

**EFFECT OF SODIUM CHLORIDE SOLUTION ON EGG
MASSES, JUVENILES AND ADULTS OF *BIOMPHALARIA*
PFEIFFERI, HOST OF *SCHISTOSOMA MANSONI***

TONNY TEYA NYANDWARO

MASTERS OF SCIENCE

(Parasitology and Entomology)

**JOMO KENYATTA UNIVERSITY OF
AGRICULTURE AND TECHNOLOGY**

2018

Effect of sodium chloride solution on egg masses, juveniles and adults of

Biomphalaria pfeifferi*, host of *schistosoma mansoni

Tonny Teya Nyandwaro

**A thesis submitted in partial fulfilment for the degree of master of science
in Medical Parasitology and Entomology in the Jomo Kenyatta University
of Agriculture and Technology**

2018

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

Signature: Date:

Tonny Teya Nyandwaro

This thesis has been submitted for examination with our approval as University Supervisors.

Signature: Date:

Prof. Zipporah Ng'ang'a, PhD

JKUAT, Kenya

Signature: Date:

Dr. Gerald M. Mkoji, PhD

KEMRI, Kenya

DEDICATION

I dedicate this thesis to my wife who with unconditional love has always been supporting me through the course of study. This thesis is also dedicated to my mother, brother Maghanga, my sisters Kambe and Wakio for their love, endless support and encouragement. With fond memories of my late father Abel Magiya

ACKNOWLEDGEMENTS

First and foremost, I express my deepest gratitude to my supervisor, Dr. Gerald Mkoji of the Kenya Medical Research Institute (KEMRI) for his support as I undertook my research. He facilitated collection of snails used in the study, provided laboratory space and the material I needed for my research, and further more gave guidance, suggestions and valuable comments that helped shape this work.

I also sincerely thank my supervisor Prof. Zipporah Ng'ang'a of College of Health Sciences COHES, at the Jomo Kenyatta University of Agriculture and Technology (JKUAT) for her guidance, encouragement and for her valuable comments during the entire study. I'm truly grateful to her for support. In spite of her busy schedule she was always available to offer guidance and support during my project.

My sincere gratitude's to staff members of the Schistosomiasis section at the Centre for Biotechnology Research and Development (CBRD) in KEMRI where I did my research project for their support and help, and in particular, I sincerely thank Mr. Martin Mutuku for his guidance on snail maintenance procedures and his valuable suggestions

My sincere appreciations to my friends, Samson and Betty for their encouragement and moral support. To all those who helped me in one way or another during my training-I say thank you for their contributions to my education. Last but not least, I sincerely thank my Mom, my brother and my sisters for their prayers, love, encouragement, and for being there for me.

TABLE OF CONTENTS

DECLARATION	ii
DEDICATION	iii
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	v
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF APPENDICES	x
LIST OF ABBREVIATIONS	xi
ABSTRACT	xii
CHAPTER ONE	1
INTRODUCTION	1
1.1 Background Information	1
1.2 Problem Statement.....	3
1.3 Justification	4
1.4 Hypothesis.....	5
1.4.1 Null hypothesis.....	5
1.5 General Objective	5
1.5.1 SPECIFIC OBJECTIVES.....	5
CHAPTER TWO	6
LITERATURE REVIEW	6
2.1 Burden of Schistosomiasis	6
2.2 Role of Snails In Schistosomiasis Transmission.....	7
2.3 Snail Control Approaches.....	8
2.4 Chemical MOLLUSCICIDES	9

2.5	Plants Molluscicides.....	10
2.6	Environmental Vector Control	12
2.7	Other Snail Control Approaches	12
2.8	Role of Salt In Snail Control.....	13
2.9	Biological Control of Schistosomiasis.....	13
CHAPTER THREE		15
METHODOLOGY		15
3.1	Study Design.....	15
3.2	Site of Study	15
3.3	Collection of Snails	15
3.4	Snail Maintenance.....	15
3.5	SALT (Nacl) PREPARATION	16
3.6	Effect Of Nacl Solution On Hatchability Of <i>B. Pfeifferi</i> Egg Masses And Their Development.....	16
3.6.1	Test Solution	16
3.6.2	Controls.....	16
3.7	Effect Of Nacl Solution On The Survival Of Neonate Of <i>B. Pfeifferi</i>	16
3.8	Effect Of Nacl On The Survival Of Adult <i>B. Pfeifferi</i>	17
3.9	Data Analysis.....	18
CHAPTER FOUR		19
RESULTS.....		19
4.1	General Observations	19
4.2	Effect of Nacl Solution On Egg Masses Of Snails.....	21

4.3	Effect of Nacl Solution On Neonates Of <i>B. Pfeifferi</i>	28
4.4	Effect of Nacl On Adult <i>B. Pfeifferi</i>	30
CHAPTER FIVE		38
DISCUSSION		38
5.1	Adults Snails	40
5.2	Neonates	42
5.3	Egg Masses	43
CHAPTER SIX		46
SUMMARY CONCLUSION & RECOMMENDATIONS.....		46
6.1	Summary	46
6.2	Conclusion.....	46
6.3	Recommendations	46
REFERENCES.....		47
LIST OF APPENDICES.....		62

LIST OF TABLES

Table 2.2:1: Schistosoma life cycle	8
Table 4.1:1: Responses of neonates & adults during the 24 hr. exposure to NaCl	19
Table 4.1:2: Effect of NaCl on the different developmental stages of <i>B. pfeifferi</i> snail	21
Table 4.2:1: Fecundity of Egg laid by adult snails exposed to NaCl	24
Table 4.4:1: Responses of Adults <i>B. pfeifferi</i> to NaCl solution	31

LIST OF FIGURES

Figure 4.2.1: Hatchability rate of eggs at different concentrations of salt solution	23
Figure 4.2.2: Control solution at 7 day, fully developed neonate snail's.....	25
Figure 4.2.3: Poorly developed embryo in egg exposed to 3200mg/L NaCl at 7 day	26
Figure 4.2.4: Undeveloped embryos in egg exposed to 5600 mg/L NaCl at 7 day	27
Figure 4.2.5: NaCl concentrations against egg mass stage of <i>B. pfeifferi</i>	28
Figure 4.3.1: Mortality in neonates at different concentration after 24 hours of exposure...	29
Figure 4.3.2: NaCl concentrations against neonate stage of <i>B. pfeifferi</i>	30
Figure 4.4.1: Effects of salinities on survival of the adults of <i>B. pfeifferi</i> after exposure...	33
Figure 4.4.2: Feeding behaviour of adult following exposure to NaCl solution.....	34
Figure 4.4.3: NaCl concentrations against Adults stage of <i>B. pfeifferi</i>	35
Figure 4.4.4: Proportion of adult <i>B pfeifferi</i> snails that fed on each of the days	36
Figure 4.4.5: Survival of adult snails after exposure to different test salinities.....	37

LIST OF APPENDICES

Appendix 1: Scientific Steering Committee Approval letter	62
Appendix 2: Journal Publication.....	63.

LIST OF ABBREVIATIONS

AFP	Attractant food pellet
Al₂(SO₄)₃	Aluminum Sulphate
CBRD	Centre for Biotechnonology Research & Developmentment
C₈H₁₆O₄	Metaldehyde
CuSO₄	Copper sulphate
DI	Deionized
FCU	Freeze-dried cow urine
FePO₄	Iron (III) Phosphate
ITROMID	Institute of Tropical Medicine and Infectious Diseases
KEMRI	Kenya Medical Research Institute
LC₅₀	Concentration lethal to 50% of the snails' population
NaCl	Sodium Chloride (salt)
NTDs	Neglected Tropical Diseases
PZQ	Praziquantel
WHO	World Health Organization
‰	Parts per thousand
µm	Micrometer

ABSTRACT

Human schistosomiasis is a parasitic disease caused by digenean trematode parasites in the genus *Schistosoma*, which live in the bloodstream of their mammalian hosts, and utilize freshwater gastropod snails as intermediate hosts. Schistosomiasis, a major public health problem in the sub-Saharan Africa, is among the neglected tropical diseases (NTDs) targeted for elimination by WHO by 2025. Although schistosomiasis control and elimination efforts, currently rely heavily on chemotherapy using the antischistosomal drug praziquantel, there is a growing consensus that chemotherapy alone will not help achieve the WHO elimination goal, and so, integrated approaches that include snail control among other strategies are being considered. However, snail control using synthetic molluscicides such as niclosamide is considered too costly for schistosomiasis endemic countries, and so, discovery of inexpensive but effective molluscicides should be explored. This study examined, under laboratory conditions, the potential of sodium chloride (NaCl) (common salt) as a molluscicide using the freshwater snail, *Biomphalaria pfeifferi*, as the test target. The molluscicidal activity of sodium chloride (NaCl) was tested against egg masses, neonates and the adults of *B. pfeifferi* snail. Field collected snails packed between layers of damp cotton gauze and transported to the lab in perforated plastic containers were used in these experiments. Prior to use the snails were screened for trematode infections and allowed to acclimatize for several days before use for experimental purposes. Only snails free of parasite infections were used directly as targets to evaluate the molluscicidal activity of sodium chloride (NaCl) solution or were allowed to lay egg masses for use in further experiments with egg masses and neonates. NaCl solution in concentrations of 3200 mg/L or less did not kill the adult snail (measuring 8mm or above in shell diameter) exposed for 24hr. However, at concentrations in the range 4400-6800mg/L, 10-100% of the adults were killed within 24 hr. of exposure. Concentrations of 1800mg/L or above killed 70-100% of the neonates snails, measuring 2mm or below in shell diameter. The concentration that killed 50% of the egg, neonates and adults was 495 mg/L, 1457 mg/L, and 5087 mg/L respectively. Interestingly, egg laying was completely inhibited in snails that had been exposed to non-lethal concentrations (5600 mg/L and above) of NaCl solution for 24hr, relative to unexposed controls. The molluscicidal effect of NaCl was significantly effective at all stages of *B. pfeifferi* eggs at 24hrs ($d.f = 6$, $X^2 = 42$; $P < 0.05$), neonates at 24 hrs ($d.f = 6$, $\chi^2 = 30$, ($p < 0.05$) and adults at 24 hrs. ($d.f = 4$, $\chi^2 = 15$, ($p < 0.05$). These results suggest that NaCl has potential as a molluscicide against freshwater snails of medical and veterinary importance, and given that it is more readily available and relatively inexpensive, further studies on it as a candidate molluscicide, and should be encouraged. In particular, further studies should be undertaken to determine the molluscicidal effects of NaCl on other snail species of medical or veterinary importance, and its effects on non-target organisms. Non-molluscicidal concentrations of NaCl will need to be tested for effects on snail fecundity, neonate growth and survival, and egg mass hatchability. Short-term or long-term effects of NaCl on the environment should also, be undertaken.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Human schistosomiasis is a parasitic disease caused by digenean trematode parasites in the genus *Schistosoma*, which live in the bloodstream of their mammalian hosts, and utilize freshwater gastropod snails as intermediate hosts (Colley *et al.*, 2014). Schistosomiasis, a major public health problem in the sub-Saharan Africa, is among the neglected tropical diseases (NTDs) targeted for elimination by WHO by 2025 (WHO, 2013). It is estimated that over 200 million people are infected globally, about 20,000 people die from complications arising from schistosomiasis every year (Wu & Huang, 2013), and an estimated 800 million people live under the risk of infection (Gray *et al.*, 2010).

Although schistosomiasis control and elimination efforts, currently rely heavily on chemotherapy using the antischistosomal drug praziquantel, it is evident that chemotherapy alone will not help achieve the WHO elimination goal (King *et al.*, 2006; Lelo *et al.*, 2014; Secor, 2014; Ross *et al.*, 2015), and so, integrated approaches that include snail control among other strategies (Rollinson *et al.*, 2013; Mo *et al.*, 2014; Secor, 2014; Xu *et al.*, 2015) are being considered. For several snail control had been ignored due to excessive reliance on praziquantel for schistosomiasis control. However, in recent years, the topic of snail control has again become relevant (Knight *et al.*, 2014; King *et al.*, 2015). While snail control using synthetic molluscicides such as niclosamide is effective, it is too costly for schistosomiasis endemic countries, and therefore, a search for safe, inexpensive, but effective molluscicides, is needed.

At least 5 schistosomes species are involved causing human schistosomiasis namely i.e. *S. mansoni*, *S. japonicum*, *S. mekongi*, *S. guineensis* and lastly *S. haematobium* (Martinovic-Vitanovic *et al.*, 2013). In Sub-Sahara Africa, *S. haematobium* and *S. mansoni* are the major species present (Adenowo *et al.*, 2015). Currently *S. mansoni* is closely related with poverty, in most endemic countries. Over 6 million people are infected intestinal schistosomiasis in Kenya (Odiere *et al.*, 2012). Schistosomiasis continues to affect many communities in developing countries of sub-Saharan Africa, where clean water supply and sanitation are poor (Nagi *et al.*, 2014; WHO, 2016). *Biomphalaria pfeifferi* is among the 18 *Biomphalaria* species acknowledged in the transmission *S. mansoni*. *B. pfeifferi* is widely distributed in most parts of sub-Saharan Africa where most of cases of *S. mansoni* are reported (Mutuku *et al.*, 2014). Usually *B. pfeifferi* exhibit elevated infection rates (of more than 50%) after exposure to *S. mansoni* in various locations all over Africa (Lu *et al.*, 2016). Based on the above reasons *B. pfeifferi* can be categorized as the world's most important intermediate host of *S. mansoni*.

Currently the schistosomiasis control gets a low operational and rationalized budget in many third world countries (Cioli *et al.*, 2014). Improved approaches to interrupt the transmission of schistosomiasis that are eco-friendly and low cost are required to complement the deworming programs being conducted worldwide (Njenga *et al.*, 2014). Such approaches for snail eradication would be especially useful. Donnelly *et al.*, (1983) suggested that salinity can negatively impact freshwater snails thus, can potentially limit snail population in natural snail's habitats.

This study evaluated the potential of using NaCl in the control of *B. pfeifferi* snails in laboratory conditions with a view to determining its use as a complementary approach to schistosomiasis control especially in resource poor countries. NaCl is readily available, it is inexpensive, and environmental friendly. There is no study done on the molluscicidal effects

of sodium chloride (NaCl) common salt against the egg, juveniles and adults of *B. pfeifferi* snails, host of *Schistosoma mansoni*, causal agent of intestinal schistosomiasis

1.2 Problem Statement

Schistosomiasis control relies almost exclusively on chemotherapy, using virtually the only readily available commercial antischistome drug praziquantel (Doenhoff *et al.*, 2009).

However, chemotherapy alone is not sufficient as re-infections rapidly occur after successful treatment. Furthermore, the drug is not 100% effective and retreatment become necessary (Kabuyaya *et al.*, 2017). Chemotherapy can be complemented with snail control to ensure effectiveness of control measure and to promote sustainability of the disease control efforts. Synthetic molluscicides have been used successfully for snails and Schistosomiasis control (Yang *et al.*, 2012). Chemical molluscicides can be however costly, environmentally unfriendly and difficult to sustain. Although other approaches of snails control exist such as plant molluscicides or biological control their viability and effectiveness in snail control have not been fully established and they are therefore not widely used. Snail control nevertheless remains a practical approach in the fight against schistosomiasis especially as a complement to chemotherapy.

NaCl was selected for this study based on prior studies which have suggested that salinity can greatly impact the fresh water snails (Donnelly *et al.*, 1983; Mostafa, 2009; Omole *et al.*, 2008). NaCl is also readily available, would be simple to apply and may be friendlier to the environment than conventional molluscicides. The idea of vector eradication remains attractive to the public health sector, as it will completely interrupt the transmission of schistosomiasis. This would be achieved by targeting the parasite at the intermediate stage, which is a crucial stage in the parasite life cycle. These would result in the decrease in fresh water snail population and also incidences of human infection with schistosomes.

1.3 Justification

Schistosomiasis control and elimination currently relies on chemotherapy using the antischistosomal drug praziquatel (PZQ)(Thétiot-Laurent, *et al.*, 2013). Although PZQ rapidly reduces the morbidity, cure rates are never 100% in infected individuals, and reinfections rapidly occur after successful treatment campaign in the community (Cioli *et al.*, 2014). This is largely because, the snail's role in schistosomiasis is often ignored in control campaign. Complementing chemotherapy with snail could result in control offers effective and sustainable elimination of schistosomiasis in endemic areas.

Although snail control can be easily achieved through use of chemical molluscicide, cost implications render them unusable in schistosomiasis control campaigns. Even though biological control and plant molluscicides are often discussed as possible alternatives to chemical molluscicides, they have not been universally adopted for routine use, for various reasons. Inexpensive means for snails control are still needed, urgently especially in this era when schistosomiasis is targeted for elimination. The present study is an attempt to develop an inexpensive, yet effective chemical molluscicide. *S. mansoni* is the most widespread and probably, the most important species in Sub-Sahara Africa. *Biomphalaria* snails transmit this parasite, which is especially targeted for elimination.

Therefore, use of NaCl as a snail control strategy in the maintenance stage could be considered an attractive approach. It is a low cost approach, and potentially, with minimal adverse effects to the environment; unlike molluscicides which have been implicated in causing high fish mortality and possible resistance to the molluscicide being used (Oliveira-Filho *et al.*,2010). NaCl would be cheaper and with little ecological impact to the environment

1.4 Hypothesis

1.4.1 Null hypothesis

NaCl is not effective in killing Adults, 3 Day old Neonates and Egg masses of *B. pfeifferi*.

1.5 General Objective

Effect of NaCl on eggs, neonates & adults of *B. pfeifferi*, snail host of *Schistosoma mansoni*.

1.5.1 Specific Objectives

- 1) To determine the molluscicidal activity of NaCl solution on adult snails and neonates of the snail *B. pfeifferi*, intermediate host of *S. mansoni*, causal agent of intestinal schistosomiasis in Sub-Saharan Africa.
- 2) To determine the effect of NaCl solution on hatchability of *B. pfeifferi* eggs masses.

