentrations R2 × 6concentrations R3 × 6concentrations	R1 × 6concentrations R2 × 6concentrations R3 × 6concentrations
	positive control 1	R1 × 1concentration R2 × 1concentration R3 × 1concentration	R1 × 1concentration R2 × 1concentration R3 × 1concentration	R1 × 1 concentration R2 × 1 concentration R3 × 1 concentration
	positive control 2	R1 × 1concentration R2 × 1concentration R3 × 1concentration	R1 × 1concentration R2 × 1concentration R3 × 1concentration	R1 × 1 concentration R2 × 1 concentration R3 × 1 concentration
	negative control	R1 × 1concentration R2 × 1concentration R3 × 1concentration	R1 × 1concentration R2 × 1concentration R3 × 1concentration	R1 × 1 concentration R2 × 1 concentration R3 × 1 concentration
Bulinus adults	water extracts	R1 × 6concentrations R2 × 6concentrations R3 × 6concentrations	R1 × 6concentrations R2 × 6concentrations R3 × 6concentrations	R1 × 6concentrations R2 × 6concentrations R3 × 6concentrations
	ethanol extracts	R1 × 6concentrations R2 × 6concentrations R3 × 6concentrations	R1 × 6concentrations R2 × 6concentrations R3 × 6concentrations	R1 × 6concentrations R2 × 6concentrations R3 × 6concentrations
	positive control 1	R1 × 1concentration R2 × 1concentration R3 × 1concentration	R1 × 1concentration R2 × 1concentration R3 × 1concentration	R1 × 1 concentration R2 × 1 concentration R3 × 1 concentration
	positive control 2	R1 × 1concentration R2 × 1concentration R3 × 1concentration	R1 × 1concentration R2 × 1concentration R3 × 1concentration	R1 × 1 concentration R2 × 1 concentration R3 × 1 concentration
	negative control	R1 × 1concentration R2 × 1concentration R3 × 1concentration	R1 × 1concentration R2 × 1concentration R3 × 1concentration	R1 × 1 concentration R2 × 1 concentration R3 × 1 concentration

Key R1, R2 and R3 are replicates 1, 2 and 3 respectively.

into consideration the effects of residual ethanol in the ethanol extracts. Hence, inclusive of the Thunder and the 2 controls, 15 treatments were evaluated. The molluscicidal activity assays were set up simultaneously including all treatments and controls. Therefore, the controls were common for all assays.

Snails were sorted into 3 groups as follows: *B. pfeifferi* adults, *B. pfeifferi* juveniles and *B. globusus* adults. The snails from each group were assigned to a treatment and this was replicated 3 times. For the treatments, 10 ml of the 6 dilutions of pumpkin seeds extracts were mixed with 90 ml of dam water from where the snails were sampled. This was done to minimise the number of limiting factors that could affect the snails' metabolism during the trial experiment. The duration of exposure to the molluscicide dilutions and control was 3 days. After the first 24 h, the number of molluscs withdrawn into their shells, immobile and unresponsive to vigorous action was recorded. Snails that were unresponsive to forceful, mechanical

Table 2. Showing LC50 values of pumpkin seed extract on the 5 snail classes.

Molluscicide extract and snail class exposed	LC50 values
Water extracts on *B.pfeifferi* adults	0.002 mg/ml
Water extracts on *B.pfeifferi* juveniles	0.004 mg/ml
Water extracts on *B.globosus* adults	0.004 mg/ml
Ethanol extracts on *B.pfeifferi* adults	0.002 mg/ml
Ethanol extracts on *B.globosus adults*	0.19 mg/ml
Thunder control	0.000049 mg/ml

stimulation or probing were considered as dead. To further assure that the snails were indeed dead, they were placed in distilled water and observed for 2 h.

LC 50 determination and statistical analysis

Mortality percentages (LC50) were plotted against the log-transformed values of the extract concentrations using Graph pad Prism version 7.0 software (Finney, 1971) with a 95% confidence limit. The non-linear regression lines obtained from this data were used to determine the LC50 values.

