MZUMBE UNIVERSITY

EFFICACY OF *AZADIRACHTA INDICA* PLANT LEAVES AGAINST *"BULINUS GLOBOSUS"* SNAILS UNDER LABORATORY SETTING

BY

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CERTIFICATION

The undersigned certifies that he has read and hereby recommends for acceptance by the Mzumbe University a dissertation entitled, "**Efficacy of *Azadirachta indica* plant leaves extract against *"Bulinus globosus"* snails under laboratory setting"** as partial fulfillment of the requirement Degree of Master in Health Systems Management (MHSM) of Mzumbe University.

Supervisor's signature: ……

Date: ……..

Internal examiner signature: ……..

Date…….

DECLARATION

I, Badran S. Issa, do hereby declare that this dissertation is my own work under supervision of Dr. Bunini M. Wilbert, and that it has never been presented or submitted and will not be submitted or presented to any university, institution or elsewhere, and that to the best of my knowledge and belief.

Candidate's signature:

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Badran S. Issa

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Badran S. Issa

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DEDICATION

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ABSTRACT

Niclosamide is the only chemical compound recommended for fresh- water snail control worldwide. Unfortunately, this compound has genotoxicity and carcinogenic effects. Besides, this compound is expensive and unaffordable to low income community members and developing countries like Tanzania. This study had the aim of testing the *Azadirachta indica* (neem) plant leaves extract against *Bulinus globosus* snails under laboratory setting so as to come up with alternative product to be used in the control of snails.

The objectives of this study included determination of the lethal dose at LD50 and LD95 of grinded leaves of *Azadirachta indica* plant against field collected *Bulinus* globosus and determination of effectiveness of lethal time at LT50 and LT95 of grinded leaves of *Azadirachta indica* plant against field collected *Bulinus globosus.*

Study population was snails and study design employed was experimental, which consists of two comparable groups which were an exposure and non-exposure groups containing 10 snails per batch with 266 total sample size for each group of hours.

The study revealed that *Azadirachta indica* has a molluscicidal effect against *Bulinus globosus* although at all doses no LD50 and LD95 were recorded during the 6 hours exposure despite of recorded snail mortalities. The LD50 of 28.81g/L with a 95% Confidence Interval (CI) of 39.8-54.8 was recorded at 12 hours exposure where LD95 could not be recorded at this same time. The LD95 was recorded after 24 hours of snail exposure and this was 42.99g/L with a 95% Confidence Interval of 73.9-89.7. However, the Analysis of Variance (ANOVA) showed that no statistical significance different between the mortality exerted by the three groups for 6, 12 and 24 hours ($P>0.05$). On the hand, LT50 was recorded at the dosage of 28.81g/L and LT95 at the dosage of 42.99g/L.

These findings therefore show that *Azadirachta indica* leaves extract has poison to kill *Bulinus globosus* snails because at laboratory setting experiment this was obvious. The important things noted are that to obtain high rate of mortality higher dose and longer time are needed. Furthermore, it is recommended to do the same

experiment to these snail’s species using other parts of the plant such as bark and seeds so as to make full utilization of the plant.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A.indica	*Azadirachta indica*
BoT	Bank of Tanzania
CDC	Center for Disease Control
CI	Confidence interval
g/L	Gram per Litre
Gm	Gram
HIV	Human Immune Virus
Hrs	Hours
IAMAT	International Association for Medical Assistance to Travelers
LC	Lethal Concentration
LD	Lethal Dose
LT	Lethal Time
Mg	Milligram
ML	Milliliter
NTDs	Neglected Tropical Diseases
pH	Hydrogen Ion Concentration
TPC	Tanganyika Plantation Company
USD	United States Dollar
WHO	World Health Organization

OPERATIONAL DEFINITIONS

1. **Lethal dose** (**LD**) means an indication of the lethal toxicity of a given substance or type of radiation.
2. **Lethal concentration (LC)** is a lethal dose measurement used for gases or particulates. The LD may be based on the standard person concept, a theoretical individual that has perfectly "normal" characteristics, and thus not apply to all sub-populations.
3. ***Azadirachta indica*** **(Neem)** is commonly known as neem, neem tree or Indian lilac, is a tree in the mahogany family Meliaceae. It is one of two species in the genus *Azadirachta*, and is native to the Indian subcontinent, i.e. India, Nepal, Pakistan, Bangladesh, Sri Lanka, and Maldives. It is typically grown in tropical and semi-tropical regions.
4. **Lethal Dose Fifty (LD_{50})** means an amount of dose used that causes half (50%) of the study subjects to die.
5. **Lethal Dose ninety-five (LD_{95})** means an amount of dose used that cause (95%) of the study subjects to die.
6. **Lethal Time Fifty (LT_{50})** means an amount of time taken to cause half (50%) of the study subject to die.
7. **Lethal Time ninety-five (LT_{95})** means an amount of time taken to cause (95%) of the study subjects to die.

CHAPTER ONE
INTRODUCTION

This chapter provides the background information, statement of the problem hypothesis, broad objectives and specific objectives

1.1 Background information

Schistosomiasis (Bilharzia) is a disease caused by parasitic worms. The worms that cause schistosomiasis are not found in the United States but more than 200 million people are infected worldwide (CDC, 2017). According to CDC, in terms of impact, this disease is the second only to malaria for infection and is the most devastating parasitic disease. Schistosomiasis is regarded as one of the Neglected Tropical Diseases (NTDs). It is an acute and chronic parasitic disease caused by blood flukes (trematode worms) of the genus Schistosoma. According to WHO, world estimate shows at least 206.4 million people required preventive treatment in 2016. Schistosomiasis transmission has been reported from 78 countries. However, preventive chemotherapy for schistosomiasis, where people and communities are targeted for large-scale treatment, is only required in 52 endemic countries with moderate-to-high transmission (WHO, 2016).

Schistosomiasis mostly affects poor and rural communities, particularly agricultural and fishing populations and among the affected people, 85% live in Africa (WHO, 1987; Sleigh and Mott, 1986). Vulnerability comes to those who comes in contact with the infected water with insufficient hygiene particularly children and those women with home activities including washing are likely to develop genital schistosomiasis (WHO, 2018). Movement of the people from rural to urban increases the chance of transmitting to other new areas. Increasing of the population, modification of the environment due to need of water and power supply results in increasing transmission (WHO, 2014). The rise in eco-tourism and travel increasing numbers of tourists in contracting schistosomiasis (WHO, 2018). Abnormalities including paralysis with acute and severe infection has been presented by the tourist at a times. It is believed that urogenital schistosomiasis is likely to be a woman risk factor getting HIV infection (WHO, 2016).

It has been recorded that, the first cases of schistosomiasis were reported in the early 19thcentrry (Mazigo, 2012). Moreover, The United Republic of Tanzania took second country that has the highest infection rate of schistosomiasis in the region, Nigeria being the first (Mazigo *et al*., 2012). It has been estimated that 23.8 million people with new and old cases of schistosomiasis infection reached to 51.5% in the year 1999, approximately 12.3 million people with schistosomiasis (Matovu and Nditi, 1980). Both urinary and intestinal Schistosomiasis are still a highly burden in Tanzania which cause severe disease (Mazigo *et al*., 2012). Furthermore, the recent data estimates country to have up to 53.3% prevalence (Rollinson *et al*., 2013).

Specifically, *Schistosoma haematobium* is highly available along the coastal area particularly eastern and south-eastern coasts, both islands of Unguja and Pemba (Zanzibar) and the mainland areas of the north-western zones of the Tanzanian country (Brooker Clements, 2009). The intermediate-host snail species responsible for transmission of *Schistosoma haematobium* have been identified as the coast and mainland potential areas (Brooker, 2009). Absence of *Schistosoma mansoni* intermediate host snails in the coastal area facilitate the area to be free from intestinal schistosomiasis infection (Brooker Kabatereine, 2009 & Clements ACA *et al*., 2006) but such snails prevailing along the shores and islands of Lake Victoria (McCullough FS, 1972).

Cercariae are the infective form of parasite that comes or emerges from the fresh-water snail which live in certain type of water, the water then becomes contaminated and person get infection when bear skin comes into contact with the infected water bodies (CDC, 2018). There are five species of schistosomiasis known to affect human, among others are most available in our country including *Schistosoma haematobium* and *Schistosoma mansoni*, where by *Schistosoma japonicum*, *Schistosoma intercautum* and *Schistosoma Mekongi* are prevailed in our country. Furthermore *Schistosoma haematobium, Schistosoma mansoni and Schistosoman japonicum* are mostly cause infection to human. (Larotski and Davis, 1981). *Schistosoma haematobium* cause urinary schistosomiasisa and Schistosoma mansoni cause intestinal schistosomiasis (Donohue *et al*., 20017).

