Original Research Article

Molluscicidal Assessment of Aqueous Extract of *Moringa oleifera* Lam seed on *Bulinus* Snail for the control of Schistosomiasis

ABSTRACT

Aims: This research is essentially on the Molluscicidal assessment of Aqueous extract of *Moringa oleifera* Lam seed on *Bulinus* Snail for the control of Schistosomiasis.

Study Design: This is a controlled study where a total of 810 Bulinus snails were collected from three different streams with each stream representing a community in three senatorial zones of Anambra state where schistosomiasis is endemic. Aqueous extract of *Moringa oleifera* (Lam) seed at different concentrations were used on the *Bulinus* snails to determine their molluscidal properties.

Place and duration of study: Two hundred and Seventy (270) Bulinus snails were collected from streams representing each of the three senatorial zones Of Anambra state viz; Obutu Lake, Omogho town, Orumba North Local Government Area (Anambra South), Agulu Lake, Agulu town, Aniocha Local Government Area (Anambra Central) and Omambra River , Omor town, Ayamelum Local Government Area (Anambra North) This research was carried out between November 2018 to April 2019.

Methodology: Aqueous dilutions of the grinded *Moringa oleifera* (Lam) seed were exposed to the *Bulinus* snails from the various streams for 24 hours, after which the snails were removed from the experimental test solution and washed thoroughly with dechlorinated tap water and transferred to containers with fresh dechlorinated tap water for another 24 hours of recovery. The snails were incubated at 28 ± 5 °C and fed with lettuce leaves.

Results: Aqueous *M*oringa *oleifera* Lam seed extract was toxic to *Bulinus* adult snails in a dose dependent manner and the total Lethal Concentration (LC_{50} and LC_{90}) values determined after 24 hours exposure from the whole streams were 468 ppm and 813 ppm respectively. *Moringa oleifera* seed extract was toxic to the snails even at low concentrations.

Conclusion: Aqueous seed extract of *Moringa oleifera* Lam is toxic to *Bulinus* snail even at low concentrations.

Key words: *Bulinus*, *Moringa oleifera*, Schistosomiasis, Streams, Anambra State

INTRODUCTION;

Schistosomiasis is a neglected tropical parasitic infection (1, 2), with high prevalence in Nigeria (3). It is a snail-borne trematode infection, a blood flukes from the genus *Schistosoma* which according to (4), is endemic in 74-76 developing countries especially in impoverished communities without access to a sound public health system and has been ranked second to malaria in terms of socio-economic and public health significance (5,6).

Snail intermediate hosts play active roles in the transmission of Schistosomiasis infections in Africa. They are the sites of an intense multiplication of parasites. Most of the diseases caused by snail-borne trematodes are prevalent in the tropic and sub-tropic regions of the world, and the medical and economic burden of these diseases are often neglected which is why they are called Neglected Tropical Diseases (NTDs).

A lot of control strategies have been employed in combating this disease, the snail control strategies are considered a priority for the reduction of schistosomiasis transmission. *Bulinus* is a genus of small tropical fresh water snails, function as intermediate hosts for the schistosomiasis blood fluke (7).

Control of *Bulinus spp.* comprises an important element in integrated strategies for reducing the spread of schistosomiasis, and the single molluscicide recommended by the World Health Organization is the organic compound niclosamide. This molluscicide possesses low toxicity to mammals; however, it is toxic to fishes, amphibians, and crustaceans (8,9). Also due to the high cost, environmental contamination, and possible development of snail resistance to chemical molluscicides, natural molluscicides are rapidly being developed (10). Many plant extracts are potential molluscicides that are environmentally friendly, less toxic and are less likely to cause snails to develop resistance (11).