A multifactor Analysis of Variance (ANOVA) was used to determine the significance of the effects of the C. *maxima* crude extracts on snail mortality. The ANOVA model selected allowed us to test the significance of the treatments whilst accounting for species and age. All the possible interactions were included in the model to determine whether the treatment effects varied with snail age and/or species. Non-significant interactions were discarded. An assessment of the model assumptions using plots on the residuals revealed that the assumptions were satisfied. Pairwise comparisons among the treatments were done using Tukey HSD to compare *C. maxima* crude extracts treatments and the respective concentrations against the positive control, Thunder. Data were considered statistically significant at $P = 0.05$. All tests were done using IBM SPSS Statistics (Version 25) Tables 1 and 2.

Results

Generally, *C. maxima* crude extracts induced a significant molluscicidal activity ($P < 0.00005$; $F = 27.762$; $df = 14$) (Figure 1). Furthermore, *C. maxima* crude extracts had the same molluscicidal activity regardless of species. However, the age of the snail influenced the

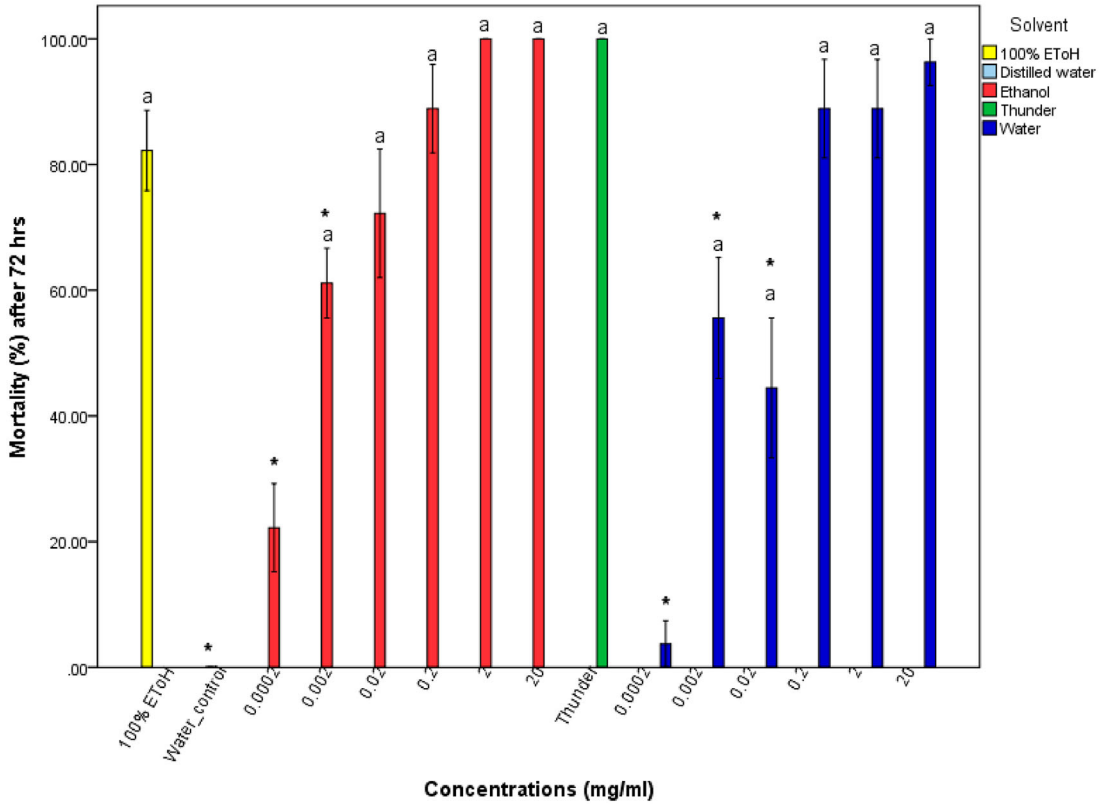

Figure 1. Molluscicidal activity of ethanol-based extracts and water-based extracts concentration of *C. maxima seeds* on *B. pfeifferi* and *B. globosus* snails. The asterisk indicates significance at $p < 0.05$ compared to the positive control Thunder. The letter 'a' indicates significance at $p < 0.05$ compared to the negative control. Error bars represent the standard error.