CHAPTER TWO

LITERATURE REVIEW

2.1 Burden of Schistosomiasis

Schistosomiasis is one of the most widespread parasitic helminth infections, and is second only to malaria in terms of socio-economic and public health importance in most tropical and sub-tropical regions of the world (Sousa-Figueiredo *et al.*, 2012). In Africa, approximately 40 million women have schistosomiasis during pregnancy (McDonald *et al.*, 2014), and over, 230 million people worldwide require treatment for schistosomiasis yearly .

Over, 30 countries in Africa share the burden of schistosomiasis. Nigeria has the highest prevalence with 28.8million cases reported, follow closely by 19 million cases in Tanzania, 15.2 million cases in Ghana, 14.9 million cases in Congo and lastly 13.2 million cases in Mozambique (Bajiro *et al.*, 2016).

In Kenya, roughly about 23 % of the total population, is infected with intestinal schistosomiasis (Rollinson *et al.*, 2013). Many control strategies geared towards controlling schistosomiasis infections in profoundly exposed populations, but much has not been achieved due to re-exposure to the infection (Adenowo *et al.*, 2015).

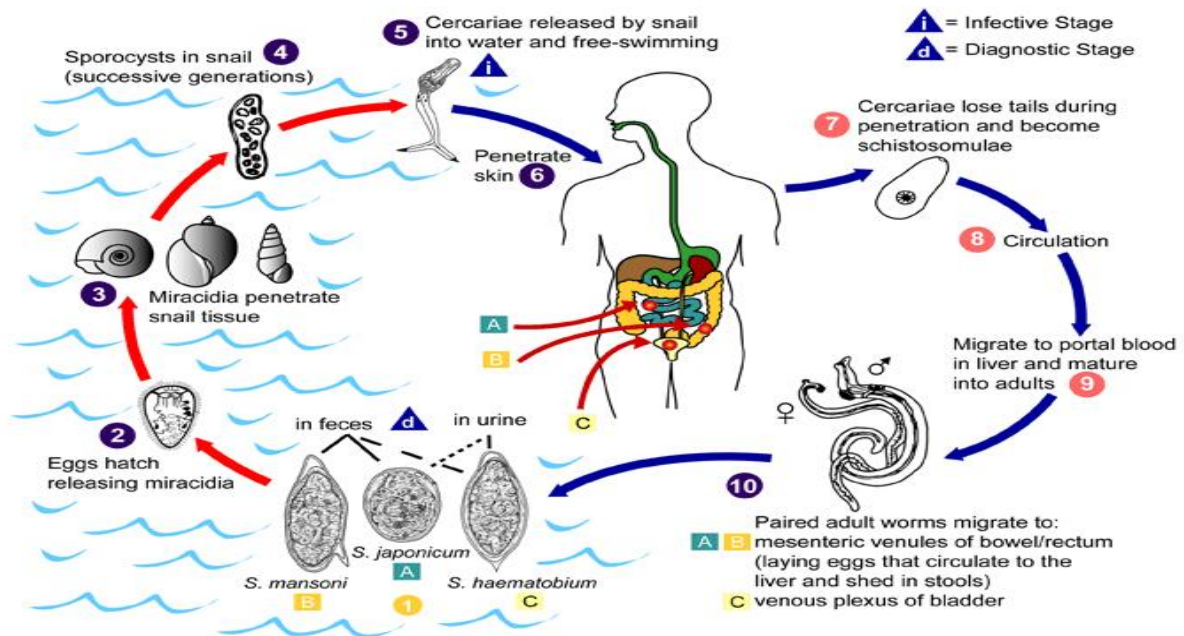
Biomphalaria pfeifferi is a freshwater snail found in most parts of sub-Saharan Africa. It is the intermediate host for the trematode parasite *S. mansoni*, which is responsible for causing intestinal schistosomiasis (Stensgaard *et al.*, 2013). Schistosomiasis is transmitted by aquatic snails in a wide variety of fresh water habitat.

Fresh water snails belong to the phylum *Mollusca*, class *Gastropoda*. The fresh water gastropod is divided into two subclasses namely the *Prosobranchia* and the *Pulmonata* (Wade *et al.*, 2006). The intermediate host plays an essential role in the parasitic life cycle of *Schistosoma*. *Schistosoma* cercariae are released from the snails into the water and penetrate the skin of susceptible individuals exposed to the water while engaged in water-based activities such as bathing, swimming, fishing and agricultural activities (Grimes *et al.*, 2015).

2.2 Role of Snails in Schistosomiasis Transmission

Schistosoma mansoni infection ranks second behind malaria in terms of public health importance in tropical regions of the world (Hua *et al.*, 2013). Its distribution is attributed to the broad geographic range of susceptible species of the freshwater snail genus *Biomphalaria* that serve as intermediate hosts for its larval stages (Opisa *et al.*, 2011). Infected fresh water snails will shed cercariae once exposed to sunlight, hence contaminating the water (El-Sherbini *et al.*, 2009). The most prevalent infections are *S. haematobium*, *S. mansoni*, and *S. japonicum* respectively; while *S. mekeongi* and *S. intercalatum* are the less prevalent species (Scholte *et al.*, 2012). A lot of scientific scrutiny has been carried out on *Biomphalaria* snails (*Basommatophora* and *Planorbidae*) to gain more insight into the distribution and exposure risk of intestinal schistosomiasis (Scholte *et al.*, 2012). Out of the 12 species of *Biomphalaria* found in Africa implicated in the transmission of *S. mansoni*, none has so far shown resistance to the infection (Pinto *et al.*, 2013). Schistosomiasis can be controlled by destroying the carrier snail and thereby breaking the life cycles of the parasite as shown figure 2.2.1

Table 2.2:1 Schistosoma life cycle (Source: <http://www.cdc.gov/dpdx/schistosomiasis/index.html>).



Schistosomes have developed very advanced mechanisms to deal with host-specific immune responses. It is documented in both snail and man hosts, schistosomes can elude the host's defense system and still maintain ability to reproduce (Fonseca *et al.*, 2015).

2.3 Snail Control Approaches

Schistosomiasis controls dates back in 1920s, when the Egyptian government put in place measures to control schistosomiasis among school going children (Appleton & Kvalsvig, 2006). Then in 1940s application of Copper Sulphate was started, together with other control methods (Blankespoor & Reimink, 1991). In 1955, Sodium Pentachlorohenate (NaPCP) a new and a more efficient molluscicide replaced Copper Sulphate (Komiya, 1961). From then, it has generally been agreed that effective control approaches should consist of snail control, mass drug administration and sanitation.

In 1980s, an expert committee of the WHO introduced a new schistosomiasis control strategy, which shifted attention from transmission control to morbidity control (Savioli *et al.*, 2004)

Efforts to effectively and sustainably control schistosomiasis have failed partly because the role played by snails in the transmission is often ignored (Grimes *et al.*, 2015). One approach of dealing with the problem of schistosomiasis is through destroying the snails' intermediate host, and thus removing an essential link in the life cycle of the fluke (Ajay *et al.*, 2013). This can be achieved by use of synthetic or plant molluscicide, but the use of synthetic molluscicide has been complicated due to its high costs, development of resistance and toxicity to non-target organism (Hamed, 2011). This has called for more research on environmental friendly molluscicides.

2.4 Chemical MOLLUSCICIDES

Towards the end of the 19th century and the beginning of 20th century readily available materials like carbolic acid were recommended molluscicide. Compounds such as Bordeaux mixture (which was a suspension of Cupric Hydroxide) were endorsed as molluscicidal dusts and sprays (Lovett & Black, 1920). Several types of chemical molluscicides were developed and tested for snail control. For example in the 1950s Copper Sulphate was used as a molluscicide. It was toxic to snails even in small concentrations and accumulated in the snail's body causing death (Jose, 1956). Niclosamide has been used worldwide for snail control, it is still in use due to its high efficiency and low toxicity to humans. However, it's expensive and has poor water solubility (Berrios-Duran *et al.*, 1968). Sodium Pentachlorophenate is known to cause dermatitis in man and animal, kills aquatic plants and animals including fishes, it is rapidly inactivated by the ultraviolet ray (Ishak *et al.*, 1972).

The inorganic fertilizer compounds like super-phosphate, potassium sulfate, ammonium sulfate, ferrous sulfate and copper sulfate formulated in a solution of 1.5% (w/v) with an adhesive substance, showed good properties as repellent, and were toxic to *Theba pisana* snails (Wakil, 1999). Phenolic compounds; flavonoids, unhydroxylated flavone and flavanone hesperidin have been shown to have molluscicidal properties against *Bulinus truncatus* (Lahlou, 2004). Edward & Sogbesan, (2007) studied acute toxicity of Temophos (organophosphate larvicide) against *Lymnaea natalensis* and *Bulinus globosus*. Abate was observed to be ovicidal to eggs masses of the two snail species at 0.03 and was found to be toxic to both *L. natalensis* and *B. globosus* snail hosts of *Fasciola* and *schistosoma* respectively.

Other major chemical molluscicides are carbamate, organophosphate and synthetic pyrethroid (Duke *et al.*, 2010). Molluscicidal effects of metaldehyde have been found to largely depend on ambient temperature and humidity (Singh *et al.*, 2013). The organophosphates are powerful inhibitors of cholinesterase, and are less similar to the mode of action of carbamates (Yadav *et al.*, 2017). Inhibition by organophosphorus is irreversible; pyrethroids mainly affected the nerve membrane by altering its permeability to Na⁺⁺ and K⁺ (Čolović & Krstić, 2013).

2.5 Plants Molluscicides

Plant molluscicides are considered to be potential alternatives to synthetic molluscicides because of their high target toxicity, low mammalian toxicity, low cost, solubility in water, bio-degradability. Plant molluscicide are readily available in endemic areas (Daffalla & Amin, 1976). Although, considerable research on plant molluscicide has been undertaken, a number of compounds are currently being evaluated as potential molluscicides. However, none has reached the market yet. Plant molluscicides have been suggested as possible alternatives to chemical molluscicides and indeed several plants with molluscicidal activity

have been tested (Olajumoke & Morenikeji, 2012). However, none is available for routine application.

Methanol extracts of *Euphorbiales Schimperiana* have been suggested to have molluscicidal potential and remain stable over a wide range of temperatures, (Al-Zanbagi *et al.*, 2001).

Organic solvent extracts of latex of *Euphorbia pulcherima* and *Euphorbia hirta* have been tested against snails and aquatic pests (Singh *et al.*, 2004). *Lantana camara*, Kalmi, (*Ipomoea aquatic*) and Haicha (*Alternanthera sessilis*), when formulated into dusts, showed molluscicidal properties against *L. auricularia*, *L. luteola* and *I. exustus* (Al-Zanbagi *et al.*, 2001). Volatile oils from *Cymbopogon nervatus* and *Boswellia papyrifera* have been were evaluated against *B. pfeifferi* and *Bul. truncatus* under laboratory conditions, and were found to be active against both snails (Molla *et al.*, 2013).

Abdalla *et al.*, (2011) proved *Calotropis procera* and *Nicotiana tabacum* and the seed of *Trigonella foenum* plants have molluscicidal effect against *Bul. truncatus* snails. *C. procera* plant had the highest activity, followed *N. tabacum* plant and lastly by *T. foenum*.

Tiwari, (2012) observed that different concentrations *Azadirachta indica* bark powder, *Annona squamosa* seed were highly toxic to *L. acuminata*. When giant grey slug *Limax maximus* and tree slug *L. marginatus* were exposed to Za'ater plant *Origanum syriacum* *L. marginatus* was more susceptible to the molluscicide effects of Za'ater plant than *L. maximus* (Aa, 2014). Methanol extracts of *Callistemon viminalis* fruits, bark and leaves were tested for molluscicidal activity against *Bio. alexandrina*. Lethal Concentration 50 values for *C. viminalis* fruits, bark and leaves were 6.2, 32 and 40 ppm respectively. Histopathological studies proved that the site of action of all tested extracts was localized in the digestive system and hermaphrodite gland (Ahmed *et al.*, 2014). Dried methanol extracts of 4 plants namely *Pterocarpus angolensis*, *Sclerocarya birrea*, *Pappea capensis* and *Commiphora*

Africana were screened for molluscicidal activity against *Lymnaea natalensis* and *Helisoma duryi*. *Pterocarpus angolensis* was the most effective, while *C. africana* was the least effective. Extract of *P. angolensis* was selectively toxic to *L. natalensis* (Ndabamba *et al.*, 2015)

2.6 Environmental Vector Control

Environmental management for vector snails include, modifying and the environment, with the aim of minimizing or pathogen propagation (Hamed, 2011). In the case of snail control the aquatic environment is modified to an extent that the snail population will not readily thrive or survive in a habitat. Environmental modification by fluctuating water levels in canals, clearing of vegetation and engineering modification of perceived habitat has been used to render habitat inhabitable by the snails vector (Deelen, 2013). Disadvantages of environmental vector control as applied to snails include; interference with fish in particular rivers (El-Sherbini *et al.*, 2009). Also increases in rate of water flow may inhibit snails, but the snail habitat may be rendered suitable for other medically important organisms to thrive for example the larval forms of the black fly *Simulium* could easily colonize a ravine habitat with fast-flowing water (Qiu *et al.*, 2014).

2.7 Other Snail Control Approaches

A vaccine ultimately, is anticipated to be the most effective form of Schistosomiasis treatment and control. If proved to be effective and inexpensive enough for worldwide distribution, it would eliminate the need for snail and reservoir host control. So far, the vaccine based on irradiated cercariae offers almost complete protection in experimental animals only (Mohamed, 2011). Different formulation of frozen dried cow urine (FCU) of different Indian breeds like Shahiwal Geer and Tharparkal have been found to be toxic to *L. acuminata*. Additionally, boiled urine was noted to have molluscicidal properties (Kumar *et al.*, 2012).

2.8 Role of Salt In Snail Control

NaCl can be used in snail vector control, because of its effect on the physiology of different life stages of the snails (Donnelly *et al.*, 1983). Nagabhushanam, (1979) observed that when *Indoplanorbis exustus* (Deshayes) were exposed to different salt concentration. The lethal concentration was 0.5% and the snails lost substantial weight due to loss of water when exposed hypertonic salt concentrations. In the presence of the NaCl solution distressed snails attempted to crawl out of the test solution and retracted into the shell or showed abnormal body movements suggesting that they may have been distressed (Donnelly *et al.*, 1983). Clark & Appleton, (1996) also, observed that such responses are characterized dying snails exposed to a molluscicide. Mostafa, (2009) observed that *B. arabica* snails remain alive when exposed to 5‰ NaCl concentration, and that 100% mortality occurred at 7.2‰ of NaCl concentration. *Tarebia granifera* can survive high salinity over a long period of time, however higher salinity adversely affected the *T. granifera* population. When *T. granifera* was suddenly transferred to 30 psu, 100% mortality was observed within 48hrs; activity also declined with increasing salinity (Miranda *et al.*, 2010). Al-Yaquob, (2011) observed that egg masses and hatchlings of *Lymnaea auricularia* (*Lymnaeoides*) were more sensitive to salinity than the adult snails, in which increasing salinity caused a progressive and significant reduction in both the rate of hatching and the mean percentage egg hatching was noted.

2.9 Biological Control of Schistosomiasis

Biological control (or biocontrol), once recommended by many as an environmentally friendly approach for solving pest problems, lately has attracted criticism because of the potential for biocontrol agents to permanently harm or even cause the extinction of native non-target species (Cowie, 2001). Biological control ought to be considered during integrated pest management strategies, due to its specificity on the pest species and due to its environmental friendly characteristics (Xiaonong *et al.*, 2002).

Biological control (or biocontrol) involves use of predatory, competitive or pathogenic organisms to regulate or eliminate snail populations (Symondson *et al.*, 2002). While biological control has great potential, it has been rarely used for routine snail control. When competitive interactions between *B. globosus* and *B. tropicus* were investigated, the latter was found to out compete the former (Chingwena *et al.*, 2002). Apparently *B. tropicus* had a higher reproductive rate than the *B. globosus* and also preyed upon the eggs masses of *Bul. globosus*. Fish like *Trematocranus placodon* have been used as biological control against *Bul. Nyassanus* (Evers *et al.*, 2006). *Metriaclima lanisticola*, a native cichlid of Lake Malawi was observed to predate three snails of (*Bulinus* spp). *B. globosus*, *B. nyassanus* and *B. tropicus* (Lundeba *et al.*, 2007). Gashaw *et al.*, (2008) evaluated potential of the snails *Physa acuta* and *Melanoides tuberculata* and the African catfish *Clarias gariepinus* as biological control agents against *B. pfeifferi* under laboratory conditions. Based on their findings the two competitor snails and African catfish could be used as biological control agents against *B. pfeifferi*.

Chimbari, (2012) suggested that the fish *Sargochromis codringtonii* and the duck *Cairina moschata* can be used as biological, particularly in irrigation schemes. These biological agents have wide variety of diets, which will allow them to switch to another less preferred prey if the preferred one is absent. Schistosomiasis infection in Puerto Rico declined after introduction of *Thiara granifera*, a snail which competes with the *B. glabrata* (Mouhamadou *et al.*, 2014).

CHAPTER THREE

METHODOLOGY

3.1 Study Design

This was a laboratory based experimental study of *B. pfeifferi* snails.

3.2 Site of Study

B. pfeifferi snails used in the experiments described in this thesis were all collected from streams and canals in Mwea irrigation scheme, Kirinyaga County

3.3 Collection of Snails

Snails were collected from streams using a standard flat wire-mesh snail scoop with a mesh size of 2 mm, and occasionally were picked up from the stream using hand-held long metal forceps (Gbalégba *et al.*, 2017). They were then packed between layers of damp cotton gauze and transported to the central lab in Nairobi in perforated plastic containers the same day they were collected. In the lab they were screened for trematode infections and kept in plastic aquaria and allowed to acclimatize for several days before being used for experimental purposes. The collected snails were used directly as target snails to evaluate the molluscicidal activity of sodium chloride (NaCl) solution or were allowed to lay egg masses, which were then used for further experimentation using the salt solution. Neonate snails hatched from the egg masses were also used as targets in molluscicide experiments.