Moringa oleifera Lam plants have provided a number of useful clinical agents that prove to have considerable potentials as source of new drugs (12). The aqueous extract of *Moringa oleifera* Lam seeds contain bioactive molecules including saponins, lectins, (13), and volatile oils (14). There are two types of lectins: coagulant *Moringa oleifera* Lam lectin (cMoL) which is a basic protein and water-soluble; *Moringa oleifera* lectin (WSMoL) which is an acidic protein (15, 16). These proteins have ability to agglutinate erythrocytes (17) and show coagulant activity that reduces water turbidity (18). Recent studies showed that *M. oleifera* Lam lectins had ovicidal effects on Aedes aegypti (19) and insecticidal potential against *Anagasta kuehniella* (especially cMoL) (20). Also, the seed powder of *Moringa oleifera* Lam had a molluscicidal activity against the snails *Biomphalaria glabrata* and *Physa marmorata* (21, 22). Therefore, the use of medicinal plants which grow abundantly in areas where Schistosomiasis is endemic may become a useful complement either as molluscicides or chemotherapeutic for the control of the disease. Medicinal plants are promising choices that may grow the scope of molluscicides accessible for controlling of *Bulinus* snails as these plants are less costly, safer and having a high level of degradability (23).

METHODOLOGY

Study Area: The study area Anambra state is made up of three senatorial zones viz: North, Central and South. The senatorial districts were used as yardstick for partitioning of the study area. Anambra state is in the south eastern part of Nigeria and lies on the latitude 5° 40′ N and 6° 45N and 6° 35′E and 6° 30′E). Hydrologically, Anambra composition include rivers , streams, lakes and ponds with River Niger and Anambra River being the largest and longest with several tributaries that give rise to many streams and streamlets. Thus the study was conducted in three selected communities from the three L.G.A's. The towns were Agulu (6.0923°N, 7.0411°E), Omogho 6.3642°N, 6.8163°E) and Omor (6.5224°N, 6.9281°E) Figure 1. Other rivers and streams include; Nkissi, Anakwaeze, Ulasi, Otolo, Ekulo, Aghammiri, Mamu, Obizi, Egi, Oyi, Ariba, Aroka.

Ethical Consideration: A letter was obtained from the Head of Department of Biological Sciences Chukwuemeka Odumegwu Ojukwu University to Anambra State Ministry of Education. Informed consents were obtained from the various community leaders through a letter from the state Ministry of Education to assist in the collection of materials/samples for the research work.

Study Design

Sources of Bulinus Snail: A total of 810 Bulinus snails were used for the study. Two hundred and Seventy (270) of them from streams representing each of the three senatorial zones of Anambra state viz; Obutu Lake, Omogho town, Orumba North Local Government Area (Anambra South), Agulu Lake, Agulu town, Aniocha Local Government Area (Anambra Central) and Omambra River , Omor town, Ayamelum Local Government Area (Anambra North).

Collection of Snails: The snails were collected from the streams/rivers in each of the senatorial zones with the help of the scoop net. The snail usually attaches itself beneath aquatic plants like water lettuce. This snail collection was made possible with the assistance of a wooden canoe. The scoop net was placed under the aquatic plants and intermittently checked for trapped snails. This was repeatedly done until the numbers of snails needed were obtained.

Criteria for Snail Selection: The snails must be from the above streams representing the three senatorial zones in Anambra State, the snail must be motile, Only adult snails between 10 – 13 mm were collected, embryos and small sized snails were left out. Only those snails free from any infection and devoid of any strange colors were used.

Procession of Snails: Adult *Bulinus* snail which is a fresh water snails were used for this study. This was carried out in in a semi controlled environment with the average daily temperature range of 28 °C to 33 °C in open plastic tanks measuring 50 × 23 × 17 cm containing filtered de-chlorinated fresh water.
The snails were fed daily on dry previously boiled lettuce. The snails from the various streams were reared in separate tanks in the laboratory. Water in bowls was changed twice a week to eliminate faecal droppings and fouling. The snails were kept for a period of two weeks to acclimatize to laboratory conditions before use in the experiment.
After 24 hours challenge with the aqueous *Moringa oleifera* Lam, the dead snails were counted and the still viable snails were recovered and introduced into fresh water for two weeks to check for after effects of the aqueous *Moringa oleifera* Lam exposure. Within the 2 weeks dead as well as unhealthy, algal infected snails were removed as soon as discovered.

Sources and Preparation of the *Moringa oleifera* Lam seeds; The seeds used in the study were collected from a 4 year's old *Moringa oleifera* plant. The *Moringa oleifera* seeds were collected washed and air dried. The seed-lobes were grinded into powdery form. 10 g of powdery seed were weighed and added into 1000 mL of dechlorinated water. It was shaken vigorously and allowed to stand for 3 hours. The mixture was filtered and the filtrate collected and oven dried for 24 hours. It was filtered to get the solid concentrate. The seed powder was weighed and added directly to 1000 mL of filtered water in individual beakers at eight different concentrations:

Molluscidal Bioassay: The *Bulinus spp* adulticidal bioassay was performed according to the methods of the (24) following the recommendation of the Expert Committee on the Bilharzia.