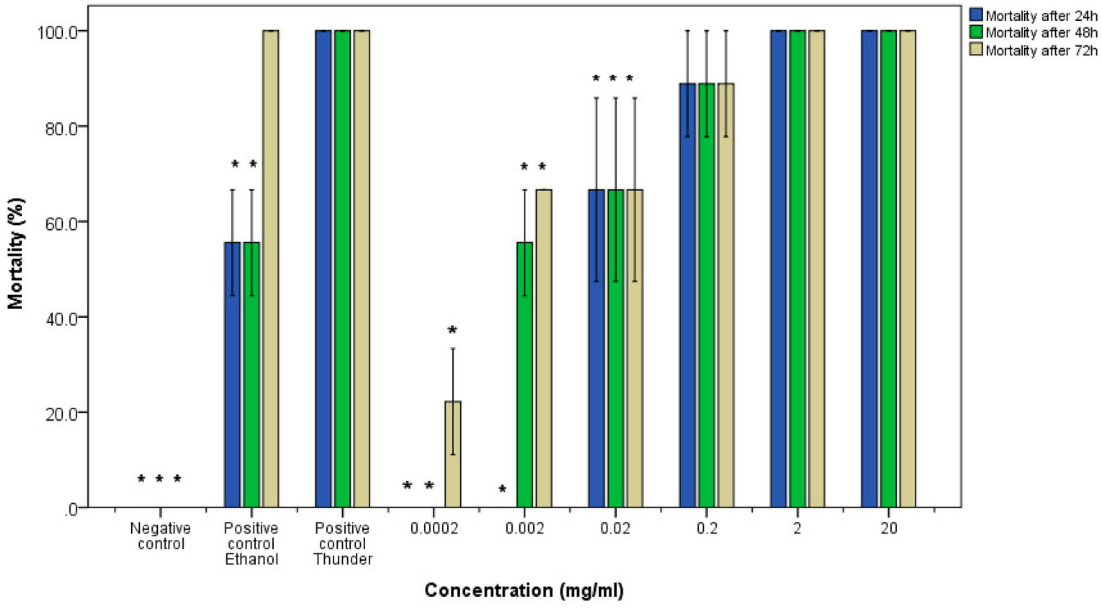

Figure 2. Molluscicidal activity of ethanol extracts of pumpkin seeds on adult *B. globosus* snails. The asterisk indicates significance at $p < 0.05$ compared to the positive control Thunder. Error bars represent standard error.