There are two main genera of fresh water snails which are of public health importance in Africa and America. They are: *Bulinus and Biomphalaria*. The former is an intermediate host of *Schistosoma haematobium and Schistosoma intercalutum* whereas the latter is an intermediate host for *Schistosoma mansoni* (Cook, 1996; WHO, 2003).

Snails belong to the phylum of Mollusca that is basically divided into six classes, namely: *Monoplacophora*, *Amphineura*, *Scaphopoda*, *Lamellibranchiate Cephalopoda* and *Gastropoda*. Among them, the *Gastropoda* class is the most important in public health point of view. The class comprises of about 75,000 species and have adapted themselves in almost all kinds of habitats e.g. in the sea on land freshwater (Kristensen, 1989). Fresh water snails are distributed all over the world. Their sizes vary from 1cm to 30cm in length. The most characteristic traits in the structure of these snails are the spiral shell, the soft slimy body with a well-developed head and foot. Gastropoda differ from most other animals being asymmetrically built of the body's normal opening in place in the middle line; all others are placed asymmetrically on one side of the body. The class is basically divided into three subclasses i.e. *Pulmonate, Prosobranchia* and *Opistobranchia* (Krinstensen, 1989).

The *pulmonate* snails living in fresh water constitute the most important group of gastropoda being of great medical importance in Africa, America and the Indian continent. They are hermaphroditic i.e. (having both sex) and some species reproduce through self-fertilization, while others require cross-fertilization. Copulation may last for 3-5 hours and some time the fertilization is mutual, i.e. both snails act individually, only one the snails act as male while other is a female but then in the next mating the roles may be reversed (Madsen, 1985).

After fertilization, the eggs pass down the female tract where they are supplied with a nutritive material (albumen) and membranes eggs are deposited in groups (egg masses), which are fixed on stones, sticks plants, plastic or of others snail's shell. The egg masses of *Bulinus* are flat and firm, and the terminal part of the egg mass is attached to the first part, giving them a around or over outlined. These characteristic

differences in the morphology of egg masses differ in various *pulmonate* families except *Biomphalaria*. The hatching period depends on the temperature. For *Bulinus* species it takes 6-8 days at 25 ^{0}C to hatch. The newly hatched snails have a size of about 0.1 mm shell height. Under favorable conditions, the snails may reach maturity and commence egg lying in about 4-5 weeks after hatching. At maturity they have attained a shell height of about 5 -7 mm. During the first 6-7 weeks of egg laying each snail may on the average produce 300-1000 eggs under optimal conditionals as these species have a very high reproductive ability. Usually, however, under field conditions, the maturation period is longer, growth slower and the egg lying intensity is probably also lower (Madsen, 1985; WHO 1987).

Bulinus and *Biomphalaria* snail species are widely distributed worldwide. They can be found in almost all types of water bodies' i.e. small temporary ponds or streams to large lakes, rivers, or man-made structures like irrigation canals and dams (WHO, 1984). Both *S. haematobium* and *S. mansoni* are highly endemic in many parts in of Tanzania. However, *Schistosoma haematobium* is highly prevalent in the Coastal areas of the Indian Ocean and some area around Lake Victoria, whereas *S. mansoni* is highly prevalent around Lake Victoria (Munisi *et al*., 2016). Records shows that greater rates of new cases on the Lake Victoria and their shores islands and the east coast of the country including Tanga and Dar es salam, not only that but also in Shinyanga cases are reported. The Islands of Unguja and Pemba, schistosomiasis infection cases are reported even if control and elimination program have been implemented. Additionally, *Schistosoma haematobium* has another intermediate host called *Bulinus nasutus* while *Biomphalaria choanomphala* for *Schistosoma mansoni* respectively (IAMAT 2018).

Population density of snails varies in various locations. The main factors causing fluctuation in snail's population include physical factors, such as transport, topography and geomorphology, wave action, rainfall pattern and temperature. These factors, rainfall pattern and temperature are probably remain the main factors for fluctuations in snail's density. Importantly, rainfall pattern has a pronounced effect on the infection rate of snails (Webbe, 1958). Also various chemical compounds,

such as Calcium Carbonate, Oxygen, pH value and Carbon Dioxide have a pronounced effect on presence or absence of fresh- water snails (Madsen, 1085). Furthermore, biological factors such as: food type, vegetation, bacteria, fungi and other microorganisms do contribute to the presence of fresh water snails in many areas (Madsen, 1985).

Pulmonate snails are generally confined to shallow water although some species are found at considerable depths e. g. *Bulinus trigonus* down at 9.2 m in Lake Victoria. Water with a permanent high turbidity is generally unsuitable for culminate snails; the turbidity has several secondary ecological effects which may affect the snails. Turbid water exerts a stronger friction than clear water and thus snails can be washed out at lower speed, furthermore algal growth is inhibited when silt particles settle on water body (Madsen, 1985).

1.2 Control of schistosomiasis

There are many strategies established to control schistosomiasis, including mass treatment, improve sanitation, health education and other synthetic molluscide application (WHO, 2012). Currently, mass drug administration using praziquantel, is still being used as a major intervention measure to control schistosomiasis that has not been succeded in reducing the burden of infection (Mazigo *et al*., 2012). Due to that failure the need to revise the current approach for the successful control of the disease is needed without delay. Definately, there is need to target integrated control measures specifically the life cycle, as the only way forward that will lead to sustainability and future elimination of the disease respectively. (Inobaya *et al* 2014).

1.3 Fresh water snails control strategies

The control of snails may entail the use of environmental management, biological agents, synthetic molluscides and natural plant molluscides (Madsen. 1985, WHO, 1993). It has been reported that a complete eradications of snails in an area is unrealistic goal (DBL 1989). But reduction in the transmission of human infection remains the aim to achieve. In principles, there are many fresh water snail control strategies that are used in many countries. Some of the most common strategies are presented below.

1.4 Environmental management

Environmental management is defined as the technical and administrative intentions which modify and control environmental factors and their relations with man and vectors. The major objectives are to prevent and control vector and to reduce man - vector–pathogen contacts in order to achieve most favorable health status for a target population (WHO, 1982; Bos *et al*., 1993). Largely, the WHO (1982) has classified environmental management into three categories. These are environmental modification, manipulation and others.

1.4.1 Environmental modification

This includes grading, filing, drainage, land leveling. It is a permanent change of land, water and vegetation to prevent, reduce or eliminate vector or intermediate host breeding sites or environmental conditions which support transmission of water borne and water washed disease.

1.4.2 Environmental manipulation

It is the changes of environmental conditions tthat create temporary and adverse breeding conditions for vector breeding or transmission. Here it includes water level fluctuations, water speed changes, flushing, weed clearance and salinity changes.

Modification *or* ***manipulation*** of human habitation or behavior is any environmental modification measures to reduce man to vector and or man to pathogen contacts including personal protection, safe bathing of laundry places, latrines, and supply.

Oomen *et al*. (1990), contended that environmental management factor is a vehicle having hygiene education and community participation as well as infrastructure. Apparently environmental management strategies have been very successful in snail control operations. For example, a case study in Namwala Kilombero District Tanzania have illustrated river flushing to be very effective in fresh water snails control operations (Fritsch, 1993).

In China environmental modifications was done for permanent interrupting transmission and it was carried out in all areas with low endemicity. They were

digging new ditches and filling of old ones, build lining of irrigation canals with concrete and the modify of sluice gates to prevent snails from entering and spreading into other sections of an irrigation system also by adding a sedimentation pool has shown major effect (Chen and Feng, 1999).

1.5 Biological control

An introduction of natural enemies i.e. (Predators or parasites) or competitors of undesired species constitute biological control of fresh- water snails. For the predators their work is kill the prey species, while the parasite cause sterilization. In the case of competitors, they reduce the amount of resource available for another species or harm the other species during the process of obtaining the resources which both species are trying to gain. Infract biological has shown most promising approach to biological snail control in some areas. The most extensively studies competitors snails are *Marisa cornuaietis, Helisoma duryi* and *Thiara granifera.* For example, in Tanzania *Marisa spp* was introduced in a small dam and it has eliminated thriving populations of *Bulinus tropicus, Lymnea natalensis* and *Biomphalaria pfeifferi.* Furthermore, in 1975 Tanganyika Planting Company (TPC) in northern Tanzania released *Helisoma duryi* into the drains and maintained population over there (Madsen 1985).