3.4 Snail Maintenance

Snails were kept in a room maintained at a temperature of 26°C under natural light, in shallow plastic aquaria measuring 18 x 28 x 10 cm in aerated tap water. Aeration of tap water for 2-3 days removed the chlorine present in tap water. The adult and neonate snails were fed

on boiled green lettuce (*Lactuca sativa*) and commercial brand of flake fish food (Crop King Fish feed) supplemented with bone meal (Tamfeeds Bone Meal) as a source of Calcium for shell development. Water in the aquaria was changed once a week or whenever necessary (Donnelly *et al.*, 1983).

3.5 SALT (NaCl) PREPARATION

The different experimental NaCl concentrations were prepared by diluting analytical NaCl obtained from the supplier (Sigma Aldrich) using dechlorinated tap water. The same dechlorinated tap water was used as control.

3.6 Effect Of NaCl Solution On Hatchability Of *B. Pfeifferi* Egg Masses And Their Development

3.6.1 Test Solution

Development and hatchability of treated egg masses was investigated in saline concentrations range from 800-6800 mg NaCl. Freshly laid egg masses on colourless polythene papers were used for this experiment. Five eggs masses laid within 24 hr. prior to the experiment were transferred to 500ml plastic bowls containing dechlorinated water or the NaCl test solutions. The egg masses were then examined daily under a dissecting microscope to assess embryo development for up to 12 days. The experiment was repeated three times.

3.6.2 Controls

Control egg masses were maintained in dechlorinated water without NaCl under similar lab conditions.

3.7 Effect Of NaCl Solution On The Survival Of Neonate Of *B. Pfeifferi*

The snails were allowed to lay eggs on polythene sheets. Egg masses attached to the polythene sheets were located and isolated by cutting the plastic around each egg-mass with a scalpel (about 0.5-1.0cm from the egg-mass). The egg masses were immersed in Petri dishes

containing clean well water to remove any debris and transferred to containers containing 200 ml of dechlorinated tap water; the dishes were covered until eggs hatched into neonates. Neonate snails (24-32hrs) were used in this experiment, and in each test container 10 neonate snails were introduced using a painting brush and maintained in a volume of 1000ml test solution with control snails maintained in a similar volume of dechlorinated water. Snail responses to the test solution was recorded within the first 6hr and at 24hr following exposure. To confirm snail mortality, both test snails and control snails were transferred into fresh, clean water, and maintained for another 24hr to see whether or not they will recover from the effect of the test NaCl solution. Snails were considered dead if they did not come out of their shells during the 24hr recovery period. This experiment was repeated 3 times.

3.8 Effect Of Nacl On The Survival Of Adult *B. Pfeifferi*

NaCl solution was tested against adult *B. pfeifferi* snail's concentrations in the range of 3200-6800 mg/L. Adult snails in the size range 8-10 mm shell diameter or 13-15 mm shell diameter) were used in this experiment, in each test container 10 snails were introduced and maintained in a volume of 1000ml test solution with control snails maintained in a similar volume of dechlorinated water. Snail responses to the test solution was recorded within the first 6hr and at 24hr following exposure. To confirm snail mortality, both test snails and control snails were transferred into fresh, clean water, offered lettuce, and maintained for another 24hr to see whether or not they will recover from the effect of the test NaCl solution. Snails were considered dead if they did not come out of their shells and respond to food items during the 24hr recovery period. The snails that were not affected by the salt solution or those that recovered from the effects of the salt solution were maintained in fresh water for up to 4 days after exposure to the salt solution, and observed for their feeding habits and fecundity (egg laying capacity). This experiment was repeated 3 times.

Percent mortality was calculated using the following formula

$$\text{Killed snails} = \frac{(\% \text{ Killed by the Treatment}) \times (\text{Dead in Control})}{(100 - \% \text{ dead in control})}$$

3.9 Data Analysis

The obtained data was subjected to Excel 2013 to get LC₅₀, LC₉₀, Upper and lower confidence intervals using the linear regression analysis. Chi-square values were also obtained using Excel 2013

CHAPTER FOUR

RESULTS

4.1 General Observations

NaCl solutions affected the 3 developmental stages of *B. pfeifferi*, egg were the most susceptible to the effects of NaCl compared to the other developmental stages. Sub lethal toxic effects of NaCl on adult and neonates included, snails trying crawling out of the test solution and some showing abnormal body movements (muscular and spiral twisting of the body) as shown in Table 4.1.1. NaCl concentration of 5600mg/L > for the adult snails and 1800mg/L > for the neonates snails respectively caused death.

Table 4.1:1 Behavioural Responses of neonates & adults during the 24 hr. exposure to NaCl

Responses	Neonates	Adults
Snails floating on top of NaCl conc.	Present	Absent
Tried crawling out	Present	Present
Excessive production of mucus	Present	Present
Abnormal body movements	Present	Present
Snails retracted into their shells on coming in contact with NaCl	Present	Present

Snails body fully retracted & remained motionless	Present	Present
---	---------	---------

Snails body partially retracted & remained motionless	Present	Present
---	---------	---------

The lethal concentration that killed 50% of eggs, neonates and adult stages of the snail was 495 mg/L, 1457 mg/L and 5087mg/L respectively. While, the lethal concentration that killed 90% of eggs, neonates and adults stages of the snail were 4211 mg/L, 3007 mg/L and 6385mg/L respectively as shown in table 4.1.2. The molluscicidal effect of NaCl was significantly effective at all stages of *B. pfeifferi* {eggs at 24hrs ($d.f = 6, \chi^2 = 42; P < 0.05$), neonates at 24 hrs ($d.f = 6, \chi^2 = 30, (p < 0.05)$) and adults at 24 hrs. ($d.f = 4, \chi^2 = 15, (p < 0.05)$). The different test solution of NaCl showed that Linear equation correlation coefficient (R^2) = 0.6316, 0.9663 and 0.9157 on the eggs, neonates and adults of *B. pfeifferi* respectively.

Table 4.1:2 Molluscicidal effect of NaCl on the different developmental stages of *B. pfeifferi* snail

Stage	Regression	Chi Square value (P<0.05)	LC ₅₀ (mg/L) Upper & Lower confidence	LC ₉₀ (mg/L) Upper & Lower confidence
Eggs (24-48hrs old)	Y=0.0085x+54.207	(d.f= 6, χ^2 = 42; P<0.05)	495 (-1086) to (2106)	4211 (2640) to (5800)
Neonates (48-72hrs old)	Y=0.0258x+12.413	(d.f= 6, χ^2 = 30, (p<0.05)	1457(473) to (2441)	3007(2023) to (3990)
Adults (8-10 mm)	Y=0.0308x-106.67	(d.f= 4, χ^2 = 15, (p<0.05)	5087(3569) to (6605)	6385 (3585) to (9185)

4.2 Effect of NaCl Solution On Egg Masses Of Snails

The egg masses were the most susceptible to the effects of NaCl compared to the other developmental stages. Figure 4.2.1 shows the hatching rate of *B. pfeifferi* eggs masses exposed to NaCl in the range 800-6800 mg/L, over a 24 her period and thereafter incubated

for 12 days. Egg masses exposed to 5600mg/L or above failed to hatch completely even after incubation through 12 days. When examined under a dissecting microscope at magnification X100, there was no indication of egg/embryo development. On the other hand, 90-95% of the egg masses exposed to 1600mg/L or less hatched normally and the proportion of egg masses hatching was similar to that observed in the control egg masses, maintained in fresh water. Interestingly, a salt concentration of 3200 mg/L delayed egg mass hatching for 2 days beyond the hatching time for control egg masses not exposed to salt, and in addition, significantly reduced the proportion of egg masses developing to the level of hatching down to slightly above 40%. Exposure of egg masses to salt concentrations of 5600 mg/L and above led to 100% inhibition of egg hatching. There was a significant difference (*d.f* 6, $\chi^2 = 648$, ($p < 0.05$)) proportions of eggs hatched among the 7 treatment groups

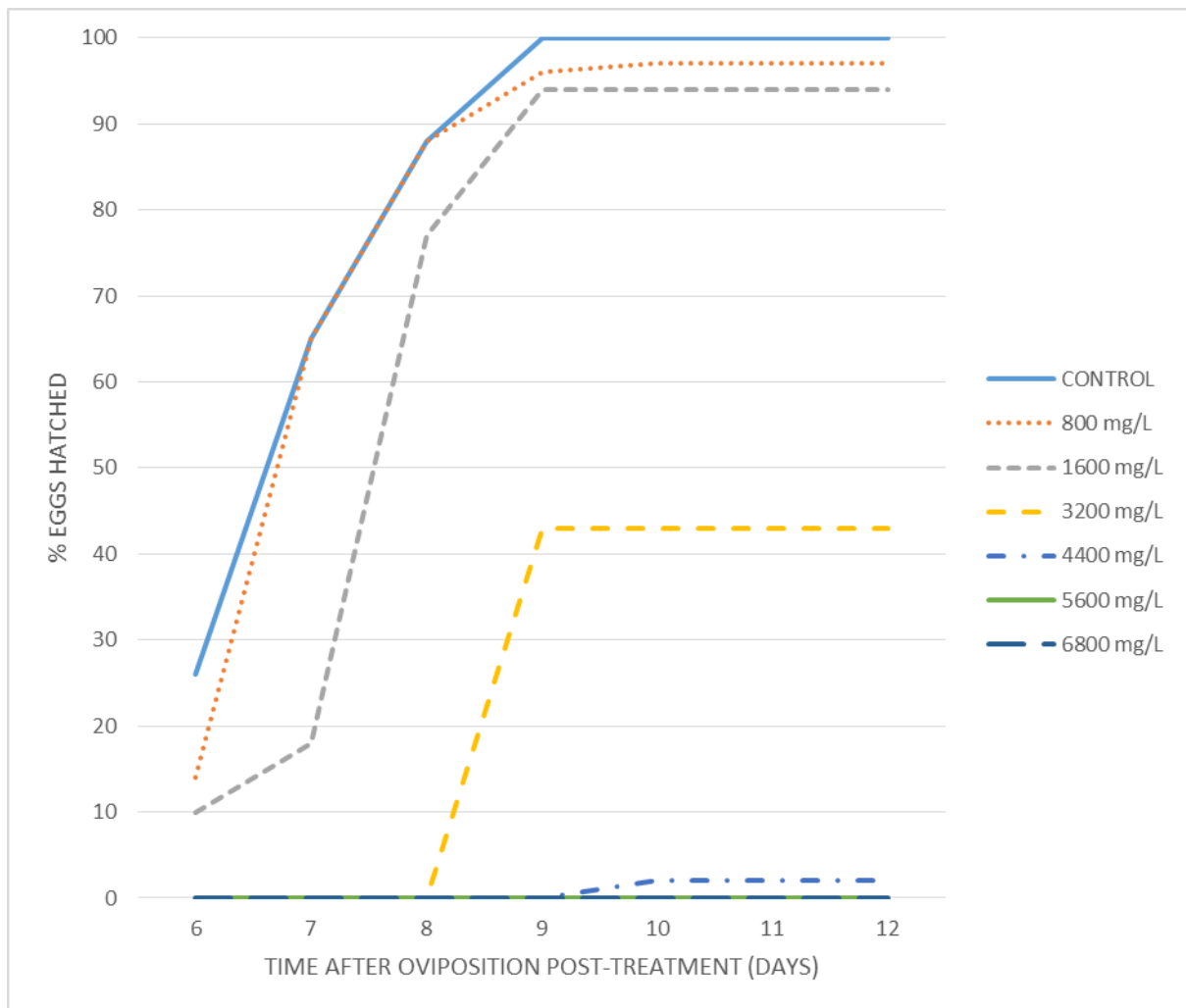


Figure 4.2.1 Hatchability rate of eggs at different concentrations of salt solution

Figure 4.2.2 shows eggs laid by snails exposed to 5600 mg/L had the lowest proportion of egg hatching compared to NaCl concentrations of 4400 mg/L and below, There was a difference ($d.f\ 3, \chi^2 = 34, (p < 0.05)$) in the proportions of snails that reproduced among the 4 treatment groups

Table 4.2:1 Fecundity of Egg laid by adult snails exposed to NaCl.

Conc. (mg/L)	No. of eggs laid at the beginning	Day 7(X)	Day 8(Y)	Hatched Proportion Day 7 (X/Z)*100	Hatched Proportion Day 8 (Y/Z)*100
Control	171	123	146	20.6	24.5
3200	191	136	169	22.8	28.4
4400	195	106	131	17.8	22.0
5600	38	24	27	4.0	4.5
Total	595(Z)				



Figure 4.2.2 Control solution at 7 days, fully developed neonate snail's ready to hatch were present in egg exposed to control

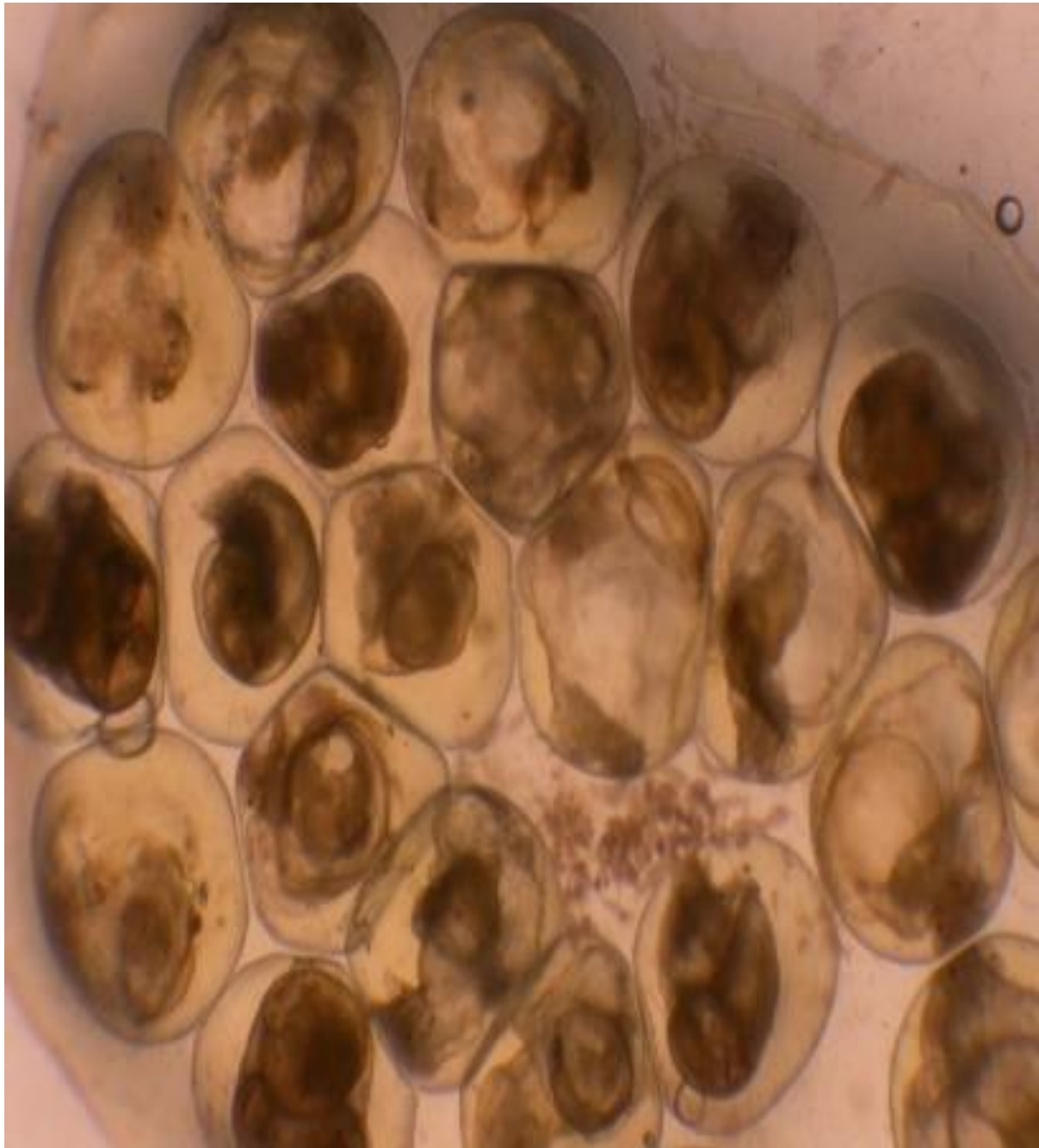


Figure 4.2.3 Poorly developed embryo and few neonates were observed in egg exposed to 3200mg/L NaCl at 7 days



Figure 4.2.4 Completely undeveloped embryos were seen in egg exposed to 5600 mg/L NaCl at 7 days