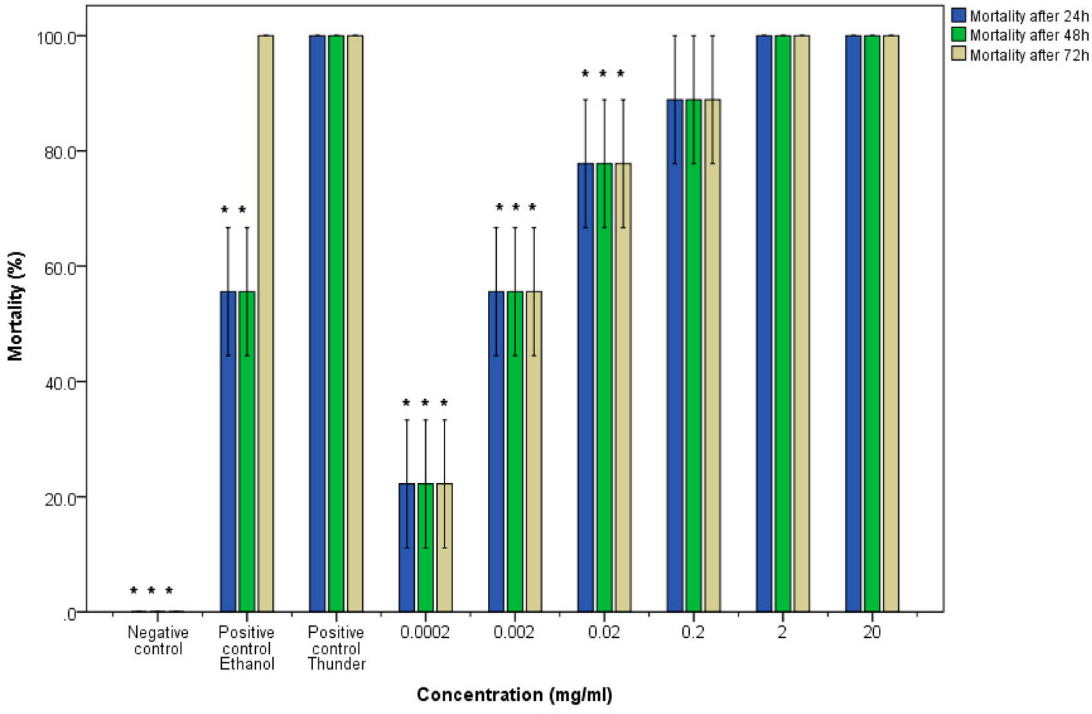

Figure 3. Molluscicidal activity of ethanol extracts of pumpkin seeds on adult *B. pfeiferri* snails. The asterisk indicates significance at $p < 0.05$ compared to the positive control Thunder. Error bars represent standard error.

efficacy of the *C. maxima crude* extracts ($P = 0.002$, $F = 9.778$; $df = 1$). The mean mortalities of snails also significantly differed according to the solvent used to prepare the *C. maxima* crude extracts ($P < 0.00005$; $F = 16.714$; $df = 4$). Ethanol-based extracts had significantly higher mean snail mortality compared to the water-based extracts. In addition, positive control of ethanol had a molluscicidal activity that was the same that of Thunder ($P = 0.311$). Four ethanol-based concentrations, 20, 2, 0.2 and 0.02 mg/mL had mean snail mortalities that were not significantly different from Thunder ($P > 0.05$). Three *C. maxima*