1.6 Chemical control

Fresh water snail control using molluscicides have been intensively used with satisfactory results in human schistosomiasis control operations (Madsen &Christensen 1992), WHO 1993, Lardans & Dissous 1998). A great number of chemicals have been used as a molluscicides in the past, but at present very few molluscicides are used in routine snail control, and virtually only one, namely Niclosamide, (Bayluscide or Mollotox) is commercially available, its effectiveness had been established before (Gonnert, 1961). This product application cause killing action on non – target plant and animals (Andrews *et al*. 1983). Moreover, this compound is known to cause genotoxicity and carcinogenic effects (Vega *et al*. 1988), the chemical has high cost (Pierri, 1995). The likelyhood of repeating of breeding grounds is great (Sarquis *et al*., 1997, 1998). The ecological toxicity of this

product was the restriction of this product on its use as an official molluscide in governmental programs to control schistosomiasis. Some of the problems related with the use of chemicals in control strategies normally described as the need for repeated application, since eradication of snail intermediate host populations is rarely achievable. The method needs sustained motivation, reliability and supervision of snail control team.

1.7 Plant mollusciding

World Health Organization (WHO) strengthen the need to promote intermediate-host snail control to prevent schistosomiasis transmission with the emphasize use of plant having mollusciadal effects (WHO, 2012a). Various plants have been studied as natural plant molluscicides (Jurberg *et al*. 1998). Many studies over 340 species pointed out that *Euphorbiaceae* and *Sapindaceae* plant families have the greatest number of species with the effective molluscicidal activity. The following species of plant have been studied including *Anacardium occidental* (Pereira & Souza, 2006). *Euphorbia pulcherrima, Euphorbia splendens, Caesalpinia peltophoroides* and *Stryphnoden dronadstringens (Mendes et al*. 1984). Of these, *E. splendens has shown* its molluscidal activity in doses under 0.5mg/L, eight times smaller than the lethal does for fish (Vasconcellos & Schall 1986). It is furthermore reported by Lugt, (1986) that the efficacy of the *Phytollaca deodcandra (Endod) in* killing fresh water snails is unquestionable.

The basic questions that have arisen in the selection of plant molluscides on its uses, such as: toxicity, availability of the plant, annual growth, adaptability to different local conditions, and site of molluscidal activity in parts of the plant that easily renew, such as the leaves. To be appropriate for use, a product must be storable and remain workable for at least one year; be physically and chemically stable, also must have ethno botanical value; and be easy to extract and apply, preferably in water extracts (Kloos & McCullough 1982).

Consideration has been made for all these criteria, in 1998 by the Oswaldo Cruz Institute (Fiocruz, RJ) which obtained a biotechnology patent on a method to collect, extract and apply *E. splendens var. hislopiilatex* as molluscide (Vasconcellos, 2000).

This study, is an experiment which was designed to test efficacy of *Azadirachta indica* (Neem) in controlling *Bulinus* snails under laboratory settings. The use of this plant has its taxonomic classification as illustrated below.

Azadirachta indica (Neem)-Scientific name, *Azadirachta indica*, common name "mwarubaini" in (Kiswahili, Family name *Meliaceae*. *Azadirachta indica* is invasive in parts of Tanzania Kenya, and Uganda (A.B.R) Witt pers. obs). Tanzania and Kenya coasts is spreading rapidly. The locations within which *Azadirachta indica* is naturalized include Indian Sub- continent (Indian and Bangladesh) and South- East Asia, Northern Australia, Tropical Asia, Africa, Fiji, Mauritius, Puerto Rico, the Caribbean and many countries in South and Central America.

1.8 Taxonomical Classification of Neem

Neem tree is a member of the Mahogany family. The plant has related properties to its close relative, Melia azederach. This word "*Azadirachta*" is derived from the Persian azaddhirakt which mean 'noble tree' (Kanagasanthosh, Kavirajan, & Shanmugapriyan, 2016).

The taxonomic positions of neem are as follows:

Order: Rutales

Suborder: Rutinae

Family: Meliaceae

Subfamily: Melioideae

Tribe - Melieae

Genus: Azadirachta

Specie: Indica

Latin: Azadirachta indica (Ogbuewu *et al* 2011).

Azadirachta indica is introduced and grow well in East Africa particularly in parts Kenya, Tanzania and Uganda and many other African countries, it is along parts of the Kenya and Tanzania coasts Zanzibar inclusive and it has been spreading rapidly. https://keys.lucidcentral.org/keys/v3/eafrinet/weeds/key/weeds/Media/Html/Azadirachta_indica_(Neem).htm.

Figure 1.1: A Neem tree, serrated leaves and seedlings

1.9 Description of *Azadirachta indica*

The plant can grow and invade shrub lands, open woodlands, grasslands, floodplains, banks of watercourses, coastal sites and others. It is a fast- growing tree that can reach a height of 15-20 m, though it rarely reaches 35-40 m. It is evergreen, but in severe drought it may shed most or nearly all of its leaves. The branches are spread wide. The fairly dense crown is roundish or oval and may reach the diameter of 15-20 m in old, free- standing specimens the opposite, simple pinnate (Once- divided) leaves are 20-40cm long with 20-31 medium to dark green leaflets about 3-8cm long. The leaf stalks (petioles) are short. Very young leaves are reddish to purplish color. The shape of nature leaflets is more or less asymmetric, and margins are toothed (dentate) (Azadirachta, 1963).

According to the keys and facts sheet, neem flowers is white and aromatic arise from the connection of the stem, normally in more-or-less drooping flower clusters which are up to 25cm long. These branching inflorescences, stand from 150 to 250 flowers. The flower is about 5-6mm long and 8-11mm wide (FACTSHEET, 2011).

Its fruit is smooth (glabrous) olive- like drupe which varies in shape from elongate oval to nearly roundish, and when ripe are 1.4-2.8 x1.0-5 cm. The fruit skin is thin and turns yellow when ripe. The bitter-sweet pulp is yellowish-white and very fibrous. The pulp is 0.30- 0.5 cm thick white, hard inner shell of the fruit encloses one, rarely two or three, elongated seeds (Kernels) having a brown seed coat (FACTSHEET, 2011).

Uses of *Azadirachta indica*

In Tanzania it is known as "Mwarobaini", and other Indian Ocean, neem tree is known as 'the panacea'. It is believed to cures forty diseases, *A. indica* is considered to be very effective in the treatment of contagious disease like scabies, despite the fact that, only beginning scientific evidence based, which still has to be supported, and is recommended for those who are sensitive to permethrin, a known insecticide which might be an irritant. There is also subjective evidence of its effectiveness in treating infestations of head lice in human. By using neem oil, cats and dogs are sprayed against flea as well as in some beauty products. on the other hand, extended internal use (ingestion) may cause irritation of the liver and kidneys, amazingly, the neem believed to cure forty diseases but even discovered to cure one hundred health problems including other uses as pesticides (Azadirachta, 1963).

1.10 Statement of the problem and justification

To date Niclosamide is only chemical compound recommended for fresh- water snail control worldwide (Gonnert, 1961, & WHO, 1987, Sturrock, 2000). Unfortunately, this compound has genotoxicity and carcinogenic effects (Vega *et al*, 1988). Importantly, the compound is expensive (>USD 33.80/kg) DBL, 1985, and Pieri, 1995). Thus, it may be unaffordable to low income community members and developing countries, Tanzania inclusive because of priority setting.

This situation has negative implications to the control of schistosomiasis in developing countries. Apparently, the use of plant molluscides is being advocated by the World Health Organization (WHO, 1987) because it can be easily available to the local environment. To date *Phytollaca deodcandra* (Endod) is the only plant molluscide which is recommended for use. However, the compound is only available in Ethiopian region (WHO, 1987). Therefore, there is a need for alternative plant molluscides which are available in the country.

It appears that, all parts of *Azadirachta indica* (leaves, bark, and seeds) have mollusciadal effects to a wide range of vectors and vermin. The leaves are believed to have mollusciadal effects to fresh-water snails as experience shows that they have been used by local people in Pemba and Unguja islands. However, the extent to which such leaves may kill *Bulinus* snails is not well established and documented for references. Fortunately, this plant may grow well in many places in the United Republic of Tanzania. Therefore, the use of such plant leaves in control of fresh water snails can be experimented to point out its molluscidal effects so that control it could be used by local people in a proper way.