In each assay, ten healthy adult snails with uniform size (shell diameter of 10 –13 mm) were immersed for 24 hours in a glass jar containing 200 ml of water. The treatments were: seed powder (0.2, 0.4, 0.6, 0.8, 1.0, 1.5 and 2.0 g/l, copper (II) carbonate (50 µg mL^{-1}; positive control), and just filtered tap water (negative control). After the exposure period of 24 hours, the snails were removed from the experimental test solution and washed thoroughly with dechlorinated tap water and transferred to containers with fresh dechlorinated tap water for another 24 hours of recovery. The snails were incubated at 28 ± 5 °C and fed with lettuce leaves.

Evaluation of snail survival was made after 14 days (2 weeks) and survival rates (%) were obtained for each treatment. Snails were considered dead if they could not move or retracted well into or hanging out of the shell, with the body and shell discolored (24).

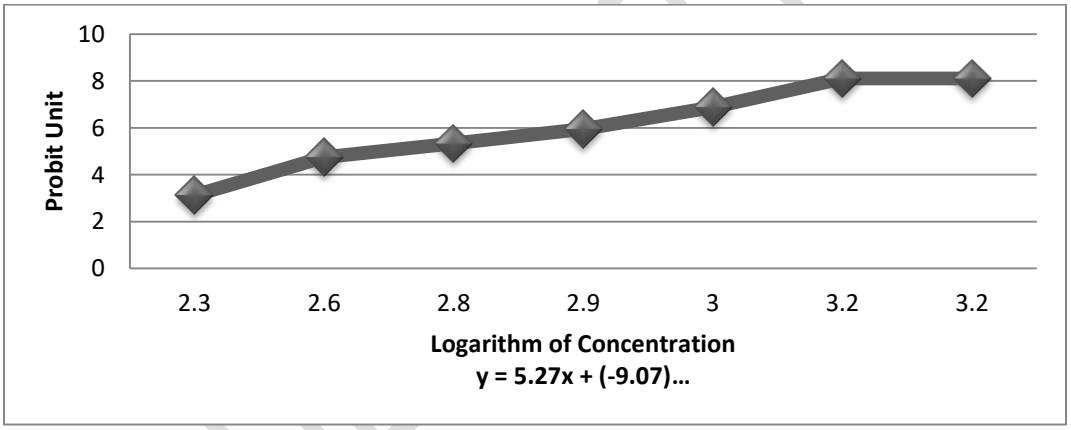
And then, the percentages of observed mortalities were recorded. Bioassays were performed in triplicate for each concentration.

Calculation of Lethal Concentration (LC): this was done using a combination of Microsoft Excel, Probit table Data Analysis and Regression

Statistical Analysis: The data collected were analyzed using SPSS version 17. For the bioassay, Probit analysis by (25) was used to calculate the lethal Concentration (LC). Data analysis was also done using Regression. Significant differences between treatment groups used in the bioassays were analyzed using Student's *t*-test (significance at $P < 0.05$).

RESULTS

Figure 1 presents the average Percentage death of *Bulinus* spp treated with different concentrations of Aqueous *Moringa oleifera* Lam seed in Omogho town, Orumba North Local Government Area of Anambra south Senatorial Zone. The various logarithm of concentrations were obtained, likewise the Probit Unit values for the average death per each concentration were also obtained. The graph in figure 1 shows the logarithm of the different concentrations plotted against the different probit units to compute the LC_{50} and LC_{90} values. After the computation, the LC_{50} and LC_{90} for aqueous *Moringa oleifera* Lam seed extract in Omogho Senatorial zone were calculated to be 467.7 ppm and 812.8 ppm respectively.