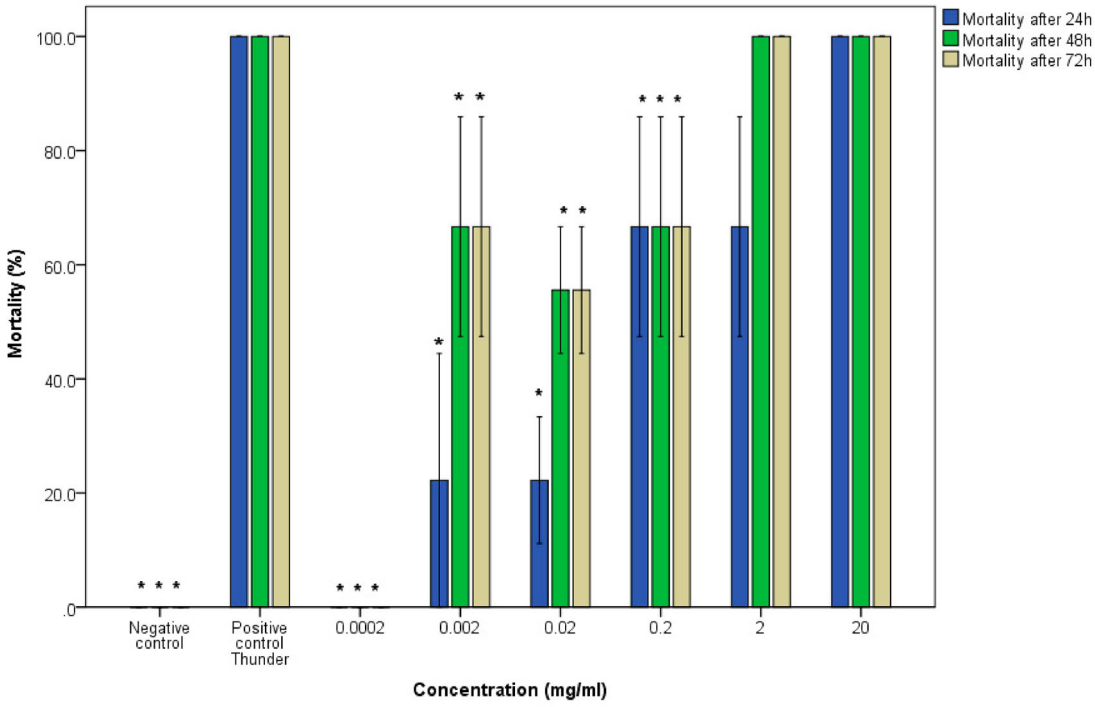

Figure 4. Molluscicidal activity of water extracts of pumpkin seeds on adult *B. globosus* snails. The asterisk indicates significance at $p < 0.05$ compared to the positive control Thunder. Error bars represent standard error.

water-based concentrations, 20, 2 and 0.2 mg/mL had mean snail mortality that was comparable to the positive control (Figure 1). Based on Pairwise comparisons using Tukey's HSD, the water extracts 20, 2 and 0.2 mg/mL had snail mortalities that were not significantly different from Thunder ($P > 0.05$) (Figures 2–4).

Results also showed that there was no significant difference between the survival rates of juvenile and adult *B. pfeifferi* snails exposed to water extracts (U = 123.5; $P = 0.197$; CI = 95%). Furthermore, there was no significance in the difference of species exposed to the water extracts (i.e. *B. pfeifferi* and *B. globosus*) ($U = 314.5$; $P = 0.854$; CI = 95%). The mortalities of *B. pfeifferi* adult snails were concentration-dependent and the molluscicidal activity of water extracts decreased with concentration with 0.0002 mg/mL showing no activity at all (Figure 5). *B. pfeifferi* juvenile snails that were exposed to water extracts of pumpkin seeds did not show uniform concentration-dependent mortalities with 0.2 mg/mL and 0.002 mg/mL dilutions causing abnormally higher mortalities than the subsequent stronger more concentrated dilutions (Figure 6). The average mortalities of the snails exposed to the positive and negative controls were uniform in all the trials.

The results also showed that there was no significant difference between the effects of the water and ethanol extracts on adult *B. pfeifferi* ($P = 0.875$; CI = 95%). The mortalities of *B. pfeifferi* adult snails exposed to ethanol extracts were concentration-dependent and the molluscicidal activity decreased with the extract concentration as shown by 0.0002 mg/mL showing no activity (Figure 5). Water extracts of pumpkin seeds induced concentration-dependent mortalities on the *B. pfeifferi* juvenile snails with the lowest concentration of 0.0002 mg/mL causing mortality.

Discussion

The search for bioactive plant components that can be used as non-conventional molluscicides and anti-helminths has received considerable attention in recent times because of the increasing development of resistance to chemical synthetic molluscicides in snail populations. At present, there is an expanded consideration for the use of new molluscicides which are profoundly successful, rapidly biodegradable, more affordable, and easily accessible with uncomplicated application procedures (Jia et al. 2019). However, scientific evidence to validate the use of plant-based molluscicides remains limited and