The study was therefore designed to assess the lethal effect of plant leaves of *Azadirachta indica* killing *Bulinus* snails under laboratory settings. The findings from the study will be used for designing field experimental studies which may suggest its proper use by local people and plan for future product development. This strategy may help to reduce transmission of urinary schistosomiasis in endemic parts of Tanzania.

1.11 Hypothesis

Null hypothesis

Azadirachta indica plant leaves extract has no significant lethal effect in killing *Bulinus globosus* snails at given dose and time.

Alternative hypothesis

Azadirachta indica plant leaves extract has a significant lethal effect in killing *Bulinus globosus* snails at given dose and time.

1.12 Research questions

1.12.1 General research question

What are the lethal doses and time of grinded leaves of *Azadirachta indica* plant in the killing of the field collected *Bulinus globosus* snails under laboratory setting.

1.12.2 Specific research questions

What are the lethal doses of *Azadirachta indica* that can kill *Bulinus Globosus* snails under laboratory setting at given time?

What are the lethal times of *Azadirachta indica that can kill Bulinus Globosus snail under* laboratory setting at given doses?

1.13 Broad objective

To assess efficacy of *Azadirachta indica* plant leaves to control *Bulinus Snails* species.

1.14 Specific objectives

1.14.1 To determine the lethal dose at LD_{50} and LD_{95} of grinded leaves of Azadirachta indica plant against field collected Bulinus globosus.

1.14.2 To determine the effectiveness of lethal time at LT_{50} and LT_{95} of grinded leaves of Azadirachta indica plant against field collected Bulinus globosus.

CHAPTER TWO
LITERATURE REVIEW

Introduction

This chapter provides theoretical review, Conceptual framework and Empirical review.

2.1 Theoretical review

Mwarobaini (*Azadirachta indica*-neem tree), as the name applied in Swahili believed to cure forty disease or health problems. It is an evergreen tree mostly it is inhabitant to the Indian subcontinent. It is widely used for oral and skin sepsis for many years. Scientific studies have demonstrated neem to have, anti-oxidant, cytotoxic and anti inflammatory purposes. It is source of medicine to treat more than a 100 health problems, many bioactive mechanism usually referred to as limonoids have been isolated from various parts of the neem tree, which include azadirachtin, salannin, meliantriol, and nimbin.

Azadirachtin constitutes the major component (Schumacher, 2011). In additional Neem oil is said to be an effective pesticide that gets rid of over 200 species of insects. Some of the most common include: Aphids, Mites Scale, Leaf hoppers, White flies, Caterpillars, Mites, Mealy bugs, Thrips e.t.c *(http://www.saferbrand.com/articles/benefits-uses-neem-oil-for-plants.)*

Major chemical constituents of neem are Terpenes and Limonoids. The major active components in the Limonoids are azadirachtin, 3-deacetyl-3- cinnamoylazadirachtin, I-tigloyl-3-acetyl-II-methoxyazadirachtin, 22, 23-dihydro-23β-methoxyazadirachtin, nimbanal, 3-tigloylazadirachtol, 3-acetyl-salannoV nimbidioV margocin, margocinin, margocilin and others (Ogbuewu, 2008). Terpenoids are isoazadirolide, 6 nimbocinolide, nimbonone, nibonolone, methylgrevillate and Margosinone. Neem increases the production of Glutathione-s-transferase, thus improving the ability of the liver to detoxify itself of chemical contamination (Ogbuewu 2011).

To date, azadirachtin is among various chemical remains the most active component (Warthen *et al*. 1984; Yamasaki *et al*. 1986; Schneider and Ermel 1987). Neem

believed to have killing effect to fresh water snails according to local people in Zanzibar, and therefore in such situation, there has been increasing significance showing molluscicide properties of an interesting aspect of natural plant products, while they may be highly toxic, neem leaves, they may degrade very rapidly when released into the environment (WHO, 1983).

To search for affordable and environmental friendly molluscicides and the high cost and environmental effect of molluscicides in current use for the control of schistosomiasis has generated the specially effort to those of plant origin (Molgaad *et al.,* 1999). Plant molluscicides can prove to be an ideal source of low cost, safe and effective molluscicides (Marston and Hostettmann, 1985; Sigh *et al.*, 1996).

Many problems particularly side effects of the synthetic pesticides pushed scientists to research more environmental friendly tools, therefore kingdom Plantae has proved to be the only remaining natural products (Ahmed 2005). This situation has given to the world a greater importance in the use of plants molluscicides (Webbe and Lambart, 1983). Many plants have been screened for their natural molluscicidal properties in an effort to find satisfactory alternative to Niclosamide and which is suitable to use as a self-help schistosomiasis control programme (Rajan*e et al.*, 1998). molluscicidal compounds extracted from plants is the only cost-effective alternative to use of, especially those species that can be grown locally in schistosomiasis endemic areas of Africa and else where, due high cost of synthetic molluscicides used in the control of the intermediate fresh water snail hosts of schistosomiasis, resulted in changed interest of plant molluscicides (Clark *et al*., 1997).

Tanzania has made combined steps and efforts to control schistosomiasis over many years. The evidence indicates that regardless of significant efforts made to control schistosomiasis by using many approaches, the disease remains still public health problem and the rate of infections continues to increase with the increase in the population size (Mazigo *et al*., 2012).

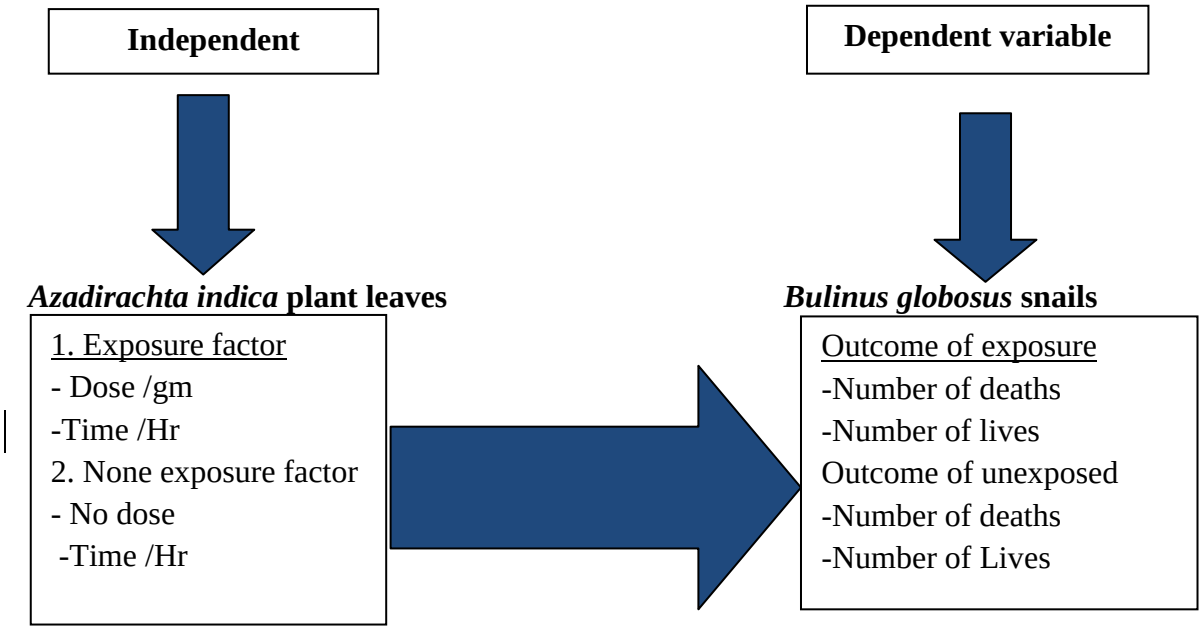
Due to those facts above it gives emphasis to me to test the efficacy of Azadirachta plant leaves against *Bulinus globosus* snails under laboratory setting that could be affordable, manageable by all stakeholders inside and outside the country respectively.