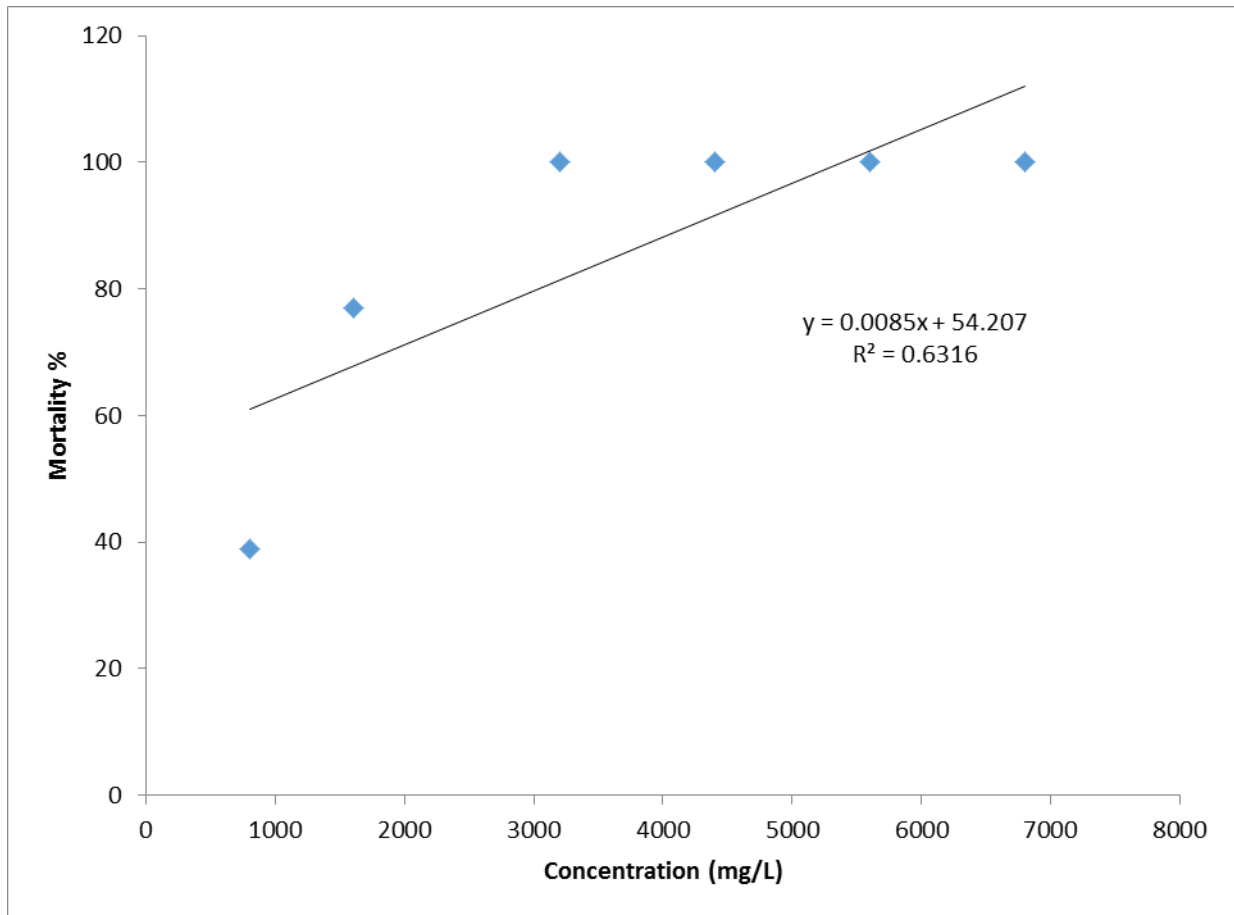


Figure 4.2.5 NaCl concentrations against egg mass stage of *B. pfeifferi*

The newly hatched neonates from eggs exposed to different concentration of NaCl were mostly attached to the walls of the container and had thin shells in comparison to the control groups. Movement of the newly hatched neonates in the treated group was slow, and the snail had smaller tentacles as compared with those in control groups. Figure 4.2.6 show that there was a moderate positive correlation between the mortalities observed in the egg masses and the different concentration of NaCl. There was a significant difference (*d.f* 6, $\chi^2 = 42$, $p < 0.05$) in the proportions of egg that failed to hatch among the 7 treatment groups.

4.3 Effect of NaCl Solution On Neonates Of *B. Pfeifferi*

When the neonate of *B. pfeifferi* were exposed to the various NaCl concentrations, the following observations were made; snails tried to escape by crawling to the side of the

containers, excessive production of mucus, abnormal body movements which included muscular and spiral twisting of the body, bodies partially retracted and motionless, and bodies both partially and fully withdrawn. These effects were more pronounced with increase in NaCl concentration.

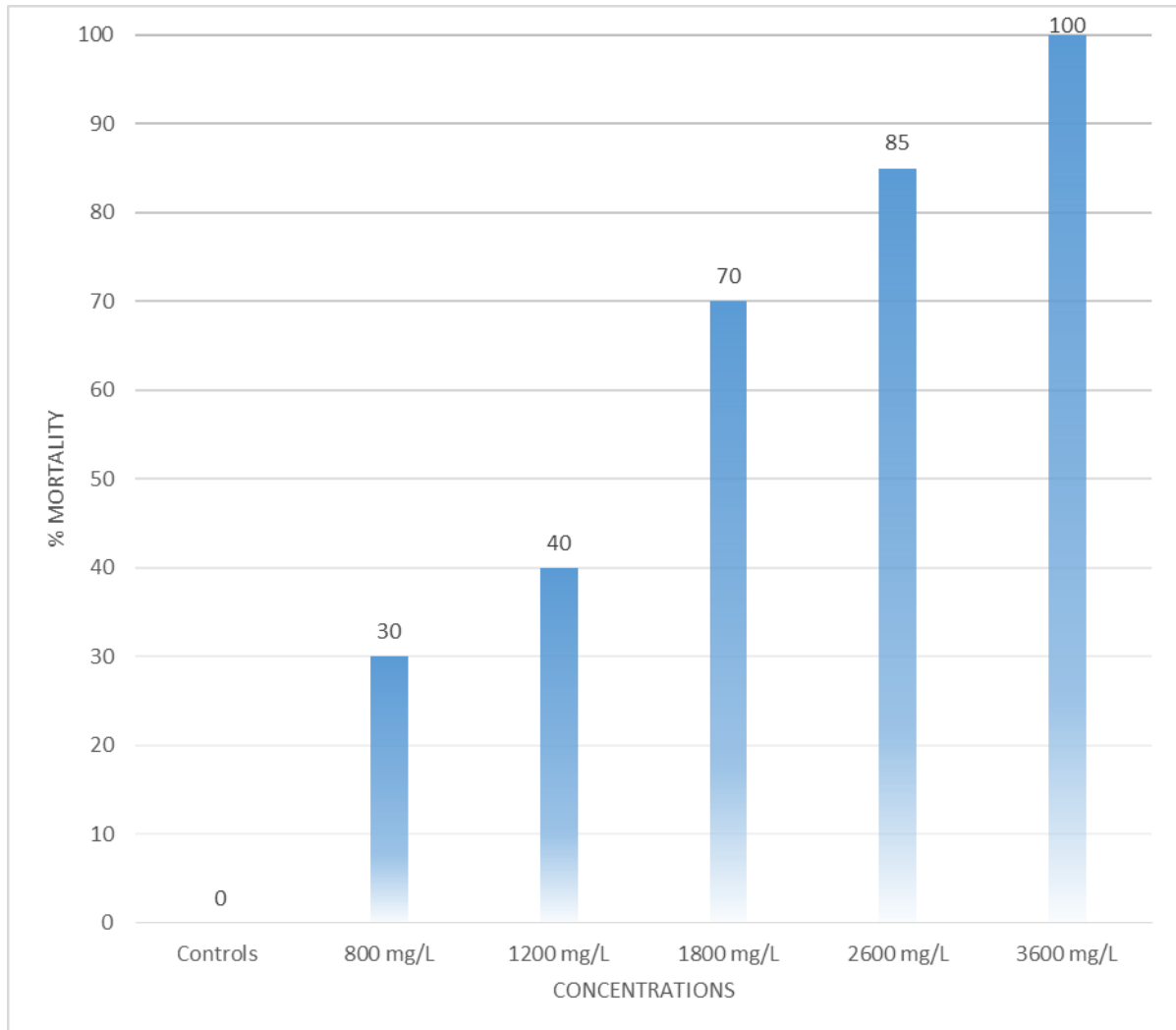


Figure 4.3.1 Levels of mortality in neonates of *B. pfeifferi* at different concentration after 24 hours of exposure

Figure 4.3.1 shows mortality percentage of the neonates after exposure and recovery period. There were no mortalities in the control group, while NaCl concentrations of < 1200mg/L caused mortalities below 40%. NaCl concentration of 3600mg/L was lethal causing 100% mortality.

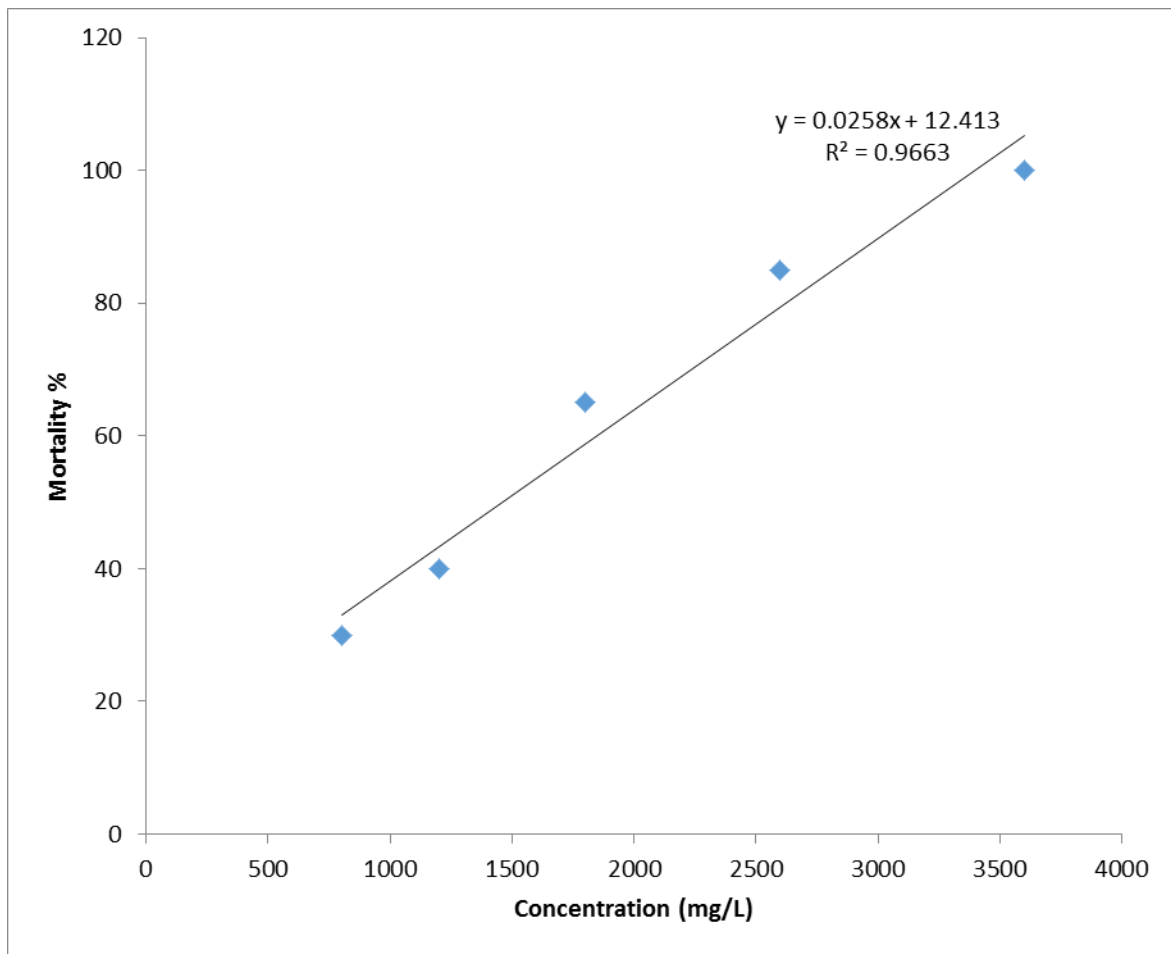


Figure 4.3.2 NaCl concentrations against neonate stage of *B. pfeifferi*

Figure 4.3.2 illustrates there was a strong positive correlation between the mortalities observed in the neonate and the different concentration of NaCl. There was a difference d.f = 5, $\chi^2 = 30$, ($p < 0.05$) in the proportions snails that dead among the 6 treatment groups

4.4 Effect of NaCl On Adult *B. Pfeifferi*

Table 4:2 shows results responses of adult *B. pfeifferi* snails to the different NaCl concentrations. When snails were initially immersed into the test salinities, they retracted into their shells and remained motionless at NaCl concentration of 3200mg/L or above, the controls snails maintained normal movements and responses to food items during the 24 hrs. observation period. Snails exposed to NaCl concentration of 3200 mg/L or above responded

by either trying to crawl out of the solution or the bodies remained retracted and motionless during the 24hr. exposure to the salt solution

Table 4.4:1 Responses of Adults *B. pfeifferi* to NaCl solution

Conc.	Snails response during 24 hr. exposure period to NaCl solution	Snails response during 24 hr. recovery period to NaCl solution	% Mortality
Control	Maintained normal movements (100%)	Exhibited normal movements and responded to food items (100%)	0
3200 mg/L NaCl	Abnormal body movements (25%) Snails tried crawling out (45%) Head fully retracted and motionless (10%) Head partially retracted and motionless (20%)	Exhibited normal movements (40%) and responded to food items (60%)	0
4400 mg/L NaCl	Abnormal body movements (55%) Snails tried crawling out (10%) Head partially retracted and motionless (35%)	Exhibited normal movements (15%) and responded to food items (75%)	10
5600 mg/L NaCl	Head fully retracted and motionless (80%) Head partially retracted and	Exhibited normal movements (10%) and responded to food items (10%)	80

	motionless (20%)	
6800 mg/L	Head fully retracted and	100
NaCl	motionless (100%)	

Control snails, showed normal movements, responded to food items during the 1st 24hr and 2nd 24 hr. period and all snails survived during the entire period

- 1) During the 1st 24hr. snails were exposed to the NaCl solution at concentration in the range 3200-6800 mg/L
- 2) During the recovery period snails exposed to NaCl solutions were transferred to fresh water and allowed to recover.
- 3) Snails were considered dead if they remained motionless during the 24hr recovery period and did not respond to food items in the test solution.

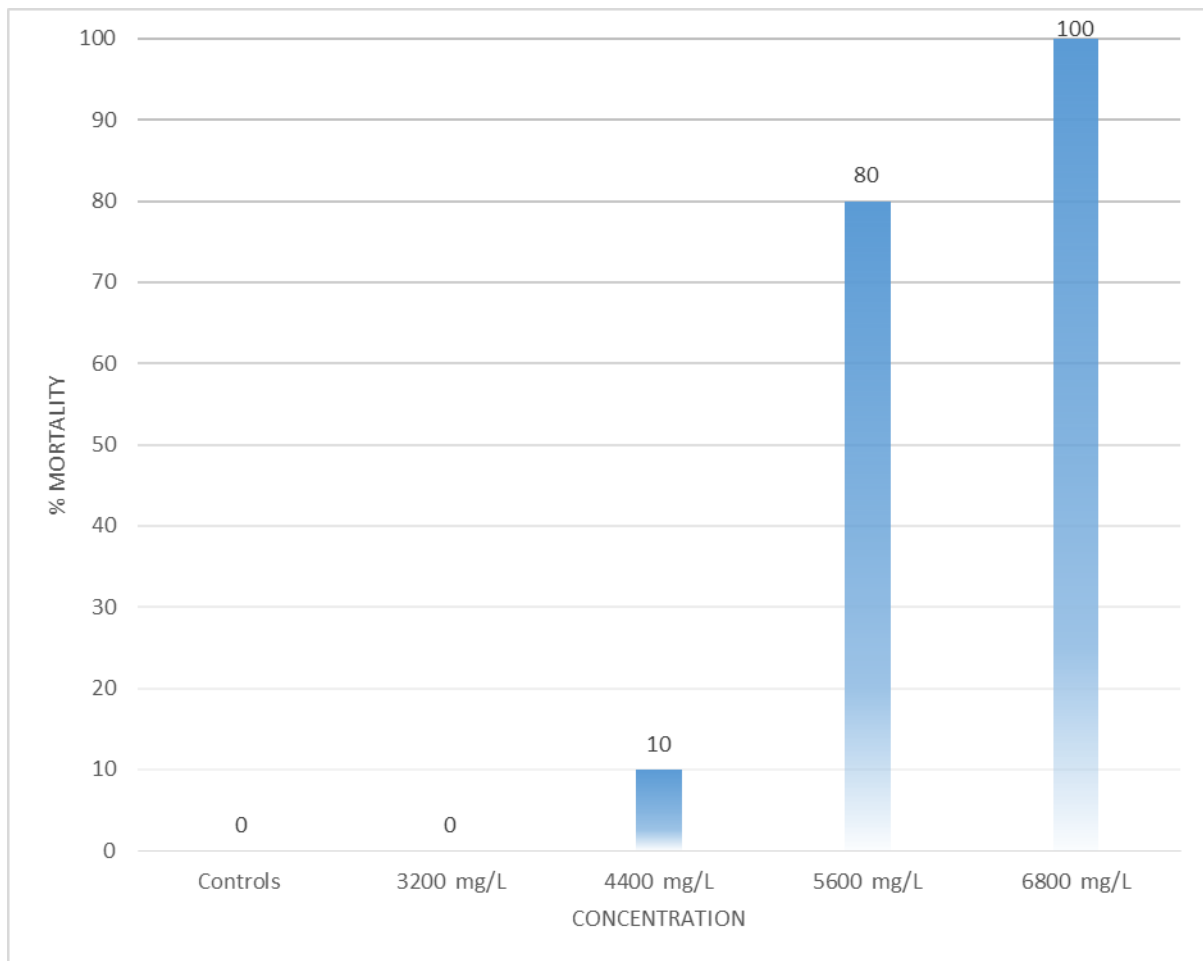


Figure 4.4.1 Effects of the different level of salinities on survival of the adults of *B. pfeifferi* after 24 hours of exposure.

Figure 4.4.1 shows mortality rate of adult snails after 24 hours of exposure and 24 hours of recovery period. Snails in the control group and those exposed to 3200mg/L had no mortalities, at concentrations of >5600mg/L mortalities were over 78%.