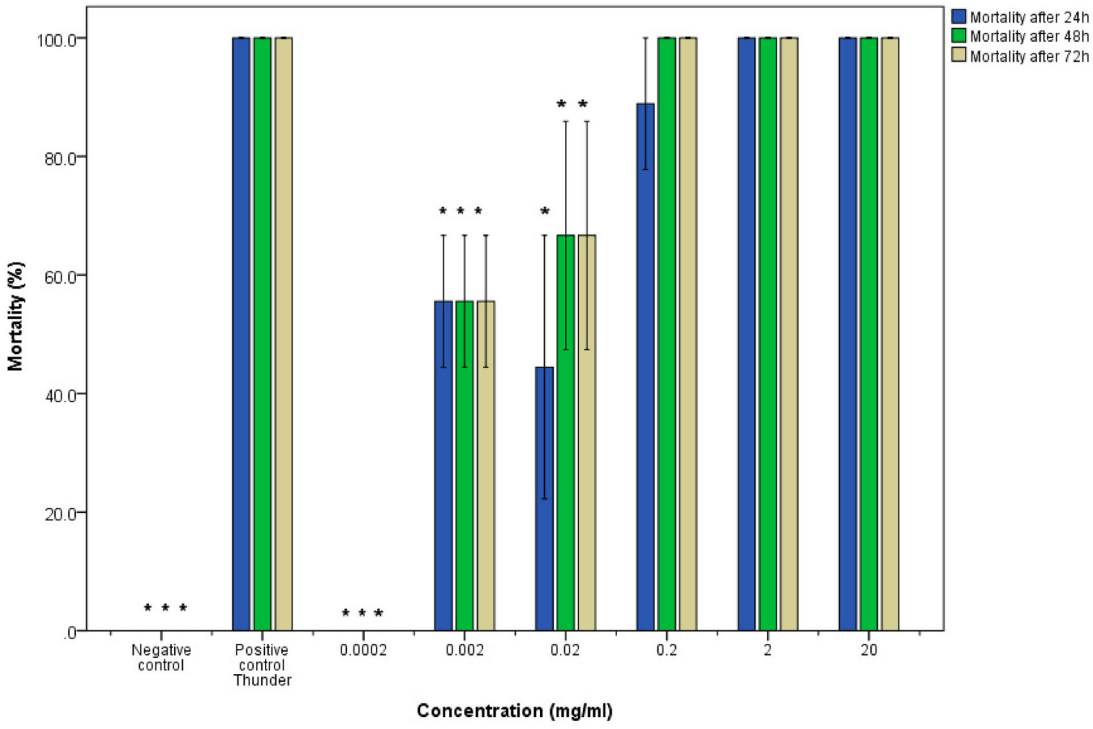

Figure 5. Molluscicidal activity of water extracts of pumpkin seeds on adult *B. pfeifferi* snails. The asterisk indicates significance at $p < 0.05$ compared to the positive control Thunder. Error bars represent standard error.