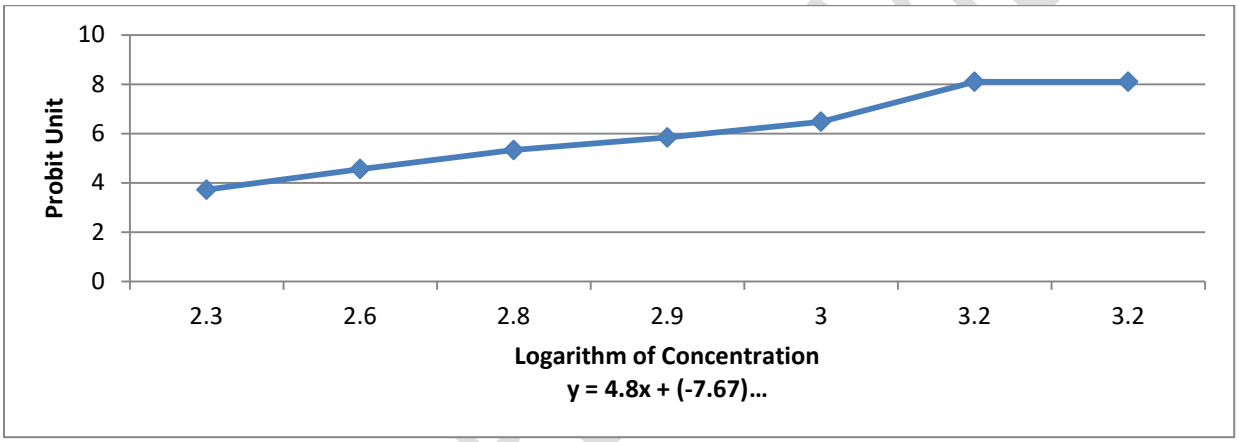


To compute LC_{50}	**To compute LC_{90}**
y = 5.27x + (-9.07)	y = 5.27x + (-8.6)
Where y = 5.0, x = 2.67	Where y = 6.28, x=2.91
Antilog 2.67 = 467.7	Antilog 2.91=812.8
LC50 = 468 ppm	LC90 = 813 ppm

Figure 1: LC_{50} and LC_{90} of *Bulinus* spp treated with different Concentrations of Aqueous *Moringa oleifera* seed in the Triple Tests done in Omogho town.

Figure 2 like its counterpart in Anambra south Senatorial Zone shows the results obtained from Agulu town in Aniocha Local Government Area of Anambra Central Senatorial Zone. The fatality of aqueous *Moringa oleifera* seeds was seen to progressively increase as the concentration of the aqueous *Moringa oleifera* seeds increased. The two apex concentrations of 1500 and 2000 ppm recorded 100% fatalities, while the least concentration of 200 ppm had an average of 3.3% *Bulinus* spp. deaths. This signifies the toxicity of aqueous *Moringa oleifera* seeds even at that low concentration.

The various logarithms of concentrations were also obtained, so were the Probit Unit values for the average death per each concentration. The graph also shows the logarithm of the different concentrations plotted against the different probit units to compute the LC_{50} and LC_{90} values obtained from the zone. After the computation, the LC_{50} and LC_{90} for aqueous *Moringa oleifera* Lam seed extract in Omogho Senatorial zone were calculated to be 436.5 ppm and 794.3 ppm respectively.



To compute LC_{50}

y = 4.8x + (-7.67)

Where y = 5.0, x = 2.64

Antilog 2.64 = 436.5

LC50 = 436,5 ppm

To compute LC_{90}

y = 4.8x + (-7.67)