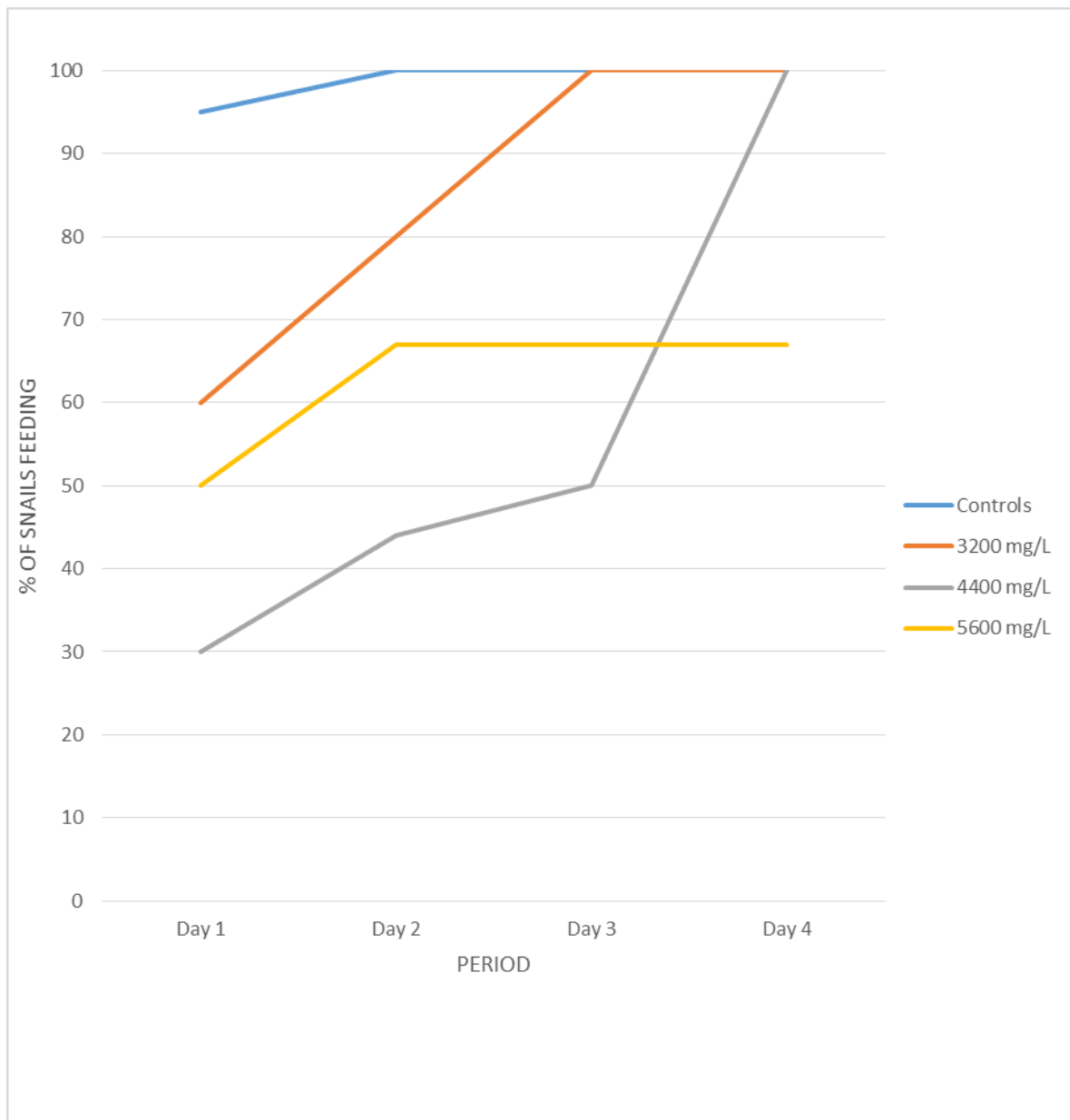


Figure 4.4.2 The Feeding behaviour of adult’s *B. pfeifferi* following exposure to different concentrations of NaCl solution

Figure 4.4.2 show 95% of snails in the control fed on day 1, and 100% fed on day 2 through day 4. In the snails exposed 3200 mg/L, 60% of the snails fed on day 1. By day 3 all the snails were feeding. However, those snails exposed to 5600mg/L did no recover fully, only 67% were feeding on day 4. There is a difference in the proportions of snails that fed on each

day {Day 1= *d.f* 3, $\chi^2 = 18$ ($p < 0.05$)}, {Day 2= *d.f* 3, $\chi^2 = 16$ ($p < 0.05$)}, {Day 3= *d.f* 3, $\chi^2 = 19$ ($p < 0.05$)} and Day 4= *d.f* 3, $\chi^2 = 16$ ($p < 0.05$)} among the four (4) treatment group.

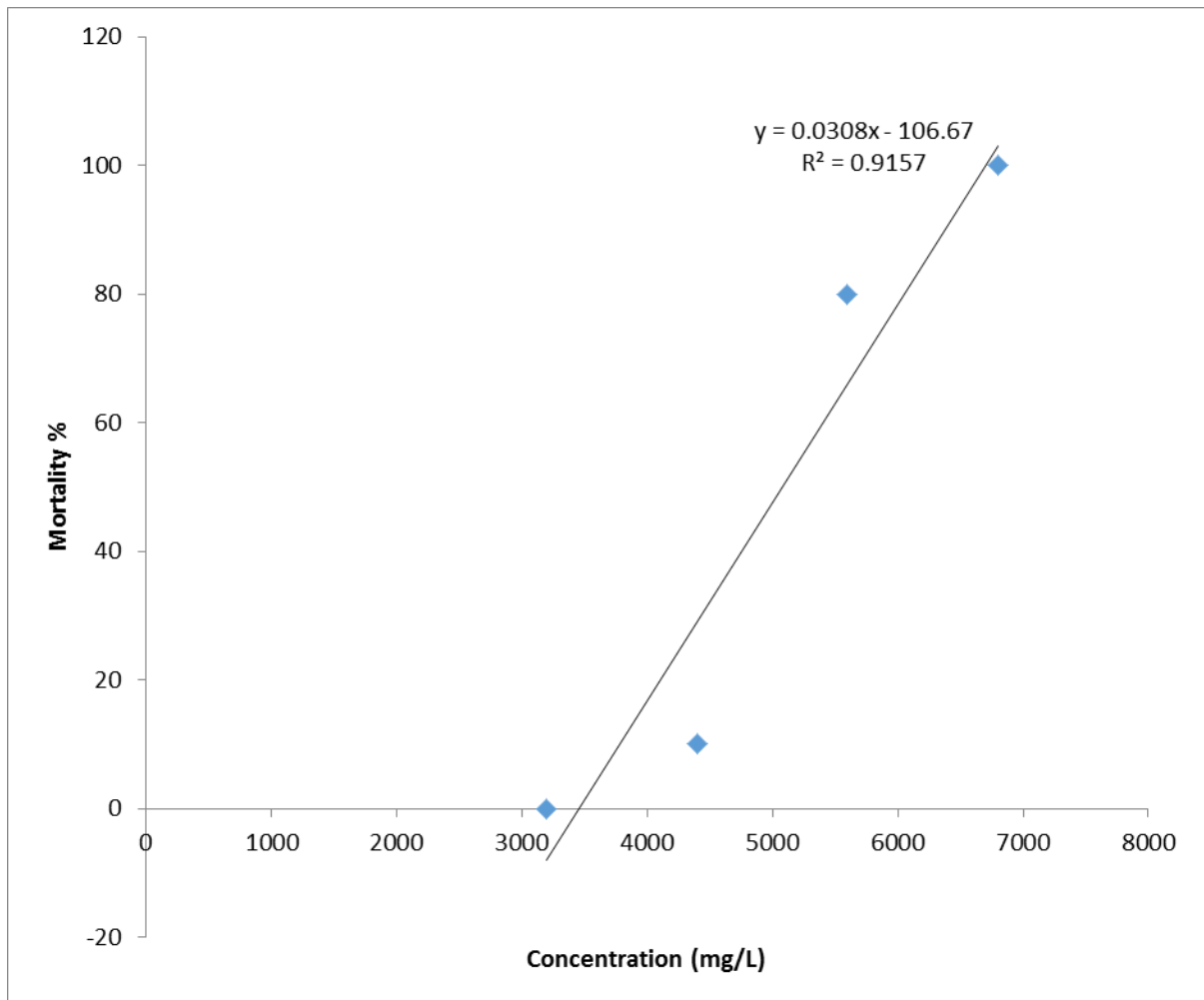


Figure 4.4.3 NaCl concentrations against Adults stage of *B. pfeifferi*

Figure 4.4.3 shows correlation between the mortalities in the adults and the different concentration of NaCl.

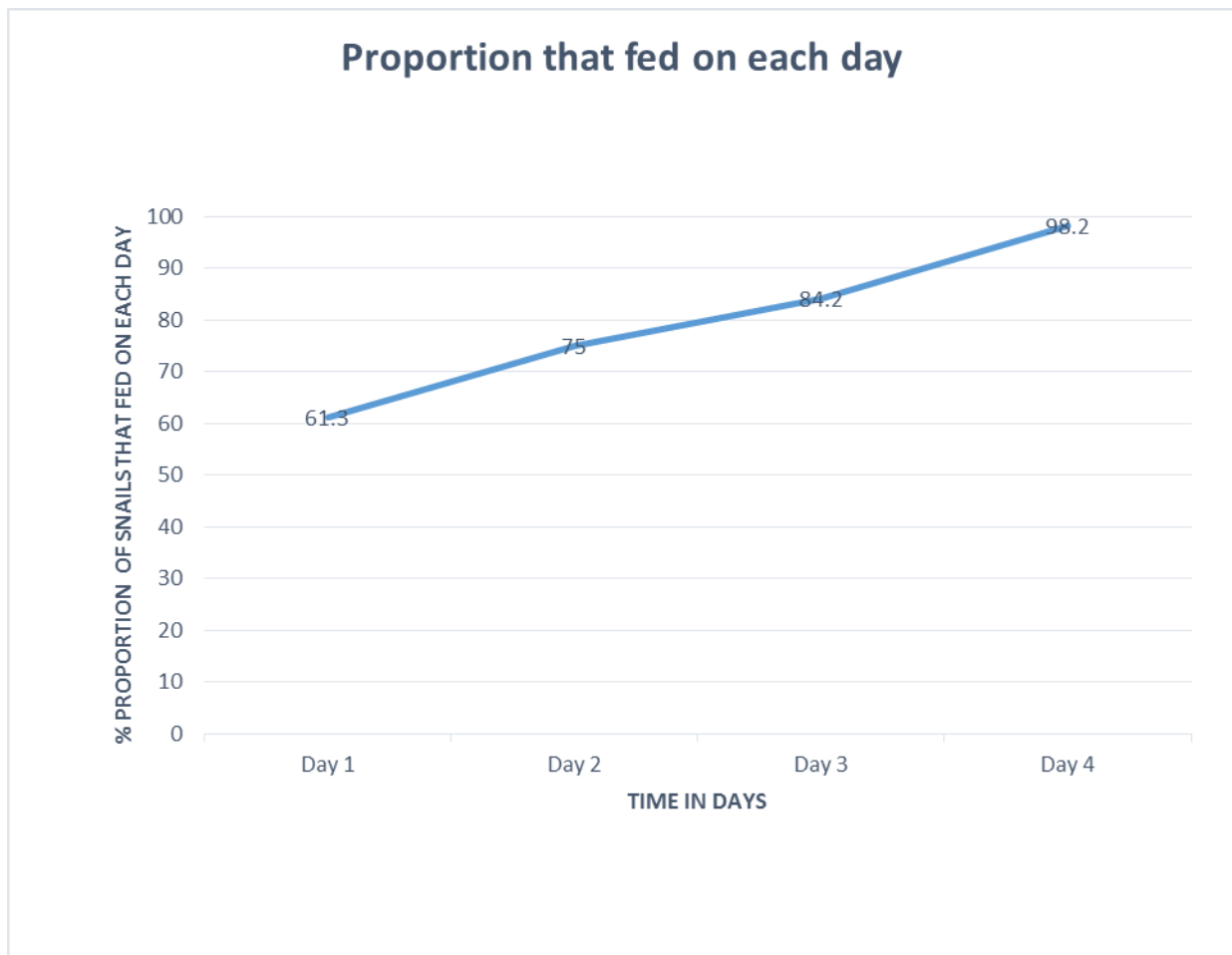


Figure 4.4.4 Proportion of adult *B pfeifferi* snails that fed on each of the days

Clearly Figure 4.4.4 shows an increasing trend in the proportion of snails that fed on a given day as the number of day's increases.

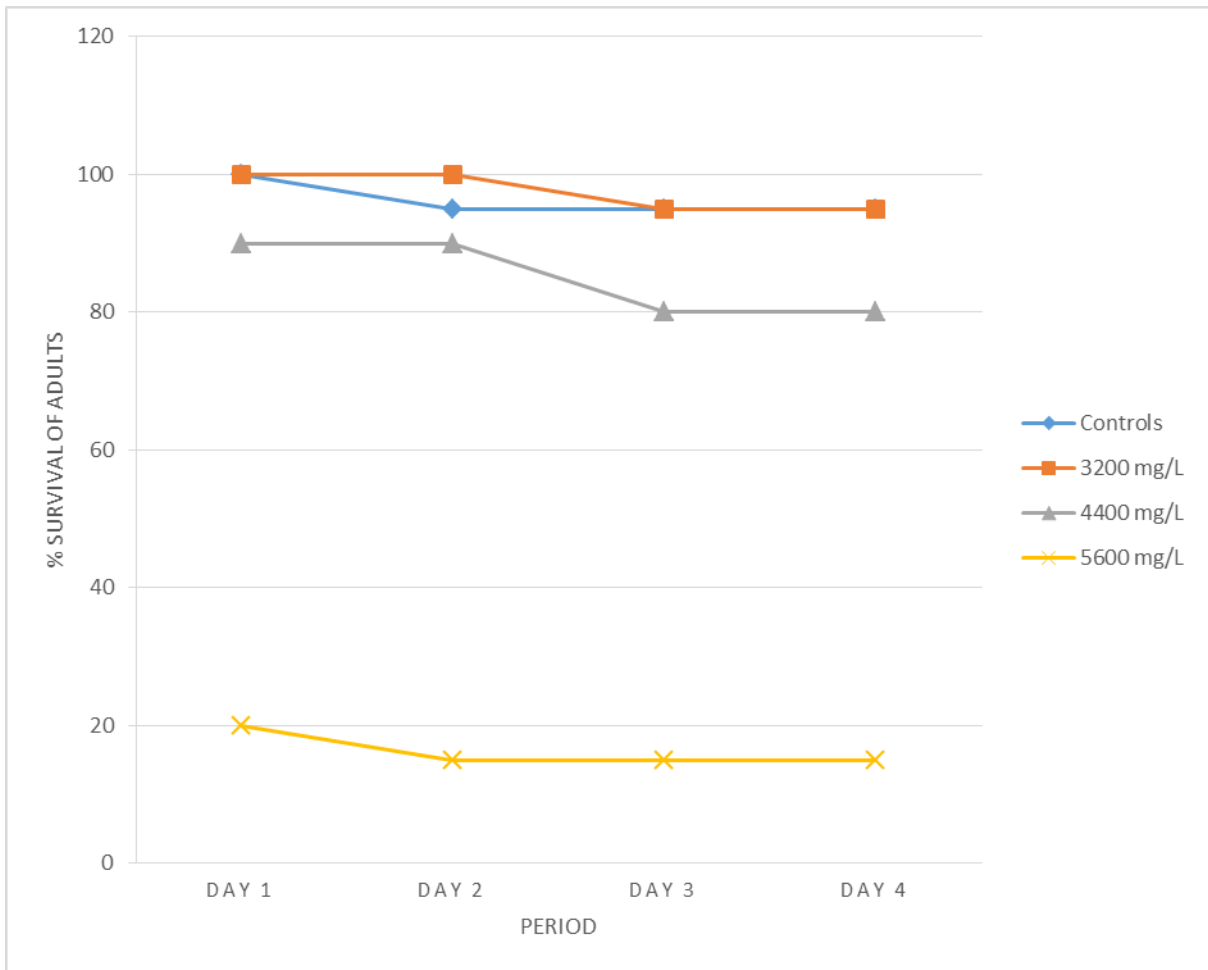


Figure 4.4.5 Survival rate of the adult snails over a 4 day period after exposure to different test salinities.

Figure 4.4.5 presents the survival rate of adult snails over a 4 day period. The results show that NaCl affected the survival of the exposed snails, an observation was which obvious on the 2nd day after treatment. Snails exposed to 5600mg/L had the lowest survival rate of below 20%, while snails exposed to 3200 mg/L had the highest survival rate of over 90%.

There is a difference ($d.f\ 3, \chi^2 = 42, (p < 0.05)$) in the proportions snails that survived among the 4 treatment groups.

CHAPTER FIVE

DISCUSSION

B. pfeifferi snails were selected because they are more readily available as a lab model and can easily be maintained under lab condition, I anticipated that the molluscicidal effects would be expected to be the same for other vector snails. Few studies have been done to evaluate efficacy of NaCl or salinity as potential molluscicide. However, none of the studies addressed *B. pfeifferi* snails in Kirinyaga County, Kenya. This study found out that NaCl concentration of 3600mg/L and 6800mg/L for neonates and adults respectively achieved 100% mortality.

Because using NaCl will definitely be harmful to the eco-system, a vector-snail-free aquatic biotope (a small-scale habitat which duplicates a biological community in an environmental condition) will be prepared beforehand. All the plants and small animals except the vector snails from the targeted river will be transferred to the biotope. Once the vector-snail-free biotope is established, salt will be applied to the river. This will not only kill vector snails but also other living-organisms in the river. After the rainy season, when the salt is washed out and the river ecosystem is conducive return to living-organisms. The river is restored by returning the aquatic plants and animals from the vector-snail-free biotope to the river. Thus the river will become free of vector snails with little impact to environment. This approach however cannot be applied on to lakes and large rivers, and also amphibious snails, the vector of *Schistosoma japonica*.