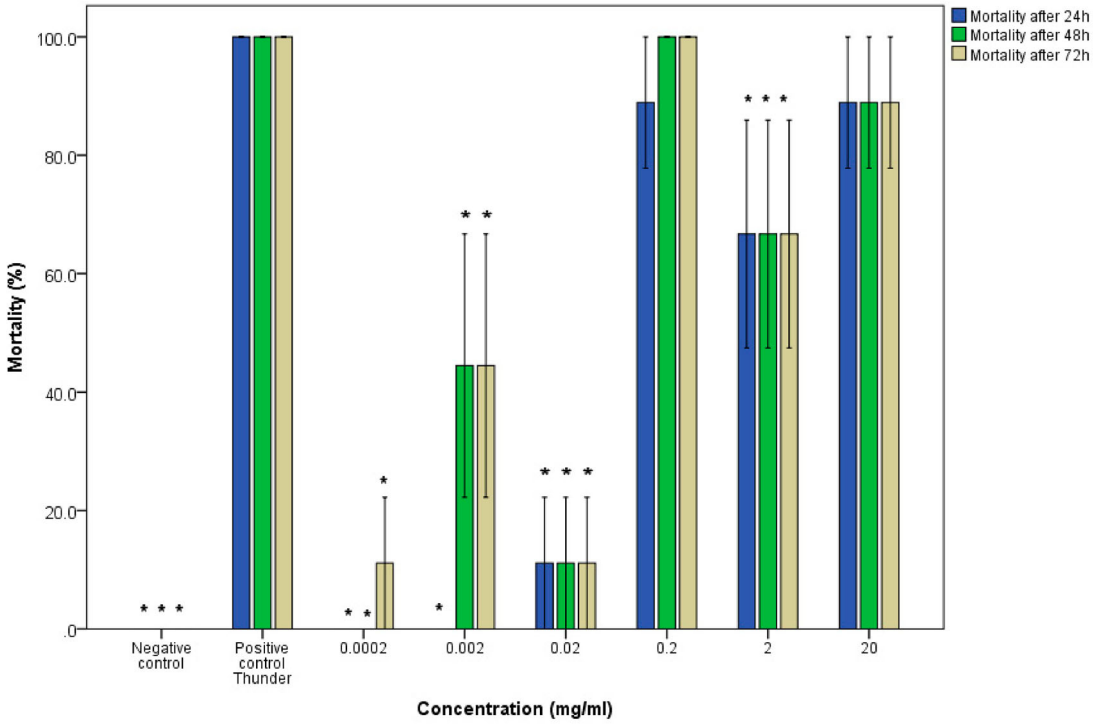

Figure 6. Molluscicidal activity of water extracts of pumpkin seeds on juvenile *B. pfeifferi* snail. The asterisk indicates significance at $p < 0.05$ compared to the positive control Thunder. Error bars represent standard error.

extensive investigations may help in understanding their properties and safety.

In the present study, we investigated the potential of pumpkin seed crude extracts molluscicide potential against adult and juvenile *B. pfeifferi* and adult *B. globosus* snails. We observed 100% mortalities in adult *B. pfeifferi* and *B. globosus* exposed to the high concentrations of both water and ethanol extracts. The potent molluscicidal activity exhibited by the *C.maxima* seed extracts in the present study is consistent with the high anthelminthic activity of *Cucurbita moschata* seed extracts (Marie-Magdeleine et al. 2009; Marie-Magdeleine et al. 2011). According to Marie-Magdeleine et al. (2009), the phytochemical analysis suggested that non-proteic amino acid cucurbitin was responsible for helminth mortalities through intoxication. Therefore, although there is paucity in literature on the molluscicidal activity of *C. maxima* seed extracts on snails, it is plausible that the mortalities observed in our study may be attributed to cucurbitin. The susceptibility of *B. pfeifferi and B. globosus* snails to the extracts could be attributed to the fact that they have no operculum; thus, their cephalopodia were continuously in contact with the molluscicide during the assays (He et al. 2017).

Molluscicides can induce death by disrupting physiological processes for example, a decrease in the heart rate, swelling of tissues, and change in the water balance (McCullough 1980; Clark and Appleton 1996). Furthermore, He et al. (2017) demonstrated that molluscicidal activity and mechanism of toxicity of a salicylanilide ester derivative against *B. pfeifferi* species could be based on an effect on neurohypophysis transmission and energy metabolism. There, however, is no current evidence to support that a common mechanism of action is responsible for these actions. Studies that evaluated the molluscicidal activity of three mangrove species (*Avicennia schaueriana*, *Laguncularia racemosa* and *Rhizophora mangle*) on the biological activities of *B. glabrata* show that sapponins and tannins are essential for effectual molluscicidal activity (Mendes et al. 2018). Further evidence is presented by studies on the molluscicidal activity of hydroalcoholic extracts of *Jatropha gossypiifolia* Linnaeus, 1753 on *B. glabrata* (Filho et al. 2014). The presence of these saponins and tannins in *C. maxima* seeds (Muchirah et al. 2018) could be a major contribution to their molluscicidal activity. However, further research is needed to discover all the specific compounds responsible for the molluscicidal activity of pumpkin seeds.