Bulinus globosus snails are among pulmonates of public health important, an intermediate host of *Schistosoma haematobium (*urinary schistosomiasis), (Madsen *et al*., 1989), where by class Pulmonata contains the family *Planorbidae* which contain the genera *Biomphalaria* and *Bulinus*. (Brown, 1980). Some species of the genus *Bulinus* distributed in Africa important intermediate hosts of *S. haematobium* include: *Bulinus truncatus; Bulinus globosus; Bulinus africanus; Bulinus nasutus*; *Bulinus senegalensis*; and *Bulinus camerunensis* (Madsen *et al*, 1989).

*Bulinu*s species are aquatic snails which are hermaphrodite. They deposit eggs in groups attached to plants, sticks or even shells of other snails. The eggs of both species hatch in 6-8 days at 25C° under favorable conditions. The snails may reach maturity and commence egg lying after 4-5 weeks. During the first 6-7 weeks, each snail may on the average, lays 300-1000 eggs under optimal conditions (Danish Bilharsiasis Laboratory, 1989).

2.2 Conceptual framework

Figure 2.1: Conceptual framework



2.3 Empirical review

Molluscicide means substance used to kill *molluscs* (snails), that essential play important role for the control of schistosomiasis. The need to use plant molluscicides has received increased interest due to an inexpensive technology and because of the high cost of synthetic compounds for snail control of those endemic areas of poor nations of the world Tanzania inclusive. Many plants are known to have molluscicidal activity while it provide cheap, safely, biodegradable and effective control agents in rural areas of developing countries (Marston, 1993).

The synthetic molluscide of choice Bayluscide (Niclosamide) has gained access into the market of chemical molluscicides. Although the toxicity of Bayluscide was low, the amphibian and fish are very sensitive to Niclosamide and the chemical has strong irritant effect on mucosal membranes. It fulfill most of the characteristics of the ideal molluscicide, as stated by the World Health Organization (Sahar, 2005). But kingdom Plantae become increasingly emphasized due to its value economically socially and biodegradable in nature.

It has been recorded that a total of 1,426 species of plants had been tested in order to find out a plant molluscicide against the vectors of *Schistosoma mansoni*, not only mansoni but also *Schistosoma haematobium* vectors including *Bulinus truncates* where by a series of laboratory experiments was conducted to explore the influence of water extracts of Azadirachta indica (Neem) on the snail intermediate hosts of schistosomiasis *Bulinus truncates*, *Biomphalaria pfeifferi, Chaetogaster limnaei* and selected non-target aquatic animals and the results has shown that, that the toxicity of the leaf extracts was greater to all tested organisms than seed extracts (Ahmed, 2016).

Other plants that shows molluscidal activity includes*; Moringa oleifera* where by the study reveals leaves of *Moringa oleifera* have a moderate molluscicidal activity and promising cercariaecidal activity in-vitro (WHO 2010). On the other hand, other study which was done in Brazil describes the effectiveness of the latex of *Euphorbia splendens* var. *hislopii* on species of the *Bulinus* and on *Biomphalaria pfeifferi*, the results showed that this compound has proved to be one of the most effective and specific plant molluscicides exposed, lead to usefulness in terms of application so that it could be recommended to be used in programs involving all communities with their participation in all endemic areas both Brazil and Africa (Able, 1998).

Continuing with the effort of finding effective plant molluscicide, other study of plant origin has been tested to assess molluscicidal efficacy of water extracts of certain medicinal plants conducted at Makueni County in Kenya which was done with the objective testing aqueous extracts of five medicinal plants, the collected parts of the plant include: leaves of *Aloe secundiflora*, *Balanites roots aegyptiaca,* leaves of *Aspilia pluriseta* and *Amaranthus hybridus*, and leaves and roots of *Psidiumgua java* together with *Azadirachta indica*. The study results detected that only the *Aloe secundiflora, Aspilia pluriseta*, *Balanites aegyptiaca, Azadirachta indica* and *Amaranthus hybridus* showed molluscicidal activity and therefore the study revealed that mortality increased with increasing concentration of the plant extracts. The active ingredients released slowly so that the effect on snail population is delayed, here there is no dought that plant act and release ingridients slowly to the

environment and making snail population to be affected slowly according to time exposed. The promising results in terms of toxicity to the vector snail were first ranked as *Amaranthus hybridus* followed by *Azadirachta indica.* The compound of these two plants could be more ideal for poducing molluscicide as they successfully resulted in high number of dead snails.

Various concentrations of the aqueous plant extracts was assessed to determining the ability Molluscicidal activity to kill adult *Biomphalaria pfeifferi*, the intermediate host of *Schistosoma mansoni* (Mwonga *et al*., 2015).

Finally, Different studies have been done to assess molluscicide activity of the plant origin against fresh water snails of different species, but unfortunately no study has been found to assess efficacy of *Azadirachta indica plant leaves against* intermediate host of schistosomiasis i.e. *Bulinus globosus snails,* and this has apparently emphasized me to undergo the study to assess efficacy of *Azadirachta indica* plant leaves against *Bulinus globosus* snails under laboratory settings.

CHAPTER THREE
METHODOLOGY

Introduction

This chapter provides study area, study population, study design, sample size determination and sampling procedures/technique

3.1 Study area

The study was conducted at Zanzibar Helminthiasis Control Program Laboratory which is in Urban District, West Urban Region. According to Garmin GPSMAP, it is near Malawi/Bububu the road from Zanzibar town and sea port, and Bank of Tanzania (BOT) building. The laboratory is situated about 2 Kilometer from Zanzibar Town. In the north it is bordered by Garage and Lumumba Secondary School, south by rain and storm water drainage, east by playground, west by main road and BOT building. The average temperature ranges from 25° and 29°C (75° and 81°F) throughout the year and relative humidity is high about 80% on average. The annual rainfall is estimated to be more than 1,600 mm (more than 60 in) according to Zanzibar Meteorological Department, 2018.

Figure 3.1: The site in Unguja Town

3.2 Study population

The study population comprised of *Bulinus Globosus* snail species which were collected with the same size from the same field namely "MTO PWEZA" to reduce bias.

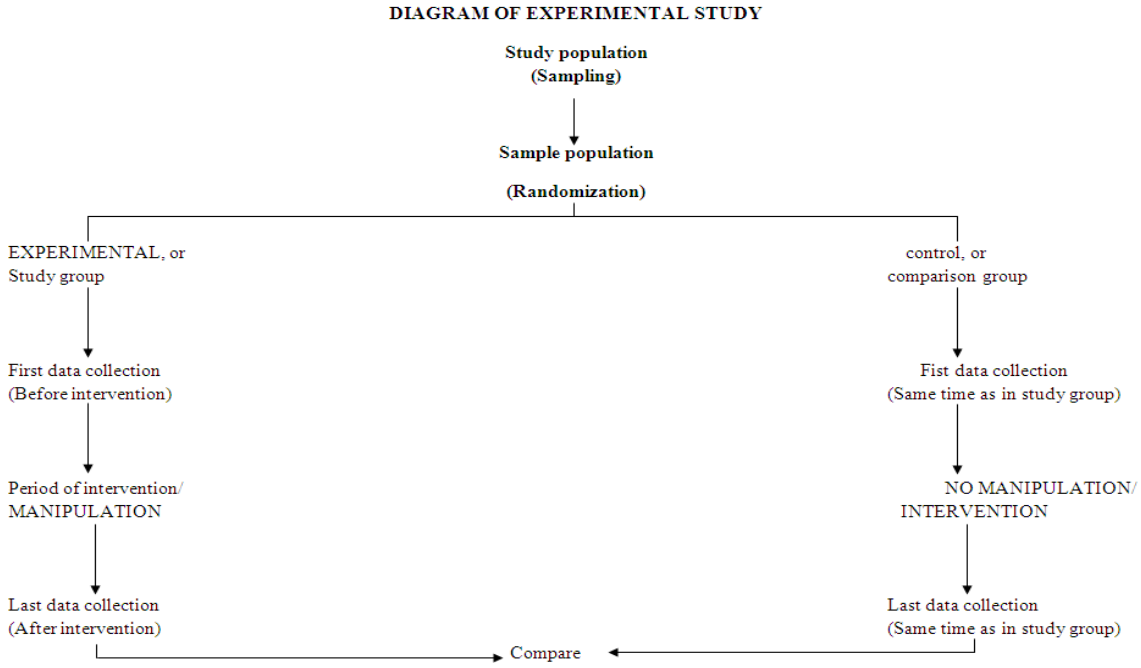
Figure 3.2: *Bulinus globosus* snails

3.3 Study design

Experimental design which consists of two comparable groups: -

1. An exposure group.
2. A non-exposure group.

Figure 3.3: Diagram of experimental study



The tested snails were subjected to various crude determined concentrations at 1g/L to 50g/L of water of the tested grinded plant leaves solution and mortality of the tested animal were recorded over time.