Exposure of snails to higher NaCl solution resulted in dehydration (loss of fluid) that lead to snail death. Body fluid of snails are known to be hypertonic (having a higher osmotic) to the external environment (Donnelly *et al.*, 1983). Higher concentrations of the external environment result in the body fluids being hypotonic in nature (Salawu & Odaibo, 2011). In

the present study snails exposed to NaCl concentration of 6800 mg/L failed to recover, even when they were transferred into fresh water during the recovery period possibly, because of ion imbalance between the body fluid of the snails and the test concentrations. The end result was movement of ions and body fluids into the test concentration leading to dehydration and death.

In the present study snails retracted into the shells and closed their operculum, once they came in contact with the different NaCl concentrations. A study conducted by (Oplinger, *et al.*, 2009) also showed that when the New Zealand mud snail was exposed to different solution of NaCl, they retracted into their shells within 1 min of exposure. According to (Ebenso *et al.*, 2005) retracting into their shells and closing their operculum (in the case of *Prosobranch* snails), the snails reduce their physiological activity. This is allowed the snails to survive under unfavourable condition and resume normal activity when conditions are favourable. Snail aestivate when exposed to stressful environment, they reduce their metabolic rate to a minimum so as to save energy (Aardt & Frey, 1979). Christian & Tesfamichael, (2000) observed that when the land snail *Helix aspersa* underwent aestivation, oxygen consumption was depressed to 16% of the control values. According to (Cheng & Sullivan, 1977) land snails respiration through both cutaneous and through a modified vascularized area in the mantle cavity. The different NaCl concentrations may have damaged cutaneous surface, resulting in alteration in oxygen uptake.

Snails exposed to lower concentration regained their normal activity after being transferred into fresh dechlorinate tap water. This suggests that removal of the snails from stressful environment to new favourable environment, greatly improved the accessibility of oxygen to the snail's tissues and organs. Molluscicidal effect of NaCl caused different behavioural responses in the snails, included muscular, spiral twisting of the body and clamping on each other was evident in the present study. This suggests that NaCl may have acted as a

neurotoxin, thus affecting the neuromuscular system of the snails. Deshmane, (2012) observed that plant toxin from *Acacia sinuate* affected the central nervous system of fresh water snail *Viviparus bengalensis* causing abnormal tentacle movement in the snails.

(Agarwala, 1992) reported that extracts of *Euphorbia royleana* Euphorbia and *Jatropha gossypifolia* contained neurotoxins that were active on the neuromuscular system of the pulmonate snails *Lymnaea acuminata*.

Abdel *et al.*, (2005) also reported that exposure of *Biomphalaria alexandrina* to volatile oils caused them to crawl on each other and also experience muscle twisting. In the present study snails exposed to NaCl tried crawling out, in order to escape the molluscicidal effects of NaCl. Such a response provides away of escape and increase snail survival. Similar observations were made by (Olajumoke & Morenikeji, 2012). The neonate's snails were observed to float to top of the different NaCl concentrations, in an effort to obtain oxygen and to protrude the foot through the aperture and escape the effects of NaCl.

In the present study NaCl affected the feeding habits of the snails after exposure. This suggests that NaCl contains antifeed substance or it affects the physiology of the snail's organs. The present findings are in agreement with those of (Bailey, 1989) which suggested that neurotoxins may affect the feeding habits of snails.

5.1 Adults Snails

The present results showed that 100% of adult's *Biompharia* snails succumbed at NaCl concentration of 6800 mg/L and 10% at lower concentration of 4400 mg/L. Donnelly *et al.*, (1983) reported that body fluid *Bulinus physopsis* had an osmotic pressure of 3.5⁰/₀₀ (3500 mg/L), and that this is why the snails survived well in NaCl concentration of 3.5⁰/₀₀ (3500 mg/L) and below. This agrees with the findings of the present study, which also showed that at 3200 mg/L snails were able to survive well.

Vaidya *et al.*, (1980) found that when fresh water snails *Indoplanorbis exustus* were exposed to different concentrations of NaCl, lethal concentration of NaCl was 0.5 mg/mL. However, their results findings did not agree with the findings of this present study. In addition, Bagarinao & Lantin-Olaguer, (2000) did a study and found out that mud snails *Cerithidea cingulata* were killed within 7 day in salinities of 48–70 ‰ (4800-7000 mg/L). El-shazly, (2012) studied the effect of different salts on the land snail *Monacha cantiana*; the lethal concentration of NaCl after 72 hours was 2090 mg/L

In the present study *B. pfeifferi* exposed to the NaCl solution at concentrations in the ranges of 3200-6800 mg/L retracted their bodies into shells on coming in contact with NaCl solution, which seems to be a common response in snails; when they are distressed or when exposed to a chemical. It can be used as an indicator for molluscicidal activity for a test substance. These findings are in agreement with those (Oplinger *et al.*, 2009), *Potamopyrgus antipodarum* snails retracted into their shells on coming contact with high concentrations of NaCl. There were some similarities in the findings of both experiments, even though different units of concentrations were used.

In the present study the molluscicidal property of NaCl inhibited feeding or rather reduce feeding ability in the exposed snails. (Pereira Filho *et al.*, (2014) noted that the feeding ability of the snail *Bio. glabrata* was completely suppressed when exposed to the hydroalcoholic extract of the leaves of *Jatropha gossypifolia*. This finding are in agreement with those of (Ebenso *et al.*, 2005), who noted that Carbamate Molluscicide inhibited feeding in the African Giant Land Snail *Limicolaria Aurora* . Adult snails also exhibited abnormal body movements, whereby they were twisting and twitching when exposed to the different NaCl solution. These responses by the snails can be explained by the molluscicidal properties of NaCl to affect the locomotion mechanism of the snails. The same observations were also been made by (Ebenso *et al.*, 2005). Mello-Silva *et al.*, (2006) observed that when *Bio.*

glabrata (Pulmonata: *Planorbidae*) were exposed to sub-lethal concentrations of the latex of *Euphorbia splendens* the adults snails tried to crawling out of the test solution. (Kiros *et al.*, 2014) noted that when *Biomphalaria pfeifferi* was exposed to fruits of *Glinus lotoides*, the snails tried crawling out of the test solution to avoid further contact with the harsh environment. This finding are in agreement with the present finding, this can be attributed to the molluscicidal effects of NaCl.

Adult snails in the present study retracted into their shells and were motionless, when they came in contact with different NaCl solutions. Similar observations have also been made by (Okeke, & Ubachukwu, 2011; El-Sherbini *et al.*, 2009; Luiz *et al.*, 2013). This molluscicidal activity is attributed to the molluscicidal effects of NaCl. In the present study fecundity of the adult snails reduced with increase in NaCl concentration, with the control recording higher fecundity. This can be attributed to the toxic effect NaCl on snail hormonal balance. Similar findings were also documented by (Kushwaha & Singh, 2010; Tiwari, 2012). High mortalities were recorded in concentrations of 5600mg/L and above, because the snails were particularly immotile and hence experienced the lethal effects of NaCl concentrations. Similar observations were also been made by (Ramadan *et al.*, 2012) when comparing the molluscicidal effects of *Commiphora molmol* (myrrh) and Bayluscide against *Melanoides tuberculata* snail. The adverse effect of NaCl were reversible when the snails were introduced into NaCl free environment. This findings are in agreement with those of (El-Sherbini *et al.*, 2009).

5.2 Neonates

The present study shows that increase in NaCl concentration resulted in decreased survival in the neonates. In the present study at NaCl concentration 3600 mg/L, 100% mortality was recorded. This observations show that NaCl can be used as molluscicidal agent based on its property to kill the vector snails. Salawu & Odaibo, (2011) observed that when one-week old

Bulinus globosus were exposed to *Hyptis suaveolens* they tried escaping crawling out of the solution. Similar observations were made in the present study, neonate snails tried to crawl out of the NaCl solutions to avoid the deadly effects of NaCl and increase their survival chances.

The neonates in the present study exhibited abnormal body movements which included muscular twitching and snails become spirally twisted similar to observations have also been made by (Chauhan & Singh, 2010).

5.3 Egg Masses

In this study, the molluscicidal effect of the different concentration of NaCl on egg masses of *B. pfeifferi* was concentration dependent. A study by (Mohamed, & Upatham, 1986) showed the maximum concentration of NaCl tolerated by *Bulinus Abyssinicus* was 2800mg/L, while in this study maximum concentration of NaCl tolerated by *Bulinus africanus* was 3200 mg/L. NaCl inhibited hatching of snails at certain concentrations, and therefore these properties make it a potential molluscicide. The ability of egg masses exposed to lower concentrations hatching was, because the concentrations of NaCl were not sufficient enough to interfere with normal embryonic development. Egg masses exposed to the 5600 and 6800mg/L were unhatched and contained dead embryos at the pre hatch snail stage of development. (Oniya, & Fajiwe, 2010) observed the calcium hypochlorite retarded embryo development of egg masses of *Bulinus globosus* at higher concentrations. Death of embryo at higher concentrations could be attributed to the interference of the embryo physiology by the high concentrations of NaCl. The ability of egg masses to survive and hatch in some NaCl concentrations, is due to presence of jelly layer of egg which is affected by higher NaCl concentrations. Jelly layer of egg offers some protection to the snail's egg masses such as exposure to NaCl against adverse conditions at concentration of 1600mg/L and below. However, at concentrations of 3200 mg/L and above the egg failed to hatch as they were

damaged beyond recovery (Przeslawski, 2004). In the higher NaCl concentrations possibly caused loss of water from the embryo leading to embryo death as observed by (Mohamed *et al.* 1986).

Little is understood about osmotic and ionic regulation in the eggs and embryos of freshwater snails (Marois & Croll, 2011). Reviews done on this subject have generally concluded that, presence of protective membrane is evident in the egg and embryo of fresh water snails (Beadle, 1969). Every egg mass normally possesses a capsular membrane which maintains a nutritive medium around each egg and offers protection against attacks from micro-organism. However, it does not provide protection from diffusion of water and ions (Pechenik, 1982). The vitelline membrane which surrounds the egg is the only medium between the egg and the outside water. This membrane maybe involved in the control of exchange of water and ions (Beadle, 1969). Results in this experiment therefore suggest that egg masses are more sensitive to effects of NaCl compared to both the adult and juvenile snails.

Encapsulated snail embryo depends on a number of mechanisms to protect themselves from harmful environment conditions. The encapsulating structures are suggested to help reduce desiccation and rate of salinity change (Woods & Robert, 1997). The difference in the tolerance level of the egg masses, 3 day old neonate and adult *B. pfeifferi* to NaCl is mainly attributed to their different abilities to tolerate osmotic stress.

Present findings are also in agreement with suggestions of (Donnelly *et al.*, 1983) that salinity has ovicidal effects on snail eggs, retarding the fecundity by reducing the number of egg masses rather than the number of egg cells. Egg masses laid by the experimental group had egg masses ranging between 11-13 egg cells per egg mass. The number of egg masses laid differed greatly depending on concentration of salinities the snail was exposed to prior. Singh *et al.*, (2012); Ukwandu *et al.*, (2011) reported that caudodorsal cells in the brain of *Lymnaea*

Acuminata released ovulation hormone, which control reproduction activity in the snails. It may be possible in the present study that NaCl affected caudodorsal cells. This in turn affected the release of the ovulation hormone and hence the decreased fecundity in the experimental group.

CHAPTER SIX

SUMMARY CONCLUSION & RECOMMENDATIONS

6.1 Summary

From the present study it can be concluded that NaCl had reasonable molluscicidal activity against all life stages of *B. pfeifferi*. The results indicate NaCl affected fecundity of the eggs, as well as feeding in the exposed snails.

6.2 Conclusion

These findings suggest that NaCl can be exploited as an alternative molluscicide against means of snail control, in schistosomiasis eradication programs. The present study demonstrates that the

- 1) LC₅₀ of *B. pfeifferi* egg masses at 495mg/L.
- 2) LC₅₀ for neonates was 1457 mg/L.
- 3) LC₅₀ For Adult's *B. Pfeifferi* Was 5087 Mg/L.

6.3 Recommendations

The findings of this study suggest that this approach could be useful for vector control when combined with chemotherapy. Further studies are recommended on ecological impact on the environment. One advantage of NaCl as a molluscicide, if proven usable is that it is readily available and is inexpensive

REFERENCES

- Aa, A.-H. (2014). Cytotoxicity of Plant Extract *Origanum syriacum* on Gametogenesis of Two Terrestrial Slugs, Using TEM. *Journal of Cytology & Histology*, 05(03), 5–8.
- Abdalla, M.A. El-Malik, K.H. and Bayoumi, R. A. (2011). Application of Some Aqueous Plant Extracts as Molluscicidal Agents on *Bulinus truncatus* Snails in Sudan. *Journal of Basic and Applied Scientific Research*, 1(2), 108–117.
- Abdel Kader, A., Hamdi, S. H., & Rawi, S. M. (2005). Biological and biochemical studies on *Biomphalaria alexandrina* snails, treated with low concentrations of certain molluscicides (synthetic and of plant origin). *Journal of the Egyptian Society of Parasitology*.
- Adenowo, A. F., Oyinloye, B. E., Ogunyinka, B. I., & Kappo, A. P. (2015). Impact of human schistosomiasis in sub-Saharan Africa. *The Brazilian Journal of Infectious Diseases*, 19(2), 196–205.
- Agarwala, A. S. & R. A. (1992). Toxicity of the Latex of Euphorbiales. *Biological Agriculture & Horticulture: An International Journal for Sustainable Production Systems*, 8(3), 211–219.
- Ahmed A Gohara, G. T. M., Gadaraa, S. R., & El-Shazlyb, W. S. A. and A. M. (2014). Molluscicidal Activity of the Methanol Extract of *Callistemon viminalis* (Sol. ex Gaertner) G.Don ex Loudon Fruits, Bark and Leaves against *Biomphalaria alexandrina* Snails. *Iranian Journal of Pharmaceutical Research*, 12(October 2012), 505–514.
- Ajay Singh, S.K. Singh, R. P. Yadav, V.K. Srivastava, D. S., & Tiwari, and S. (2013). Eco-friendly molluscicides, piscicides and insecticides from common plants. *Trends in Agriculture and Soil Pollution Research*, 5, 205–230.

- Al-Yaquob, A. J. (2011). Effect of salinity and pH on hatchability and survival of the snails *Lymnaea auricularia*. *Marsh Bulletin*, 6(1), 62–72.
- Al-Zanbagi, N. A., Barrett, J., & Banaja, A. A. A. (2001). Laboratory evaluation of the molluscicidal properties of some Saudi Arabian euphorbiales against *Biomphalaria pfeifferi*. *Acta Tropica*, 78(1), 23–29.
- Appleton, C. C., & Kvalsvig, J. D. (2006). A school-based helminth control programme successfully implemented in KwaZulu-Natal. *The Southern African Journal of Epidemiology and Infection*, 21(2), 55–67.
- Bagarinao, T., & Lantin-Olaguer, I. (2000). From triphenyltins to integrated management of the “pest” snail *Cerithidea cingulata* in mangrove-derived milkfish ponds in the Philippines. *Hydrobiologia*, 437, 1–16.
- Bailey, S. E. R. (1989). Foraging behaviour of terrestrial gastropods: Integrating field and laboratory studies. *Journal of Molluscan Studies*, 55(2), 263–272. <https://doi.org/10.1093/mollus/55.2.263>
- Bajiro, M., Dana, D., Ayana, M., Eman, D., Mekonnen, Z., Zawdie, B., ... Zeynudin, A. (2016). Prevalence of *Schistosoma mansoni* infection and the therapeutic efficacy of praziquantel among school children in Manna District, Jimma Zone, southwest Ethiopia. *Parasites and Vectors*, 9(1), 4–9.
- Beadle, B. Y. L. C. (1969). Salt and water regulation in the embryos of freshwater pulmonate molluscs. *The Journal of Experimental Biology*, 50, 491–499.
- Berrios-Duran, L. a, Ritchie, L. S., & Wessel, H. B. (1968). Field screening tests on molluscicides against *Biomphalaria glabrata* in flowing water. *Bulletin of the World Health Organization*, 39(2), 316–320.

- Blankespoor, H. D., & Reimink, R. (1991). The Control of Swimmer ' s Itch in Michigan : Past , Present , and Future The Control of Swimmer ' s Itch in Michigan : *Michigan Academician*, XXIV, 7–23.
- Chauhan, S., & Singh, A. (2010). Molluscicidal potential of Lantana indica and Alstonia scholaris plants against freshwater snail Lymnaea acuminata. *Internet Journal of Toxicology*.
- Cheng, T. C., & Sullivan, J. T. (1977). Alterations in the osmoregulation of the pulmonate gastropod Biomphalaria glabrata due to copper. *Journal of Invertebrate Pathology*. (77)90179-3
- Chimbari, M. J. (2012). Enhancing schistosomiasis control strategy for Zimbabwe: Building on past experiences. *Journal of Parasitology Research*,
- Chingwena, G., Mukaratirwa, S., Kristensen, T. K., & Chimberu, M. (2002). Susceptibility of freshwater snails to the amphistome Calicophoron microbothrium and the influence of the species on susceptibility of Bulinus tropicus to Schistosoma haematobium and Schistosoma matthei infections. *The Journal of Parasitology*.
- Christian FA and Tesfamichael TM. (2000). Processes contributing to metabolic depression in hepatopancreas cells from the snail Helix aspersa. *The Journal of Experimental Biology*, 203(23), 3603–3612.
- Cioli, D., Pica-Mattocchia, L., Basso, A., & Guidi, A. (2014). Schistosomiasis control: Praziquantel forever? *Molecular and Biochemical Parasitology*, 195(1), 23–29.
- Clark, T. E., & Appleton, C. C. (1996). The physiological effects of aqueous suspensions of plant molluscicides on Helisoma duryi (Gastropoda: Planorbidae). *Journal of Molluscan Studies*, 62(4), 459–476.
- Čolović, M., & Krstić, D. (2013). Acetylcholinesterase inhibitors: Pharmacology and toxicology.