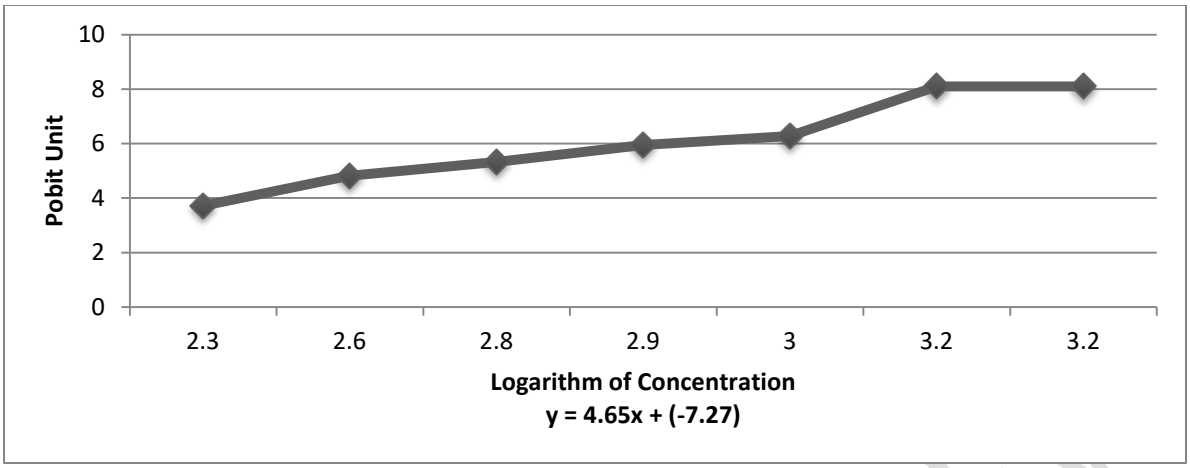
Where y = 6.28, x=2.90

Antilog 2.90=794.3

LC90 = 794.3 ppm

Figure 2: LC_{50} and LC_{90} of *Bulinus* spp treated with different Concentrations of Aqueous *Moringa oleifera* seed in the Triple Tests done in Agulu town.

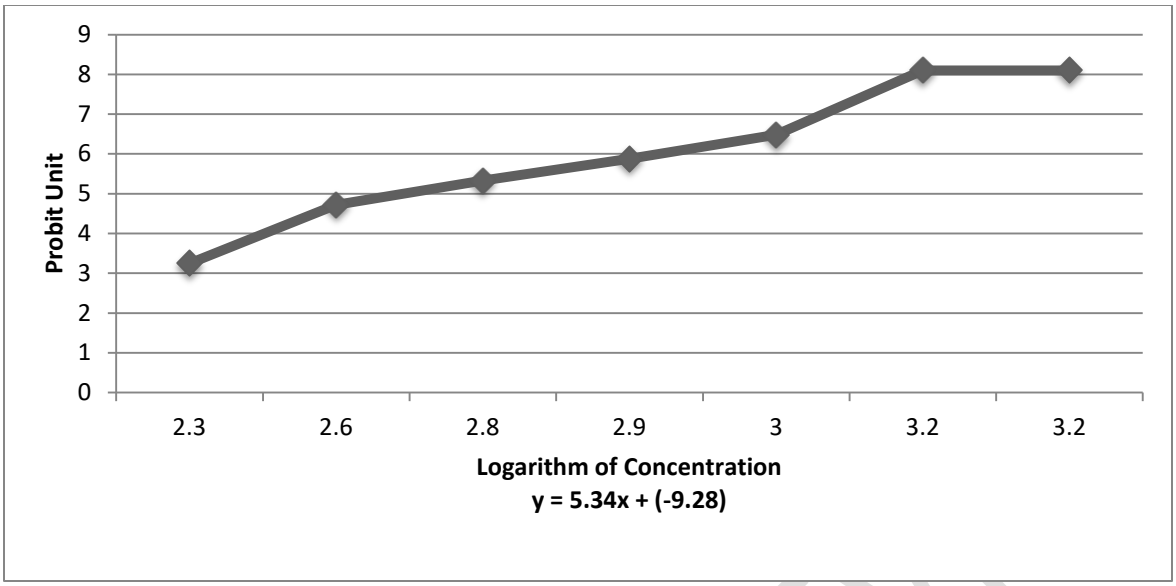
The scenario in Omor town, Ayamelum L.G.A, Anambra North Senatorial Zone was what is seen in figure 3 where the various logarithm of concentrations and the Probit Unit values for the average death per each concentration were obtained and used to calculate the LC_{50} and LC_{90} from the zone (Figure 3). After the computation, the LC_{50} and LC_{90} for aqueous *Moringa oleifera* Lam seed extract in Omogho Senatorial zone were calculated to be 426.6 ppm and 812.8 respectively.



To compute LC_{50}	**To compute LC_{90}**
y = 4.65x + (-7.27)	y = 4.65x + (-7.27)
Where y = 5.0, x = 2.63	Where y = 6.28, x=2.91
Antilog 2.63 = 426.6	Antilog 2.91=812.8
LC50 = 426.6 ppm	LC90 = 812.8 ppm

Figure 3: LC_{50} and LC_{90} of *Bulinus* spp treated with different Concentrations of Aqueous *Moringa oleifera* seed in the Triple Tests done in Omor Town.