3.4 Sample size determination

Sample size was determined using the formula for difference in proportion which was developed by Foppe I and Spiegelman D (1997) that is:-

$$n = \left(\frac{r+1}{r}\right) \frac{(\overline{P})\,(1-\overline{P})\,(z_\beta + Z_{a/2})^2}{(p_1 - p_2)^2}$$

$n = Sample\ size\ in\ the\ exposure\ group$

$r = Ratio\ of\ controls\ to\ cases$

$z_\beta = Represents\ the\ desired\ power\ (typically\ 1.28\ for\ 90\%\ power).$

$Z_{a/2}=$

$\bar{R}epresents\ the\ desired\ level\ of\ statistical\ significance\ (typically\ 2.96)$

$(P) = A\ measure\ of\ variability\ (similar\ to\ standard\ deviation)$

$(P_1 - P_2) = Effect\ size\ (the\ difference\ in\ proportions)$

This study intend to detect Odds ratio of 2.0 (20%), therefore

- For 90% power, $Z\beta$ = 1.28
- For 0.05 significance level, Z_a= 1.96
- r = 1 (equal number of cases and controls)
- the proportion exposed in the control group is 20%
- to get proportion of cases exposed:

Proportion of cases exposed = ORp control exposed/p controls exp (OR – 1) + 1

Or $P_{exp} = \frac{ORP\ control\ sexp}{p\ control\ sexp\ (ORP-1)+1}$

$P_{expo} = 2\ (0.2)\ /0.2\ (2\ -1)\ +\ 1\ =\ 0.33$

$$n\ =\ (\frac{r+1}{r})\ \frac{(\overline{P})\ (1\ -\ \overline{P})\ (z_\beta\ +\ z_{a/2})^{\ 2}}{(P_1 - P_2)^2}$$

$$n\ = 2\frac{(.265)(1-.265)1.28+1.96)^2}{(.33-.20)^2} = 242$$

Therefore, n = 242 + 10% = 266 (133 exposures, 133 non exposure)

3.5 Extracting chemical material from leaves *Azadirachta indica*

3.5.1 Obtaining the *Azadirachta indica* plant leaves stock solution

Leaves were collected from the plants cultivated at "Unguja Island" washed with water and dried at room temperature. A 100gm of leaves by using electric grinder were grinded into fine powder, two days before the experiment. The powder was sieved by using two combined kitchen sieve to get very fine powder and then used to make concentration of 1% to 50% stock solution (1-50 gram powder in every100ml distilled water) prepared by hand shaking after every 30 minutes for six hours in

flasks. After 24 hours the solution was filtered through two combined kitchen sieve, stored in stopper bottles that were used within three days of storage as described by Ahmed (2016). The stock solution comprising the following concentrations 1%, 5%, 10%, 15% to 50% of such stock solutions was prepared from grinded powder of neem leaves.

3.5.2 Determining the lethal concentrations of Azadirachta indica plant leaves solution

From prepared stock solution, such concentration was used in this experiment. The concentrations that were tested include 1%, 5%, 10%, 15% up to 50%/litre. White polyethylene trays of 32.5cm × 24.5cm × 24.5m × 6cm was used with the volume of 1 liter of water with different concentrations. Ten specimen of treatment arm were placed at each concentration and exposed to observe mortality of 6 hours, 12 hours and 24hours. One polyethylene tray with the same volume of water and snails without concentration (Treatment) was used for non-exposure group in each concentration (control group). All trays were labeled according to dose and time of exposure and observation. After every exposure time, those specimen that found dead were removed, washed by unchlorinated water and kept in another tray with the new volume of water of 1000ml for 24hours for confirmation and those not died after exposure time were treated the same as those died being washed by unchlorinated water and kept the same 24 hours for confirmation if they were died or not. A snail was considered dead when it floats to the surface with its shell becoming translucent or lay with neither movement on the bottom nor muscular activity. After that they were recorded as dead or alive. All tests of different concentrations were made simultaneously with the recorded Temperature °C, Relative Humidity % and pH of water.

3.6 Collecting *Bulinus globosus* snails from the field

All specimens were collected from the same sources of streams namely “Mto pweza” North Region, North “A” District, Shehia of Kikobweni Unguja. The stream where sample were collected is located from S-0.5° 57.896’, E-0.39° 180.340’ at 59 meters above sea level using Garmin GPSMAP. All specimens were sorted to the same size

and put into the white polyethylene tray. Small fish also were collected at that source. The sample was then directly transported to the laboratory. Sampling of Bulinus Globosus snails was done a day prior to experiment to control their lives from any disturbance during sampling and transportation, and to make necessary exclusion for those that would die.

Figure 3.4: Mto pweza collection site

3.7 In the laboratory

The samples were kept and maintained in different aquarium of 20inx8inx10in supplied with air and 2 liters of water each, the snails were fed with dried lettuce leaves after every second day and some vegetation from their habitats. Before the experiment, water was changed after every two days to remove faces and other unwanted impurities. Efforts were made to minimize stress on these field collected fresh-water snails including: giving food, removing dead snails soon as possible to prevent decomposition and finally the sample was allocated randomly during the experiment to avoid bias. Temperature, relative humidity, and pH were recorded daily till the end of experiment.

Figure 3.5: In the laboratory with different items; aquarium, digital weighing machine, thermometer with relative humidity, polyethylene tray with snails and food, air machine and polyethylene trays with snails and polyethylene trays with different concentration of azadirachta indica plnt leaves solution.

3.8 Sampling procedures/Techniques

Sampling method

Non-probability sampling was used according to the nature of the study of which no chance selecting other unrelated unit.

Purposive sampling

Sample was selected purposely because they have certain predetermined characteristics.

Sampling technique

Observation technique was carried out to collect the data, i.e. freely and direct observation of an event to take place personally.

Data collection tool

An excel sheet was used to record the finding according to the nature of the study.

3.9 Data analysis

Data were analyzed using Microsoft Excel in the computer software for data analysis. Univariate analysis and Analysis of Variance (ANOVA) were used to determine the p-value at 95% confidence level. Confidence intervals (C1) for each group was calculated after determining the prevalence. A result with a value of $p < 0.05$ was considered statistically significant. Univariate descriptive statistics can summarize large quantities of numerical data and reveal patterns in the raw data. In order to present the information in a more organized format, it was better to start with univariate descriptive statistics for each variable. The confidence interval (CI) gives the interval at which results would range in case of further similar experiments. Confidence intervals are about risk. The CI considers the sample size and the potential variation in the population and gives us an estimate of the range in which the real answer lies.

3.10 Ethical considerations

Permission to conduct study was requested at Mzumbe University and Ministry of Health Zanzibar. Four research assistants were recruited. Two among them for the field collection of the snails and other two for the laboratory works. The research assistants were trained about ethics of research and how to seek for consent together with the aim and objectives of the study. All of them were given protective gears i.e. (long gumboot, gloves and forceps) to protect them from being infected in case of affected snails.

3.11 Training of research assistants

Training was conducted at Unguja "Zanzibar Schistosomiasis Control Programme" with four selected experienced staff as research assistants, two among them for the field collection of the snails and other two for the laboratory works. Under laboratory setting one among them was a laboratory technician with experience of work in that schistosomiasis laboratory, while others were more experience for working within the program particularly under field works. Two days before, research assistants were reminded on how to collect fresh water snails at the field. This training was

carried out for two days, one day for field visit and the remaining is for the laboratory works.