- Current Neuropharmacology*, 11, 315–335.
- Cowie, R. H. (2001). Can snails ever be effective and safe biocontrol agents? *International Journal of Pest Management*, 47(1), 23–40.
- Daffalla, a a, & Amin, M. a. (1976). Laboratory and Field Evaluation of the Molluscicidal Properties of Habat-El-Mollok (Croton spp.). *East African Journal of Medical Research*, 3(4), 185–195.
- Deelen, J. J. Van. (2013). *Management in freshwater ponds and shallow lakes in the Netherlands A review of the effects on emergent vegetation diversity and terrestrialisation*.
- Deshmane, J. (2012). Behavioral Responses of Freshwater Snail , *Viviparus bengalensis* to Plant Toxin in Fruits of *Acacia sinuate*. *Biological Forum — An International Journal*, 4(1), 18–22.
- Doenhoff, M. J., Hagan, P., Cioli, D., Southgate, V., Pica-Mattoccia, L., Botros, S., ... Engels, D. (2009). Praziquantel: its use in control of schistosomiasis in sub-Saharan Africa and current research needs. *Parasitology*, 136(13), 1825–1835.
- Donnelly, F. A., Appleton, C. C., & Schut, H. J. (1983). The influence of salinity on certain aspects of the biology of *Bulinus (Physopsis) Africanus*.species. *International Journal for Parasitology*, 13(6), 539–545.
- Duke, S. O., Cantrell, C. L., Meepagala, K. M., Wedge, D. E., Tabanca, N., & Schrader, K. K. (2010). Natural toxins for use in pest management. *Toxins*, 2(8), 1943–1962.
- Ebenso, I. E., Ita, B., Umoren, E. P., Ita, M., Binang, W., Edet, G., ... Ukpong, E. E. (2005). Effect of Carbamate Molluscicide on African Giant Land Snail *Limicolaria Aurora* ABSTRACT : *
- Corresponding author : *Journal of Applied Sciences and Environmental Management*, 9(1), 99–102.
- Edward, J. B., & Sogbesan, O. A. (2007). Toxicity Effect of Temephos on *Bulinus globosus* and

- Lymnaea natalensis. *Advances in Biological Research*, 1(3–4), 130–133.
- El-shazly, M. M. Y. (2012). Lethal Effect of Salts on the Terrestrial Snail Monacha Cantiana. *The Online Journal of Science and Technology*, 2(2), 44–50.
- El-Sherbini, G. T., Zayed, R. A., & El-Sherbini, E. T. (2009a). Molluscicidal Activity of Some Solanum Species Extracts against the Snail Biomphalaria alexandrina. *Journal of Parasitology Research*, 2009.
- Evers, B. N., Madsen, H., McKaye, K. M., & Stauffer, J. R. (2006). The schistosome intermediate host, Bulinus nyassanus, is a “preferred” food for the cichlid fish, Trematocranus placodon, at Cape Maclear, Lake Malawi. *Annals of Tropical Medicine and Parasitology*.
- Fonseca, C. T., Oliveira, S. C., & Alves, C. C. (2015). Eliminating schistosomes through vaccination : what are the best immune weapons ? *Frontiers in Immunology*, 6(March), 1–8.
- Gashaw, F., Erko, B., Teklehaymanot, T., & Habtesellasi, R. (2008). Assessment of the potential of competitor snails and African catfish (Clarias gariepinus) as biocontrol agents against snail hosts transmitting schistosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 102(8)
- Gbalégba, N. G. G. C., Silué, K. D., Ba, O., Ba, H., Tian-bi, N. T. Y., Yapi, G. Y., ... Koudou, B. G. (2017). Prevalence and seasonal transmission of Schistosoma haematobium infection among school-aged children in Kaedi town , southern Mauritania. *Parasites & Vectors*, 10(353), 1–12.
- Gray, D. J., McManus, D. P., Li, Y., Williams, G. M., Bergquist, R., & Ross, A. G. (2010). Schistosomiasis elimination: Lessons from the past guide the future. *The Lancet Infectious Diseases*.
- Grimes, J. E., Croll, D., Harrison, W. E., Utzinger, J., Freeman, M. C., & Templeton, M. R. (2015).

- The roles of water, sanitation and hygiene in reducing schistosomiasis: a review. *Parasites & Vectors*, 8(1), 1–16.
- Arthur, W. H., & Robert J. L., (1997). Egg-Mass Gel of *Melanochlamys diomedea* (Bergh) Protects Embryos from Low Salinity. *Biological Bulletin*, 193(3), 341–349.
- Hamed, M. A. (2011). Strategic control of schistosome intermediate host. *Asian Journal of Epidemiology*, 3(3), 123–140.
- Hua, H. Y., Wang, W., Cao, G. Q., Tang, F., & Liang, Y. S. (2013). Improving the management of imported schistosomiasis haematobia in China: Lessons from a case with multiple misdiagnoses. *Parasites and Vectors*, 6(1), 2–7.
- Ishak, M. M., Sharaf, A. a., & Mohamed, A. H. (1972). Studies on the mode of action of some molluscicides on the snail, *biomphalaria alexandrina* ☆: I. Effect of bayluscide, sodium pentachlorophenate, and copper sulphate on succinate, glutamate, and reduced TMPD oxidation. *Comparative and General Pharmacology*, 3(12), 385–390.
- Jose Antonio, J. M. . (1956). *Use of Molluscicides in the Control of Biharziasis in Venezuela*.
- Kabuyaya, M., Chimbari, M. J., Manyangadze, T., & Mukaratirwa, S. (2017). Efficacy of praziquantel on *Schistosoma haematobium* and re-infection rates among school-going children in the Ndumo area of uMkhanyakude district, KwaZulu-Natal, South Africa. *Infectious Diseases of Poverty*, 6(1), 1–9.
- Kiros, G., Erko, B., Giday, M., & Mekonnen, Y. (2014). Laboratory assessment of molluscicidal and cercariacidal effects of *Glinus lotoides* fruits. *BMC Research Notes*, 7(1), 220.
- Komiya, Y. (1961). The study and application of molluscicide. *Bulletin*, 25, 573–576.
- Kumar, S., Singh, D. K., & Singh, V. K. (2012). Tertiary combination of freeze-dried urine of Indian

- breeds of cow with plant products against snail *Lymnaea acuminata*. *Pakistan Journal of Biological Sciences*, 15(20), 992–996.
- Kushwaha, V. B., & Singh, A. (2010). Effect of Uscharin on the Reproduction of the Snail *Lymnaea Acuminata*. *World Journal of Zoology*, 5(1), 15–19.
- Lahlou, M. (2004). Study of the Molluscicidal Activity of Some Phenolic Compounds: Structure-Activity Relationship. *Pharmaceutical Biology*, 42(3), 258–261.
- Lovett, A. L. & Black, A. B. (1920). *The gray garden slug : with notes on allied forms. Bulletin of the Oregon Agricultural College Experiment Station.*
- Lu, L., Zhang, S. M., Mutuku, M. W., Mkoji, G. M., & Loker, E. S. (2016). Relative compatibility of *Schistosoma mansoni* with *Biomphalaria sudanica* and *B. pfeifferi* from Kenya as assessed by PCR amplification of the *S. mansoni* ND5 gene in conjunction with traditional methods. *Parasites and Vectors*, 9(1), 1–13.
- Luiz, C., Ayres, P., Silva, T., & Fernandes, D. (2013). Molluscicidal activity of *Moringa oleifera* on *Biomphalaria glabrata* : integrated dynamics to the control of the snail host of *Schistosoma mansoni*. *Rev Bras Farmacogn*, 23, 848–850.
- Lundeba, M., Likongwe, J. S., Madsen, H., Stauffer, J. R., & Prochazka, K. (2007). Potential of *Metriaclima lanisticola* (Teleostei: Cichlidae) for biological control of schistosome intermediate host snails. *African Zoology*, 42(1), 45–49.
- Marois, R., & Croll, R. P. (2011). Hatching asynchrony within the egg mass of the pond snail, *Lymnaea stagnalis*. *Invertebrate Reproduction and Development. Rehovot*. Retrieved from http://www.physiology.dal.ca/people/faculty/pubs/11_422.pdf%5Cnpapers3://publication/uuid/61FF7275-967F-4171-B64C-DB4129AFC0DB

- Martinovic-Vitanovic, V. M., Rakovic, M. J., Popovic, N. Z., & Kalafatic, V. I. (2013). Qualitative study of Mollusca communities in the Serbian Danube stretch (river km 1260–863.4). *Biologia*, 68(1), 112–130.
- McDonald, E. A., Pond-Tor, S., Jarilla, B., Sagliba, M. J., Gonzal, A., Amoylen, A. J., ... Friedman, J. F. (2014). Schistosomiasis japonica during pregnancy is associated with elevated endotoxin levels in maternal and placental compartments. *Journal of Infectious Diseases*, 209(3), 468–472.
- Mello-Silva, C. C., De Vasconcellos, M. C., Pinheiro, J., & De Lurdes De Azevedo Rodrigues, M. (2006). Physiological changes in *Biomphalaria glabrata* Say, 1818 (Pulmonata: Planorbidae) caused by sub-lethal concentrations of the latex of *Euphorbia splendens* var. *hislopii* N.EB (Euphorbiaceae). *Memorias Do Instituto Oswaldo Cruz*, 101(1), 3–8.
- Miranda, N. A. F., Perissinotto, R., & Appleton, C. C. (2010). Salinity and temperature tolerance of the invasive freshwater gastropod *Tarebia granifera*. *South African Journal of Science*, 106(3–4).
- Mohamed A. Dagal, E. Suchart Upatham, M. K. and V. V. (1986). Effects of some physio-chemical factors on the hatching of egg masses and on the survival of juveniles and adult snails of *Bulinus* (*Physopsis*) *abyssinus*.pdf. *Journal of the Science Society of Thailand*, 12, 23–30.
- Mohamed, R. (2011). Impact of profenophos (pesticide) on infectivity of *Biomphalaria alexandrina* snails with *Schistosoma mansoni* miracidia and on their physiological parameters. *Open Journal of Ecology*, 01(02), 41–47.
- Molla, E., Giday, M., & Erko, B. (2013). Laboratory assessment of the molluscicidal and cercariacidal activities of *Balanites aegyptiaca*. *Asian Pacific Journal of Tropical Biomedicine*, 3(8), 657–662.
- Mostafa, O. M. S. (2009). Effect of salinity and drought on the survival of *Biomphalaria arabica*, the intermediate host of *Schistosoma mansoni* in Saudi Arabia. *Egyptian Academic Journal of*

Biological Sciences, 1(1), 1–6.

- Mouhamadou Diaby, Abderrahman Iggidr, Mamadou Sy, A. S. (2014). Global analysis of a schistosomiasis infection model with biological control. *Applied Mathematics and Computation*, 247, 1–10.
- Mutuku, M. W., Dweni, C. K., Mwangi, M., Kinuthia, J. M., Mwangi, I. N., Maina, G. M., ... Mkoji, G. M. (2014). Field-derived *Schistosoma mansoni* and *Biomphalaria pfeifferi* in Kenya: A compatible association characterized by lack of strong local adaptation, and presence of some snails able to persistently produce cercariae for over a year. *Parasites and Vectors*, 7(1), 1–13.
- Nagabhushanam, D. P. V. (1979). Influence of salt concentrations on survival, body weight and blood chloride of the freshwater snail, *Indoplanorbis exustus* (Deshayes). *Hydrobiologia*, 63(3), 195–198.
- Nagi, S., Chadeka, E. a., Sunahara, T., Mutungi, F., Dan Justin, Y. K., Kaneko, S., ... Hamano, S. (2014). Risk Factors and Spatial Distribution of *Schistosoma mansoni* Infection among Primary School Children in Mbita District, Western Kenya. *PLoS Neglected Tropical Diseases*, 8(7), 1–10.
- Ndabamba M, Basopo N, Nkiwane LC, N. Y. (2015). The molluscicidal efficacy of methanol extracts of *Pterocarpus angolensis*. *African Journal of Aquatic Science*, 40(1), 103–104.
- Njenga, S. M., Mutungi, F. M., Wamae, C. N., Mwanje, M. T., Njiru, K. K., & Bockarie, M. J. (2014). Once a year school-based deworming with praziquantel and albendazole combination may not be adequate for control of urogenital schistosomiasis and hookworm infection in Matuga District, Kwale County, Kenya. *Parasites & Vectors*, 7(1), 74.
- Odiere, M. R., Rawago, F. O., Ombok, M., Secor, W. E., Karanja, D. M. S., Mwinzi, P. N. M., ... Won, K. (2012). High prevalence of schistosomiasis in Mbita and its adjacent islands of Lake Victoria, western Kenya. *Parasites & Vectors*, 5(1), 278. <https://doi.org/10.1186/1756-3305-5-278>

- Okeke, O. C., & Ubachukwu, P. O. (2011). Molluscicidal Effects of *Talinum triangulare* on *Bulinus*. *Nigerian Journal of Biotechnology*, 22, 13–16.
- Olajumoke M., & Benson . O. (2012). Molluscicidal Effects of Aqueous and Ethanolic Extracts of Lemongrass (*Cymbopogon*. *New York Science Journal*, 5(8).
- Oliveira-Filho, E. C., Geraldino, B. R., Coelho, D. R., De-Carvalho, R. R., & Paumgarten, F. J. R. (2010). Comparative toxicity of *Euphorbia milii* latex and synthetic molluscicides to *Biomphalaria glabrata* embryos. *Chemosphere*, 81(2), 218–227.
- Omole, A. J., Ogbosuka, G. E., Oredehin, A. O., & Osayomi, J. O. (2008). Effect of varying levels of salt (sodium chloride) on the performance characteristics of snails (*Archachatina marginata*). *Nutrition & Food Science*, 38(6), 555–558.
- Oniya, O.J and Fajiwe, M. . (2010). Evaluation of the Ovicidal Activity of Calcium Hypochlorite on the Eggs of *Bulinus globosus*, Intermediate Host of *Schistosoma haematobium* in Nigeria. *The Zoologist*, 8.
- Opisa, S., Odiere, M. R., Jura, W. G. Z. O., Karanja, D. M. S., & Mwinzi, P. N. M. (2011). Malacological survey and geographical distribution of vector snails for schistosomiasis within informal settlements of Kisumu City , western Kenya. *Parasites & Vectors*, 4(1), 226.
- Oplinger, R. W., Brown, P., & Wagner, E. J. (2009). Effect of Sodium Chloride, Tricaine Methanesulfonate, and Light on New Zealand Mud Snail Behavior, Survival of Snails Defecated from Rainbow Trout, and Effects of Epsom Salt on Snail Elimination Rate. *North American Journal Of Aquaculture*.
- Pechenik, J. a. (1982). Egg capsules of *nucella lapillus* (l.) Protect against low-salinity stress. *Journal*

of Experimental Marine Biology and Ecology, 71(2), 165–179.

Pereira Filho, A. A., França, C. R. C., Oliveira, D. D. S., Mendes, R. J. D. A., Gonçalves, J. D. R. S.,

& Rosa, I. G. (2014). Evaluation of the molluscicidal potential of hydroalcoholic extracts of

Jatropha gossypifolia Linnaeus, 1753 ON *Biomphalaria glabrata* (Say, 1818). *Revista Do*

Instituto de Medicina Tropical de São Paulo, 56(6), 505–510.

Pinto, H. A., Mati, V. L. T., & de Melo, A. L. (2013). The Pampulha reservoir remains a potential

urban focus of schistosomiasis mansoni in Brazil: Changes in the occurrence patterns of

Biomphalaria species and a new record of the parasite. *Revista Da Sociedade Brasileira de*

Medicina Tropical.

Przeslawski, R. (2004). A review of the effects of environmental stress on embryonic development

within intertidal gastropod egg masses. *Molluscan Research*, 24(1), 43–63.

<https://doi.org/10.1071/MR04001>

Qiu, J., Li, R., Xu, X., Yu, C., Xia, X., Hong, X., ... Shi, Y. (2014). Identifying Determinants of

Oncomelania hupensis Habitats and Assessing the Effects of Environmental Control Strategies

in the Plain Regions with the Waterway Network of China at the Microscale. *International*

Journal of Environmental Research and Public Health, 11(6), 6571–6585.

Ramadan, R. A. M., Gama, R. M. Al, & Gad, D. M. (2012). On behavioral pattern and molluscicidal

effect of commiphora molmol (myrrh) in comparison with bayluscide against melanoides

tuberculata snail (thiaridae), in abbasa , egypt Ramadan et al. *Suez Canal Medicine Journals*,

(2).

Rollinson, D., Knopp, S., Levitz, S., Stothard, J. R., Tchuem Tchuenté, L. A., Garba, A., ... Utzinger,

J. (2013). Time to set the agenda for schistosomiasis elimination. *Acta Tropica*, 128(2), 423–

440.

- Salawu, O. T., & Odaibo, A. B. (2011). The molluscicidal effects of *Hyptis suaveolens* on different stages of *Bulinus globosus* in the laboratory. *African Journal of Biotechnology*, *10*(50), 10241–
- Savioli, L., Engels, D., ROUNGOU, J. B., Fenwick, a, & Endo, H. (2004). Schistosomiasis control. *Lancet*, *363*, 658.
- Scholte, R. G. C., Carvalho, O. S., Malone, J. B., Utzinger, J., & Vounatsou, P. (2012). Spatial distribution of *Biomphalaria* spp., the intermediate host snails of *Schistosoma mansoni*, in Brazil. *Geospatial Health*, *6*(3 SUPPL.), 95–101.
- Singh, Vatsala nd Tiwar, J. (2012). Biological and Chemical Sciences Effects of Synthetic and Extracts of Plant on Reproduction of Snail *Lymnaea*. *Research Journal of Pharmaceutical* , *3*(4), 705–710.
- Sousa-Figueiredo, J. C., Gamboa, D., Pedro, J. M., Façonny, C., Langa, A. J., Magalhães, R. J. S., ... Nery, S. V. (2012). Epidemiology of malaria, schistosomiasis, geohelminths, anemia and malnutrition in the context of a demographic surveillance system in northern Angola. *PLoS ONE*, *7*(4).
- Stensgaard, A. S., Utzinger, J., Vounatsou, P., Hürlimann, E., Schur, N., Saarnak, C. F. L., ... Kristensen, T. K. (2013). Large-scale determinants of intestinal schistosomiasis and intermediate host snail distribution across Africa: Does climate matter? *Acta Tropica*, *128*(2), 378–390.
- Symondson, W. O. C., Sunderland, K. D., & Greenstone, M. H. (2002). Can Generalist P Redators Be Effective Biocontrol Agents ? *Annual Review of Entomology*, *47*, 561–594.
- Thétiot-Laurent, S. A. L., Boissier, J., Robert, A., & Meunier, B. (2013). Schistosomiasis chemotherapy. *Angewandte Chemie - International Edition*.