Figure 4 is the collation of Percentage death of *Bulinus* spp treated with different concentrations of aqueous *Moringa oleifera* Lam seed in all the Senatorial Zones. Also, the various logarithm of concentrations and the Probit Unit values for the average death per each concentration were obtained and used to calculate the LC_{50} and LC_{90} from the zone. Like the calculations from the various zones the graph in figure 4 was used to calculate the LC_{50} and LC_{90} which were calculated to be 468 ppm and 813 ppm respectively.



To compute LC_{50}

y = 5.34x + (-9.28)

Where y = 5.0, x = 2.67

Antilog 2.63 = 467.7

LC50 = 468 ppm

To compute LC_{90}

y = 5.34x + (-9.28)

Where y = 6.28, x=2.91

Antilog 2.91=812.8

LC90 = 813 ppm

Figure 4: LC_{50} and LC_{90} of *Bulinus* spp treated with different Concentrations of Aqueous *Moringa oleifera* seed in the average Triple Tests done in all the Zones.

Figure 5 shows the bar chart representation of LC_{50} and LC_{90} of the various towns from the various senatorial zones at a glance. The LC_{50} for Anambra North, Centtral and South were 427, 437 and 468 ppm respectively, while the LC_{90} were 813,794 and 813 ppm respectively.

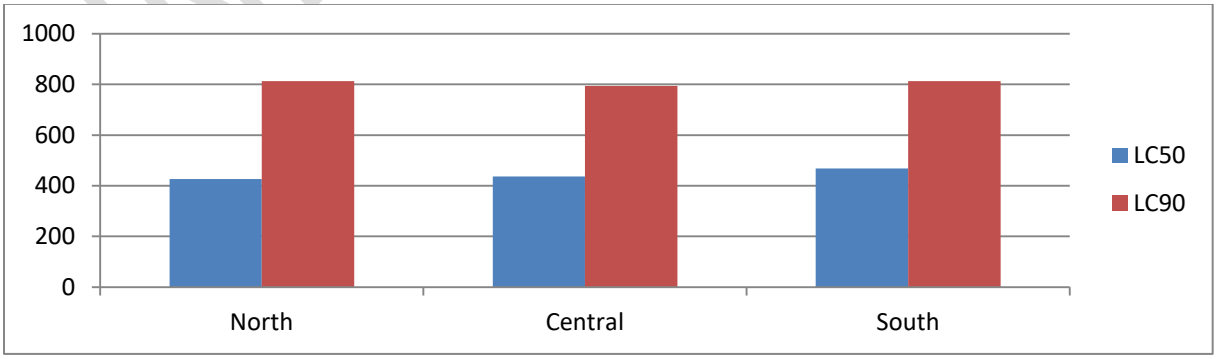


Figure 5: LC_{50} and LC_{90} of all the Zones at a glance

Figure 6 shows a graph where the total mortality rate (%) of the *Bulinus* snail was plotted against the various concentration (PPM) of aqueous *Moringa oleifera* Lam seed. The responses of snails tested in terms of probit death were found to form a linear relationship between percentage concentration (ppm) and probit unit of the extracts. Aqueous *Moringa oleifera* seeds was toxic to *Bulinus* adults in a dose-dependent manner. What this means is that as the concentration of the aqueous *Moringa oleifera* Lam seed increases, the mortality rate of the *Bulinus* snails also increases until a certain threshold was reached where all the *Bulinus* snails were ultimately destroyed.