Solvents play an important role in the extraction of phytocompounds and this may influence the activity of the compounds (Babbar et al. 2014). Accordingly, our results on the molluscicidal activity of *C. maxima* seeds showed that ethanol-based extracts overall had a higher molluscicidal activity compared to the water-based extracts. Consistently, several studies have also highlighted that ethanol extracts generally induce higher mortalities than water extracts (Singh and Singh 1998; Tripathi and Singh 2000; Elsherbini et al. 2009). In addition, given that ethanol at 5% concentration can be used as an anaesthesia in physiological studies of snails (Gilbertson and Wyatt 2016; d'D'ovidio et al. 2019), a 100% concentration of ethanol will likely be lethal. This explains why the positive control of ethanol was as effective as to the Thunder.

It is important to note that three *C. maxima* water extract concentrations (20, 2 and 0.2 mg/mL) exhibited a molluscicidal efficacy significantly similar to Thunder. Furthermore, there were no mortalities recorded for the negative control of water. This suggests that the significant mortalities observed from the snails treated with the *C. maxima* water extracts can be attributed to compounds from the *C. maxima* seeds. As such this can be an indication that water is capable of extracting phytochemical compounds concentrations that are potent. This is advantageous because the ultimate goal is to attain effective active ingredients that can be used at the most convenient and cheapest way possible by the communities.

In general, a combined analysis of *B. pfeifferi* and *B. globosus mortalities* demonstrated a significant interaction between the *C. maxima* extracts and the age of the snail. Susceptibility to plant molluscicides is influenced by size. In some cases, adult snails have been more susceptible compared to juveniles because adults have a large body which offers a large surface for absorption of plant molluscicide extracts (Rawi et al. 2011). In contrast, Syombua et al. (2013) and Obare et al. (2016) found juvenile *B. pfeifferi* snails to be more susceptible to toxic effects of extract than adults. In the present, there was no significant difference between the survival rates of juvenile and adult *B. pfeifferi* snails exposed to *C. maxima* water extracts. We postulate that lack of consensus as to whether juveniles are more

susceptible or *vice versa*, maybe to differences in the potency of plant molluscicides.

In conclusion, our results suggest that pumpkin seeds have a significant molluscicidal effect on *B. pfeifferi* and *B. globosus* snails. Throughout the study, high concentrations of both ethanolic and water extracts of pumpkin seeds showed substantial molluscicidal activities with LC50 values that were well below the upper threshold of 40 mg/L set for a potential molluscicide by the WHO (WHO, 2017). We demonstrated a huge potential of formulating an effective yet affordable and locally sourced pumpkin seed extract that can be an adopted molluscicide to be used as a snail control strategy in the reduction of Schistosomiasis transmission. Therefore, we propose that pumpkin seed extracts be categorised as one of the natural products that be considered as molluscicidal agents in a bid to control the transmission of Schistosomiasis. However, the elucidation of the molluscicidal activity requires studies that reveal details regarding the phytochemical profile of the plant. We, therefore, recommend future studies to include chromatographic analysis to analyse the phytochemical profile of the water and ethanol extracts of the pumpkin seed. Such an approach would allow researchers to identify more applications of the pumpkin seed extracts, such as the development of new drugs, and seeking molluscicidal compounds from plants which might impact transmission control of not only Schistosomiasis but other NTDs whose parasites are vectored by aquatic snails. Also it is important that further studies be carried out to test the toxicity of the pumpkin seed extracts on other taxonomic groups, e.g. invertebrates, small fish and others aquatic organisms to ensure that the extracts would not cause more ecological harm in the process of controlling snail populations.

Acknowledgements

We thank the Chinhoyi University of Technology for funding this research.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Statement of informed consent

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

Articulation of human and animal rights

Ethical clearance to conduct this study was granted by the Biology Department of the Chinhoyi University of Technology.

Funding

The research was funded by the Chinhoyi University of Technology.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analysed in this study. The paper can be understood without any further data sets other than the ones provided in the tables and figures.

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