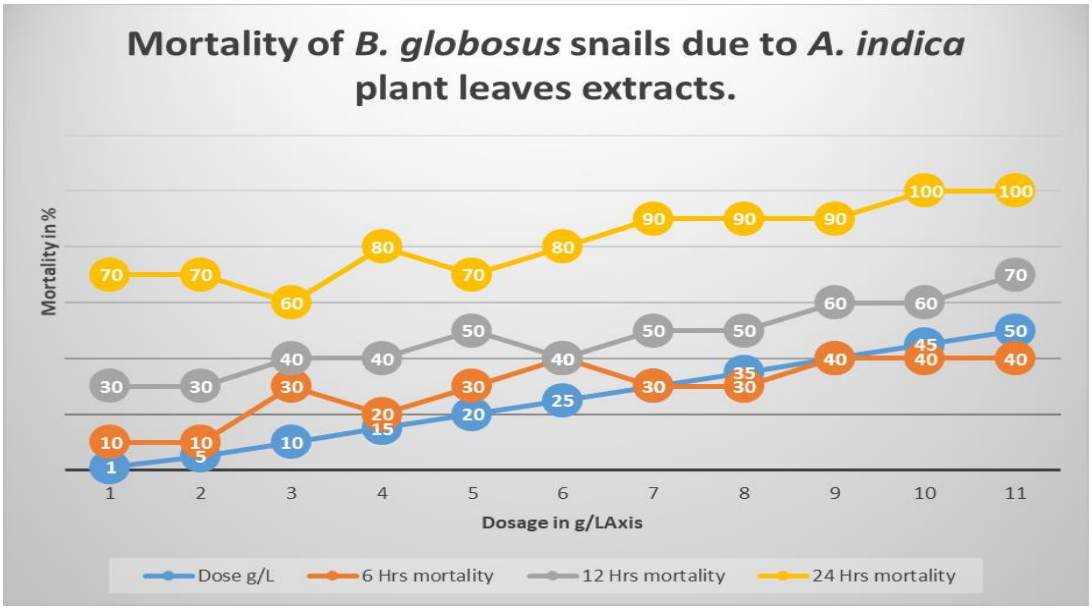
CHAPTER FOUR
PRESENTATION OF FINDINGS

4.1 Results

4.1.1 The lethal dose at LD50 and LD95 of grinded leaves of Azadirachta indica plant against field collected Bulinus globosus.

Study showed the results on effect of different doses of *Azadirachta indica* plant leaves extract and the significant behavior changes on lethality of snails in the test. The experimental groups have confirmed the molluscicidal effect. An increase in mortality rate due to exposure periods and doses of the extract as well as several factors may be acting as molluscicidal effect of the extract. Exposure of experimental snails at dosage of 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 gm/l of water at 6, 12 and 24 hours was determined. In fact, there was a relatively dose-dependent response in mortality rates of *Bulinus globosus* from 1 gm/l to 50 gm/l and the percentage of mortality reached 90 to 100% at extract doses of 35 gm/l, 40 gm/l, 45 gm/l and 50 gm/l as shown in figure 4.1 below.

Figure 4.1: Mortality of *B. globosus* snails to different doses (g/L) of *Azadirachta indica* plant leaves extract.



The line graph summarizes the results on the effect of *Azadirachta indica* plant leaves crude extract to *Bulinus globosus* snail's mortality. Results from line graph indicate that the estimate of the highest mortality after 6 hours was 40% (CI: 43.5-46.5), for 12 hours 70% (CI: 57.3-82.7) and for 24 hours 100%. This indicates that the time of exposure and dosage increase, result into more mortality.

4.1.2 Lethal dose (LD_{50} and LD_{95}) of grinded leaves of Azadirachta indica plant against field collected Bulinus globosus.

Lethal dose (LD50) is a standard measure of the toxicity of *Azadirachta indica* that cause half of the sample population to die in a specified period through exposure via ingestion, skin contact, or injection. LD50 is measured in milligrams, and is also called median lethal dose. Also, in the experiment of this study the lethal dose 95 was determined and the results of all lethal doses are as described in the table below:

Table 4.1: The lethal dose at LD50 and LD95 of grinded leaves of Azadirachta indica plant against field collected Bulinus globosus.

Time (Hours)	Mean	Confidence interval	LD_{50}	LD_{95}
6	29.1	22.6-35.6	NIL	NIL
12	47.3	39.8-54.8	28.81g/L	NIL
24	81.8	73.9-89.7	NA	42.99g/L

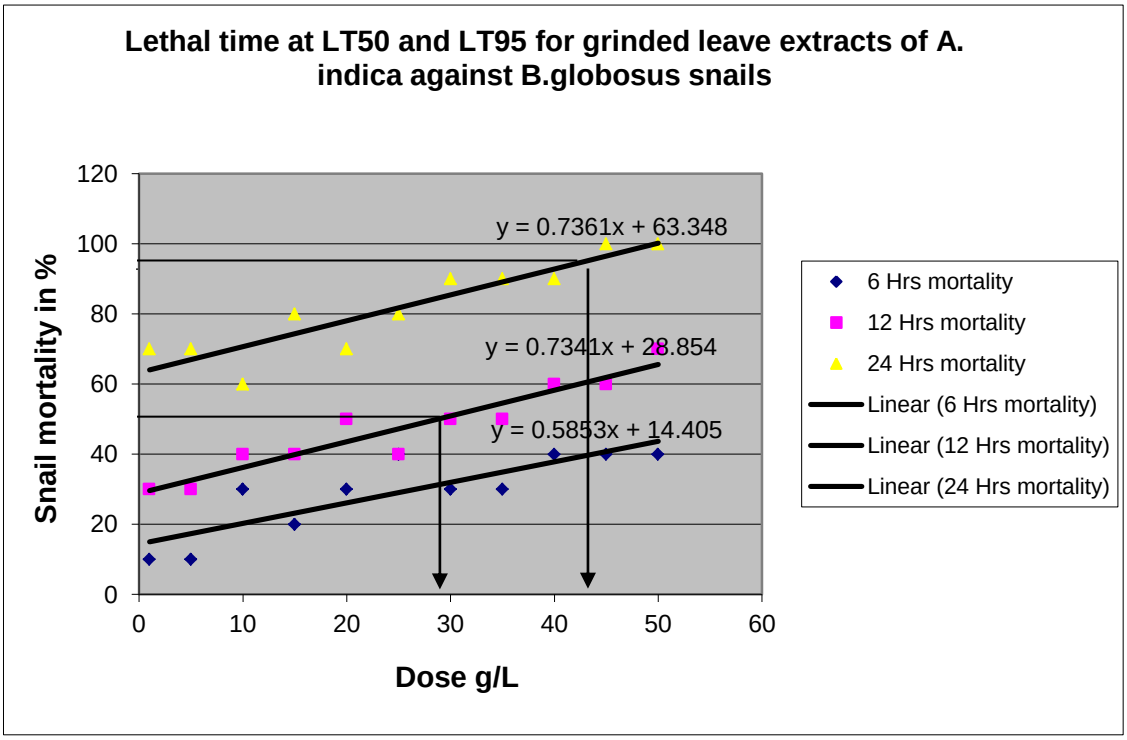
From table it is indicated that neither LD_{50} nor LD_{95} could be recorded at 6 hours of exposure time for *Azadirachta indica* leaves crude extract against *B. globosus* snail. For the 12 and 24 hours, the LD_{50} of 28.81g/L with a 95% confidence interval of 39.8-54.8 and 42.99g/L with a 95% Confidence interval of 73.9-89.7 were recorded respectively. This trend indicates that as dose concentration and time increase, mortality of snails increase as well.

4.1.3 To determine the effectiveness of lethal time at LT_{50} and LT_{95} of grinded leaves of Azadirachta indica plant against field collected Bulinus globosus snails.

In this study, the lethal time (LT_{50} and LT_{95}) were determined. The LT_{50} is commonly determined to quantify amount of stressor necessary to kill *B. globosus* snails whereas the LT_{95} was done to determine the amount of time taken to kill 95%

of the exposed *B.globosus* snails under the experiment. The figure below summarizes the findings.

Figure 4.2: Azadirachta indica plant leaves extract for the determination of LT_{50} and LT_{95} after exposure of *B. globosus* for 6, 12 and 24 hours (n=10)



At 6 hours' time, no dosage which achieved LD_{50} or LT_{50} for the plant extracts, which means application of this product need more dose and exposure time. The results above show that, the LT_{50} was obtained at the dosage of 28.81g/L of the plant extract after exposure for 12 hours. This suggests that the LT_{50} of *Azadirachta indica* plant extract against *Bulinus globosus* can be obtained at dosage of 28.81g/L of the extract in 12 hours' time. However no LT_{95} could be recorded during 12 hours period.

Regarding the LT_{95}, the results above show that, the LD_{95} was obtained at the dosage of 42.99g/L of the plant extract. This suggests that the LT_{95} of *Azadirachta indica* plant extract against *Bulinus globosus* can be obtained between 42.99g/L at 24 hours' time.