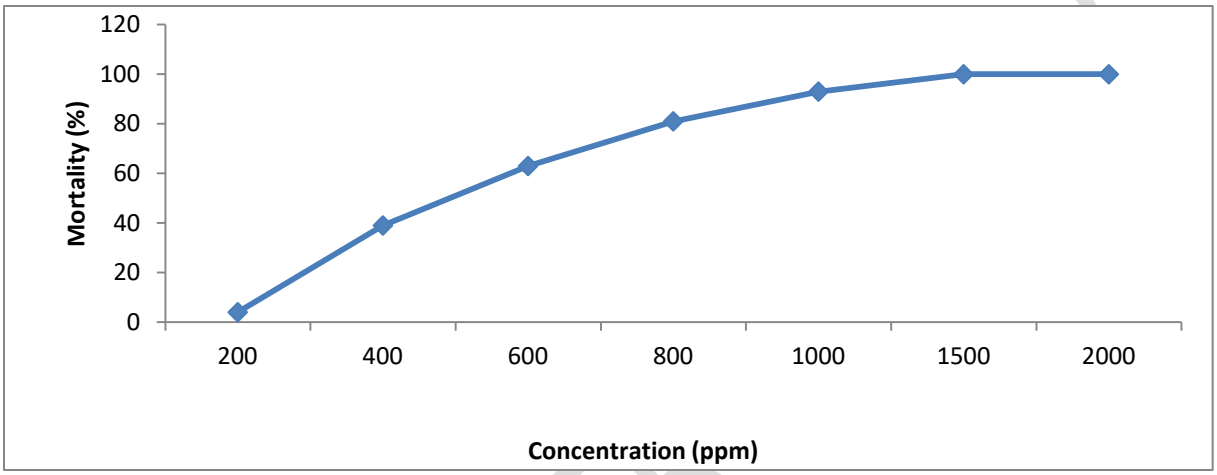


Figure 6: Mortality rates (%) of *Bulinus* at different concentrations of the Aqueous *Moringa oleifera* Seeds

Table 1 shows the survival rate of *Bulinus* snails exposed to sub-lethal concentrations of *M. oleifera* Lam seeds (<600 ppm) after exposure followed by 2 weeks of recovery. In this table there was a uniform progressive decrease in survival rate as the sub-lethal concentration increases. Most of the control recorded 100% *Bulinus* snail survival followed by concentration of 200 ppm which the *Bulinus* snail survival rate was greater than that of 400 ppm which in turn was greater than 600 ppm. This pattern showed the same uniformity in all the zones. Also the difference in the various concentrations were significantly greater to the next one preceding it (P<0.05), eg, the *Bulinus* snails in the controls were significantly greater than the *Bulinus* snails in the 200 ppm group which in turn was significantly greater to the ones in 400 ppm group etc.

Table 1: Survival rate (%) of *Bulinus* snails exposed to sub-lethal concentrations of *M. oleifera* Lam seeds after exposure followed by 2 weeks of recovery

Concentration (ppm)	Control	200	400	600
Omogho	98	93.4	89.1	53.5
Agulu	100	95.1	74	50.1
Ayamelum	100	83.8	69.1	43.7

Discussion

The study revealed that the aqueous *Moringa oleifera Lam* seeds extract have the potential of controlling *Bulinus* snails and by extension, the scourge of schistosomiasis in these senatorial zones worked on, which comprised all the senatorial zones that made up Anambra state.

The aqueous *Moringa oleifera* Lam seeds extract was toxic to *Bulinus* adult snails in a dose-dependent manner and the LC_{50} and LC_{90} in all the three senatorial zones studied. For every senatorial zone the experiment was done in triplicate, and the average probit dead recorded. As represented from figures 1 – 4, LC 50 and 90 were calculated using a complex data analysis involving regression and probit analysis. There was the same uniform toxicity pattern in all the three senatorial zones studied in that the aqueous *Moringa oleifera* Lam seeds extract indicated low toxicity at ppm of 200, and as from 1000 ppm the toxicity affected more than 90% percent of the snails. Concentrations greater than 1000 ppm were very fatal for all the *Bulinus* snails. The least concentration of 200 ppm in all the pooled *Bulinus* spp treated with *Moringa oleifera* seeds aqueous extract averaged 4%, with a progressive increase in the fatalities of the snails as the concentration increases. The LC_{50} and LC_{90} from the different zones vary, though not significantly ($P>0.05$).The difference in this variation could not be ascertained in this work since there was no death in the negative control but it might be due to the individual differences in their various ecosystem and habitat. When pooled together, the values determined from the three senatorial zones after 24-h exposure were 468 ppm and 813 ppm respectively .

Figure 6 showed that the responses of snails tested, in terms of probit death, were found to form a linear relationship between percentage concentration (ppm) and probit unit of the extracts. What this means is that as the concentration of the aqueous *Moringa oleifera* seed increases, the mortality rate of the *Bulinus* snails also increases until a certain threshold was reached where all the *Bulinus* snails were ultimately destroyed.

The above results agree with findings of (21), who confirmed that the seed powder of *Moringa oleifera* had a molluscicidal activity against the snails *Biomphalaria glabrata* and *Physamar morata* and stated that the snails were retracted into shell and suffered hemorrhage after treatment.

Table 1 shows that survival rates of adult *Bulinus* snails were markedly reduced post their exposure to sub-lethal concentrations of the aqueous seed extract. The main aim of this was to see whether the snails will recover after exposure to sub-lethal concentrations (<600 ppm) by recovering and feeding them using fresh water and boiled lettuce. From the table, two snails from the negative control died for no apparent reason from Omogho town, but the rest from other towns were intact. There was a decrease in survival after 2 weeks as the sub-lethal concentration increased which in epidemiological term signifies that some snails though alive after addition of the extract, will later succumb to the deleterious effects of the *Moringa oleifera* seed extract. On the other hand, some will shake off the deleterious effect and thrive.

Comparable perceptions were recorded by (26) who stated that exposure of *B. alexandrina* snails to methanol extracts of *Euphorbia splendens*, *Atriplex stylosa*, and *Guayacum officinalis* led to a significant decrease in their survival and growth rates. The reduction in survival rate was

due to the snails overcoming the destructive impact of these toxic compounds through discharging it to the surrounding media or by biodegrading it to non-poisonous by-products (27).

This also correlated with the work of (28) whose results demonstrated that the grinded seed of *Moringa oleifera* Lam was lethal for *B. glabrat*a (LC50 0.419 g/L and LC_{90} 1.021 g/l) and *P. marmorata* (LC_{50} 0.339 g/L and LC_{90} 0.789 g/L) but had no effect against *M. tuberculatus* . They concluded that the seed powder of *Moringa oleifera*, never tested before with this purpose, has molluscicidal activity, being lethal to the snails *B. glabrata* and *P. marmorata* and non-lethal to *M. tuberculatus*. Also (29) found that the LC_{50} and LC_{90} values for *Moringa oleifera* leaves aqueous extract on *Bulinus truncatus* were 392 ppm and 595 ppm respectively. Also The LC_{50} and LC_{90} values for *Moringa oleifera* leaves aqueous extract on cercariae of *Schistosoma heamatobium* were found to be 378 ppm and 618 ppm respectively. Their study showed that the effect of *Moringa oleifera* leaves on cercariae was more than its effect on *B. truncates*. At the highest and least concentration of *M. oleifera* leaves (750 – 250) respectively, the death rate for the snails was found to be 100% and 10% respectively, compared with 98.6% and 20% for cercariae at the same concentration. They concluded that the *Balanites aegyptiaca* mesocarp aqueous extract and *Moringa oleifera* leaves have the potential of controlling *Bulinus truncatus* and cerceriae of *Schistosoma heamatobium*.

Studies also showed that *Moringa oleifera* lectins had ovicidal effects on Aedes aegypti (19) and insecticidal potential against *Anagasta kuehniella* (20). Also, the seed powder of *Moringa oleifera* had a molluscicidal activity against the snails *Biomphalaria glabrata* and *Physa marmorata* (21, 22).

These results agree with that of (30) who stated that water extract of *Moringa oleifera* Lam seeds had lethal action against *Aedes aegypti* larvae and eggs. Also, (31) studied the ovicidal action of *Moringa oleifera* flower extract and stated that it had delayed the development of treated embryos and reasoned that the flower extract caused changes in the physiology of snails, which interfered with the production of eggs.

It can be concluded that the seed powder of *Moringa oleifera* Lam never tested before with this purpose, has molluscicidal activity, being lethal to *Bulinus* snails tested, even at low concentration .

5.1 Conclusions

Aqueous seed extract of *Moringa oleifera* Lam is toxic to *Bulinus* snail even at low concentration. Due to its non-toxic, nutritional and water purifier adjuvant characteristics The characteristics of this plant are a starter for a new line of research for more specific products in the control of aquatic snails, adding other values and use for the population, being safer and with low environmental impact, associated to the protection of biodiversity of other non-target organism.

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