CHAPTER FIVE
DISCUSSION

The literature review indicated that the compounds from *Azadirachta indica* among the others have a number of properties that are useful for controlling of pests. These include repellence, oviposition prevention and regulate insect growth activity, low mammalian toxicity and low persistence in the environment (Schmutterer, 1990; Koul, 1992). It is evident from our results that the *Azadirachta indica* that was tested in this research had a toxic effect on the snail *B. globosus*. Some test results proved the leave-extract to have molluscicidal activity. Neem leaves extract significantly reduced survival of the giant African snail *Achatina fulica* (Rao, Singh, 2000). Apart from snail's mortality, neem plant extract has shown to be effective in killing other insects as demonstrated by H. Vatandoost1 and V.M.Vaziri (2004) in their study to mosquitoes, compounds extracted from *Azadirachta indica* showed that, mortality of fourth stage larvae of Anopheles stephensi, with LC_{50} values of 60 and 43 part per million respectively. This compares with the LC_{50} and LC_{95} in our study of 28.81g/L and 42.99g/L for *Bulinus globosus.*

The Molluscicidal effects of Azadirachta indica plant leaves crude extract against Bulinus globosus by using different doses were tested. Mortality of freshwater snails after 24 hours exposure was used for monitoring the molluscicidal efficacy. These findings indicate that the exposure time and concentration of extracts affected the toxicity of neem leaves extract, as there was a significant difference between the mortality rate with exposure time and concentrations. Table presented the mortality rate of snail after exposed to *Azadirachta indica* plant leaves crude extracts for 6,12 and 24 hours. From the results, the mortality rate of *Bulinus globosus* snail were significantly increase ($p<0.05$) as dose and time increase for all neem leaves extract. The mortality of snail was gradually increased from 6 hours to 24 hours of exposures, but after 12 hours, it was observed that the mortality rate had increased rapidly where all experimental snails died at the last two dosages i.e. 45-50g/L. Similar trend of toxicity was observed in the study conducted by Rosidyani M *et al*

(2015) where the results confirmed that the molluscicidal activities of neem seed crude extract on snail were highly correlated with the concentration of extracts.

The experiments carried to other pests using *Azadirachta indica* leaves extracts showed similar results. For example, Bakr (2013) carried out a study to determine the severe toxicity of water extracts of *Azadirachta indica* leaves and seeds against *"Rattus norvegicus* where by different doses of *Azadirachta indica* extracts of leaves and seeds injected to the rats and the percentage of death was recorded over the 48 hours. The study reveal that, percentage of death in all treated rats with *Aadirachta indica* leaves extracts were increased by doses increased. Rats given higher doses of *Azadirachta indica* leaves extract and seeds showed 100% death. The LD_{50} of the extract of leaves and seeds were 6.2, 9.4 mL kg (-1), respectively. Depending on these results, it may be concluded that doses of A. indica leaves and seeds introduced to rats showed significant acute toxicity. For current study all of the treatments were resulted in significantly ($p<0.05$) killed the snail and the mortality rate was positively correlated with the extract concentrations.

At dosage of 1-50g/L for 6 hours exposure, the experiment show that no lethal dose fifty (LD_{50}) recorded. This may be contributed to some factors which could not be established during the experiment, but this could be contributed may be by snail species, environment, mixing and means of leaves extraction. In determining LD_{50} and LD_{95}, it was noted during experiment; LD_{50} happened at 28.81g/L. as stated early, at 6 hours no LD_{50} recorded but as continued with experiment, at 12 hours and 24 hours lethal dose (LD_{50} and LD_{95}) were recorded. This implies that the specie type of Neem used requires high dosage and extended time. These results are consistency to the results reported by Singh K. and Singh D.K (1996) in their study that the molluscicidal efficacy of the leaf, bark, cake, neem oil, achook and nimbecidine, was both considered as time and dose dependent. Their study reported that the toxic effect of pure azadirachtin against both the snails was greater than the synthetic molluscicides that the molluscicidal activity was both time and dose dependent and toxic effect of pure azadirachtin against both the snails was greater than the synthetic molluscicides.

In this study, lethal time (LT_{50} and LT_{95}) was noted that all happened at 12 and 24 hours respectively. The 6 hours exposure of snails could either reveal neither LT_{50} nor LT_{95}. The LT_{50} and LT_{95} were recorded at 28.81g/L and 42.99g/L respectively. These results conquer with those reported by Winkaler EU *et al.*, (2007) that the 24 hours LC_{50} of Neem leaf extract for juveniles *P. lineatus* was estimated at 4.8 g/L.

CHAPTER SIX
SUMMARY CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The objectives of this study included determination of the lethal dose at LD_{50} and LD_{95} of grinded leaves of *Azadirachta indica* plant against field collected *Bulinus globosus* and determination of effectiveness of lethal time at LT_{50} and LT_{95} of grinded leaves of *Azadirachta indica* plant against field collected *Bulinus globosus.*

It has been revealed that *Azadirachta indica* has a molluscicidal effect against *Bulinus globosus* although at all doses no LD_{50} and LD_{95} were recorded during the 6 hours exposure despite of recorded snail mortalities. The LD_{50} of 28.81g/L with a 95% Confidence Interval (CI) of 39.8-54.8 was recorded at 12 hours exposure where LD_{95} could not be recorded at this same time. The LD_{95} was recorded after 24 hours of snail exposure and this was 42.99g/L with a 95% Confidence Interval of 73.9-89.7 However, the (ANOVA) Analysis of Variance showed that no statistical significance differences between the mortality exerted by the three groups for 6, 12 and 24 hours ($P>0.05$). On the hand, LT_{50} was recorded at the dosage of 28.81g/L and LT_{95} at the dosage of 42.99g/L.

6.2 Conclusions

The products of *Azadirachta indica* are cheap and easy to prepare with environmental friendly and low-cost alternatives to synthetic chemicals. From present study findings, it was noted that the *A. indica* possess molluscide that can kill *B. globosus* snails. Based on these findings, it is therefore concluded that there is efficacy on application of *Azadirachta indica* (Neem) plant leaves extract and can be used to control fresh water snails particularly *Bulinus globosus* in ponds as environment friendly material instead of hazardous molluscides like Niclosamide which are inorganic chemicals and unaffordable to people with low income. Definitely, treatments of *Azadirachta indica*, through use of leaves extracts, cannot replace completely chemical pesticides used in snails control in the fields but the amounts of pesticide needed could be reduced, thereby decreasing the pesticide load in food chain. Also, extensive investigations are needed to establish and to provide

better information and suitable methods of application in aquatic animal production facilities in future for its safe use in public health. Moreover, the other parts of the plant such as bark, seed and roots can be tested to compare its molluscide effects to those exerted by leave extracts.

6.3 Recommendations

If full benefits are to be achieved, the followings are recommended: -

1) Further integrated support for this of all stakeholders needed from governments, policy makers, administrators, public and private organizations, national and international programs, and the donor community at large on this current strategy.

2) Since it is known to be environmental friendly, cost effective and can be manageable by local people, neem tree must be planted through integration of stake holders in schistosomiasis endemic areas around the ponds for their availability to control unwanted Schistosoma snail vectors.

3) Better extraction methods for purity and cost-effective mass production processes are recommended to be worked on to produce quality and price competitive and affordable product in the Tanzania market to satisfy the target group.
4) The laboratory study and results confined to static environment of water body and hence ponds are more relevant for the use of *Azadirachta Indica* leaves extract than in streams and therefore new study to be extended to stream environment.
5) Even if the study was experimented under static laboratory environment, further study of *Azadirachta Indica* leaves extract to be conducted under field conditions to confirm its efficacy.
6) Further Study to be conducted to confirm *Azadirachta Indica* its safety to other living organisms (selective).
7) Country must have an integrated body to establish policy from all stakeholders including researchers, legal personnel, traditional herbalist,

health care professionals, Agricultural/forest officers, policy makers, planners, social, political, religious and community level representatives responsible for all issues concerning herbal plants and their use with strategic planning on registration system for herbal medicines, manufacturing requirements, safety assessment requirements and advocating importance of herbal plants from low to high level communities.

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APPENDIX

Proposed data collection (observation) tool

Date ……/ …… / 2018

Place……

Signature ……

Exposure time of sample ……

Dose(gm)	No. of snails exposed	No. of snails died soon after exposure time	No. of snails survived soon after exposure time	No. snails died after 24 hrs. of confirmation	No. snails survived after 24 hrs. of confirmation	Remarks