- Tiwari, F. (2012). Bait Formulation Toxicity of Plant Derived Molluscicides in Attractant Food Pellets Against Vector Snail , *Lymnaea acuminata* importance because they act as intermediate hosts for. *World Journal of Zoology*, 7(1), 55–59.
- Ukwandu, N. C. D., Odaibo, A. B., Okorie, T. G., & Nmorsi, O. P. G. (2011). Molluscicidal effect of *Piper guineense*. *African Journal of Traditional, Complementary and Alternative Medicines*,
- Vaidya, D. P., Nagabhushanam, R., & Hanumante, M. M. (1980). Responses of the neurosecretory cells of the freshwater snail, *Indoplanorbis exustus* to hydrothermal stress. *Hydrobiologia*.
- Van Aardt, W. J., & Frey, B. J. (1979). Oxygen consumption and responses of the freshwater snail *Bulinus (Physopsis) globosus* to gradients of different oxygen tensions. *South African Journal of Zoology*, 14(4), 202–207.
- Wade, C. M., Mordan, P. B., & Naggs, F. (2006). Evolutionary relationships among the Pulmonate land snails and slugs (Pulmonata, Stylommatophora). *Biological Journal of the Linnean Society*, 87(4), 593–610.
- Wakil, H. B. (1999). Molluscicidal Activity and Repellency of some Inorganic Fertilizers against Terrestrial Snail , *Theba pisana* (Müller), Infesting Citrus Trees in Northern Areas , Egypt. *King Abdulaziz University Online Journal*, 11, 15–26.
- Wu, W., & Huang, Y. (2013). Application of praziquantel in schistosomiasis japonica control strategies in China. *Parasitology Research*.
- Xiaonong, Z., Minggang, C., McManus, D., & Bergquist, R. (2002). Schistosomiasis control in the 21st century: Proceedings of the International Symposium on Schistosomiasis, Shanghai, July 4-6, 2001. In *Acta Tropica* (Vol. 82, pp. 95–114).
- Yadav, J., Singh, D., Yadav, J., & Kumar, D. (2017). Organophosphates and carbamates as inhibitors

of acetylcholinesterase in *Eisenia fetida*. *Pollution Research*, 36(2), 277–281.

Evaluation Of Molluscicidal Properties Of The Common Salt Sodium Chloride (NaCl) Against Biomphalaria Pfeifferi, Snail Host Of Schistosoma Mansoni, The Causal Agent Of Intestinal Schistosomiasis

Tonny Teya Nyandwaro

Zipporah Nganga

Jomo Kenyatta University of Agriculture & Technology
College of Health Sciences, Department of Medical
Laboratory Sciences, Nairobi Kenya

Gerald M. Mkoji

Centre for Biotechnology Research and Development
Kenya Medical Research Institute, Kenya, Nairobi

Abstract: The molluscicidal activity of the common salt, Sodium Chloride (NaCl) was tested under laboratory conditions against egg masses, neonates and adult *B. pfeifferi*, snail host of the human pathogen, *S. mansoni*. NaCl concentrations of < 3200 mg/L did not inhibit hatching of snail egg masses exposed for 24hr, at concentrations in the range 800-3600mg/L, 30-100% of the neonates were killed in a 24 hr exposure assay, concentrations of 4800mg/L or above killed 80-100% of the adult snails, measuring 8mm or above in shell diameter. The LC50 of the NaCl solution for egg masses was 494 mg/L, for neonates 1457 mg/L, and for adult snails 4786 mg/L. However, egg laying and number of eggs laid were significantly reduced in a 24hr exposure to non-lethal concentrations (3200 mg/L) of NaCl solution, compared to unexposed controls. These results suggest that NaCl has potential as a molluscicide against freshwater pulmonate snails of medical or veterinary importance, given that it is readily available and relatively inexpensive, and therefore, further studies on it should be encouraged.

Keywords: Snail control, Sodium chloride, molluscicidal properties, Gastropoda, Pulmonates, Biomphalaria pfeifferi, laboratory studies.

I. INTRODUCTION

Schistosomiasis is a snail-borne tropical parasitic disease of medical significance and caused by digenean *Trematodes* in the genus *Schistosoma* (Gryseels, 2012). Considered one of the neglected tropical diseases (NTDs), the disease afflicts both humans and animals and an estimated 200 million people worldwide are infected mostly children (Colley, Bustinduy, Secor, & King, 2014), and about 20,000 people die from complications arising from the disease every year (Jenkins-Holick & Kaul, 2013). Current strategies for schistosomiasis control rely on chemotherapy, and heavily depend on the

antischistosomal drug, Praziquantel (Wang, Wang, & Liang, 2012), virtually the only drug available for use in efforts to eliminate the disease (Soukhathammavong et al., 2011). However, chemotherapy is not sustainable as re-infections do frequently occur after successful treatment, with fears that Praziquantel resistance could emerge with frequent and intensive use in global efforts to eliminate the disease (Seto, Wong, Lu, & Zhong, 2011). Consequently, alternative means or new strategies to complement chemotherapy may be necessary. For example, snail control is a practical approach for control of schistosomiasis and other snail-borne parasitic infections of medical or veterinary significance. Use of

chemical molluscicides to eliminate snails is effective, but these may not be suitable for routine use largely because they are costly to produce and apply, and may be harmful to the environment (Santos, Ferreira, Soares, & Loureiro, 2010). Low cost, environment-friendly snail control approaches are needed chemotherapy-based approaches currently being used in the fight against schistosomiasis (Rollinson et al., 2013).

(Pattanagul & Thitisaksakul, 2008) suggested that salinity can negatively impact freshwater snails thus, can potentially, limit snail populations in natural habitats. This study evaluated the potential of NaCl in the control of *B. pfeifferi* snails under laboratory conditions. NaCl has certain unique advantages over synthetic molluscicides: it is readily available, inexpensive and environment-friendly, and suitable for use in resource-limited countries.

II. MATERIALS AND METHODS

STUDY DESIGN

This was a laboratory based experimental study using *B. pfeifferi* (Family Planorbidae) snails collected from the field in Mwea Irrigation Scheme, Kirinyaga county, central Kenya, and undertaken at the Centre for Biotechnology Research and Development (CBRD), Kenya Medical Research Institute (KEMRI).

SNAIL COLLECTION

Snails were collected from streams using a standard flat wire-mesh snail scoop with a mesh size of 2 mm, and occasionally were picked up from the stream using hand-held long metal forceps. They were then packed between layers of damp cotton gauze and transported to Kenya Medical Research Institute laboratory in Nairobi in perforated plastic containers the same day they were collected. In the laboratory they were screened for trematode infections and kept in plastic aquaria and allowed to acclimatize for several days before being used for experimental purposes. The collected snails were used directly as target snails to evaluate the molluscicidal activity of sodium chloride (NaCl) solution or were allowed to lay egg masses. Neonate or juvenile snails hatched from the egg masses were also used as targets in molluscicide experiments.

MAINTENANCE SNAIL

Snails were kept in a room maintained at a temperature of 26°C under natural light, in shallow plastic aquaria measuring 18 x 28 x 10 cm in aerated tap water. Aeration of tap water for 2-3 days removed the chlorine present in tap water. The adult snails were fed on boiled green lettuce (*Lactuca sativa*). Neonate snails were fed on commercial brand of flake fish food (Crop King fish feed) supplemented with bone meal (Tamfeeds Bone Meal) as a source of Calcium for shell development. Water in the aquaria was changed once a week.

EFFECT OF NA CL SOLUTION ON DEVELOPMENT AND HATCHABILITY OF *B. PFEIFFERI* EGG MASSES

Development and hatchability of egg masses was investigated in salinities in concentrations in the range of 800-6800 mg NaCl, and control egg masses were maintained in dechlorinated water without NaCl under similar lab conditions. Freshly laid egg masses on colorless polythene papers were used for this experiment. Five egg masses laid within 24 hr. prior to the experiment were transferred to 500ml plastic bowls containing dechlorinated water or the NaCl e test solutions. The egg masses were then examined daily under a dissecting microscope to assess embryo development for up to 12 days. The experiment was repeated three times.

EFFECT OF NA CL SOLUTION ON THE SURVIVAL OF NEONATE OF *B. PFEIFFERI*

The snails were allowed to lay eggs on polythene sheets. Egg masses attached to the polythene sheets were located and isolated by cutting the plastic around each egg-mass with a scalpel (about 0.5-1.0cm from the egg-mass). The egg masses were immersed in petri dishes containing clean well water to remove any debris and transferred to containers containing 200ml of dechlorinated tap water; the dishes were covered until eggs hatched into neonates.

EFFECT OF NA CL ON THE SURVIVAL OF ADULTS *B. PFEIFFERI*

NaCl solution was tested against adult *B. pfeifferi* snails in the range of 3200-6800 mg/L. Adult snails in the size range 8-10 mm shell diameter or 13-15 mm shell diameter) were used in this experiment, and in each test container 10 snails were introduced and maintained in a volume of 1000ml test solution with control snails maintained in a similar volume of dechlorinated water. Snail responses to the test solution was recorded within the first 6hr and at 24hr following exposure. To confirm snail mortality, both test snails and control snails were transferred into fresh, clean water, offered lettuce, and maintained for another 24hr to see whether or not they will recover from the effect of the test NaCl solution. Snails were considered dead if they did not come out of their shells and respond to food items during the 24hr recovery period. The snails that were not affected by the salt solution or those that recovered from the effects of the salt solution were maintained in fresh water for up to 4 days after exposure to the salt solution, and observed for their feeding habits and fecundity (egg laying capacity). This experiment was repeated 3 times.

Percent mortality was calculated using the following formula

$$\text{Killed snails} = \frac{\% \text{ Killed by the Treatment} \times \text{Dead in Control}}{100 - \% \text{ dead in control}}$$

DATA ANALYSIS

The obtained data was subjected to Stata analysis software, version 10, to get LC₅₀ (mg/L) and LC₉₀ (mg/L), Probit regression graph, and Chi-square.

III. RESULTS

NaCl solutions of different concentrations were observed to affect the 3 developmental stages of *B. pfeifferi*. Table 1 shows the LC₅₀ and LC₉₀ values of NaCl on the three developmental stages of *B. pfeifferi*. The lethal concentration that killed 50% of egg, neonates and adult stage of the snail was 494, 1457 and 5087mg/L respectively, while the lethal concentrations that killed 90% of eggs, neonates and adults stage of the snail were 4220, 3007 and 6385mg/L respectively. The molluscicidal effect of NaCl was not significantly effective at all stages of *B. pfeifferi* eggs at 24 hrs ($\chi^2=42$; P>0.05), juveniles at 24 hrs ($\chi^2=30$; P>0.05) and adults at 24 hrs ($\chi^2=15$; P>0.05). The different test solution of NaCl showed that R² = 0.9373, 0.8343 and 0.8771 respectively.

Stage	Regression	χ^2 (P>0.05)	LD ₅₀ (C.I.) mg/L	LD ₉₀ (C.I.) mg/L
Eggs	Y=0.0085x+54.207	42.0	494 (1086-2106)	4220 (2640-5800)
Neonates	Y=0.0258x+12.413	30.0	1457(473-2441)	3007(2023-3990)
Adults	Y=0.0308x-106.67	15.0	5087(3569-6605)	6385 (3585-9185)

Table 1: Molluscicidal effect of NaCl on the different developmental stages of *B. pfeifferi* snails

The egg masses were the most susceptible to the effects of NaCl compared to the other developmental stages. The Figure 1 shows the hatching rate of *B. pfeifferi* eggs masses exposed to NaCl in the range 800-6800 mg/L, over a 24 hr period and thereafter incubated for 12 days. Egg masses exposed to 5600mg/L or above failed to hatch completely even after incubation through 12 days. When examined under a dissecting microscope at magnification X100, there was no indication of egg/embryo development. On the other hand, 90-95% of the egg masses exposed to 1600mg/L or less hatched normally and the proportion of egg masses hatching was similar to that observed in the control egg masses, maintained in fresh water. Interestingly, a salt concentration of 3200 mg/L delayed egg mass hatching for 2 days beyond the hatching time for control egg masses not exposed to salt, and in addition, significantly reduced the proportion of egg masses developing to the level of hatching down to slightly above 40%. Exposure of egg masses to salt concentrations of 5600 mg/L and above led to 100% inhibition of egg hatching

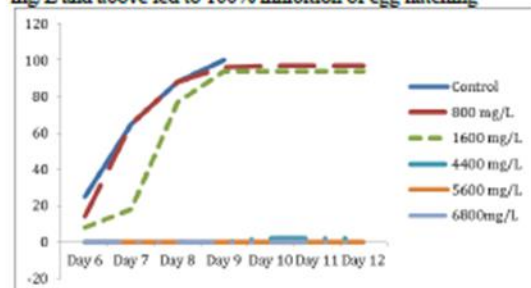


Figure 1: Rate of hatching of *B. pfeifferi* eggs after exposure to the different experimental salinities

Egg masses exposed to 5600mg/L or above failed to hatch completely even after incubation through 12 days. When examined under a dissecting microscope at magnification X100, there was no indication of egg/embryo development. On the other hand, 90-95% of the egg masses exposed to 1600mg/L or less hatched normally and the proportion of egg masses hatching was similar to that observed in the control egg masses, maintained in fresh water. Interestingly, a salt concentration of 3200 mg/L delayed egg mass hatching for 2 days beyond the hatching time for control egg masses not exposed to salt, and in addition, significantly reduced the proportion of egg masses developing to the level of hatching down to slightly above 40%. Exposure of egg masses to salt concentrations of 5600 mg/L and above led to 100% inhibition of egg hatching.

Figure 2 shows embryo development in egg masses exposed to NaCl solutions at different concentrations compared with a control egg mass maintained in tap water. Egg masses exposed to NaCl in the range 3200-4400mg/L showed delayed embryo development, whereas those exposed to NaCl concentrations of 5600 mg/L or above showed no embryo development at all.

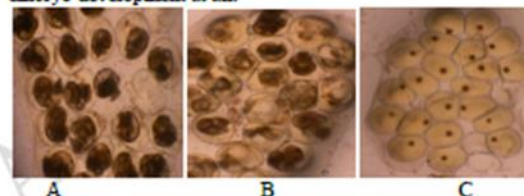


Figure 2: Photographs of *B. pfeifferi* egg masses exposed to NaCl solution over a 24hr and subsequent development

A shows a control egg mass, 7 days later with fully developed neonate snails ready to hatch; B An snail egg mass exposed to 3200 mg/L NaCl solution over a 24hr period, in its 7th day of development, showing poorly developed embryos and only a few fully developed neonates; C Egg masses exposed to salt solution at 5600 mg/L over a 24 hr. period, 7 days later with undeveloped embryos

The newly hatched neonates from eggs exposed to different concentration of NaCl were mostly attached to the walls of the container and had thin shells in comparison to the control groups. Movement of the newly hatched neonates in the treated group was slow, and the snail had smaller tentacles as compared with those in control groups.

The general 'distress syndromes' observed in the neonate after exposure to the various NaCl concentrations were: the neonates tried to escape from the NaCl concentrations by crawling to the side of the container, this effects were more pronounced with increase in concentration, excessive production of mucus was noted. Figure 3 shows mortality percentage of 3-day-old snails after exposure and recovery period. In 2600mg/L and above, mortalities of between 48% and 100% were recorded. However, no mortalities were recorded in the control group.

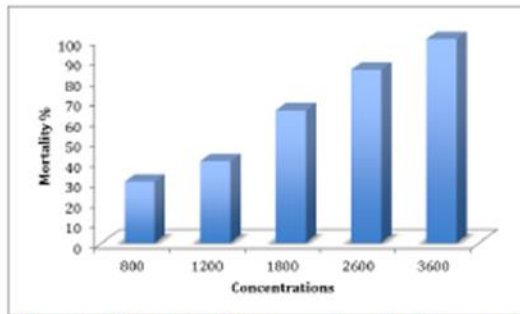


Figure 3: Effect of the different experimental salinities on 3-day-old neonates of *B. pfeifferi* after 24 hours of exposure

The following behavioral responses were observed after exposing adult *B. pfeifferi* snails to the different NaCl concentrations. In 5600 mg/L and above, the sub lethal effects included retraction into their shells (both completely and partially). The sub lethal effects at 3200mg/L included: trying to crawl out of the container, snails showed abnormal body movements (muscular and spiral twisting of the body) and clamping and crawling on each other. The figure 4 below shows mortality of adult snails after 24 hours of exposure, no mortalities were recorded in the 3200mg/L and below. At concentrations of 5600mg/L and above, the mortalities of between 78% and 100% were recorded. Mortalities recorded at 3200 and 4400 mg/L show some abnormality, they were abnormally low compared to what was expected.

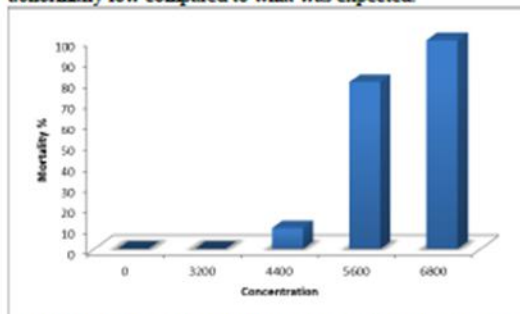


Figure 4: Effect of the different experimental salinities on adults of *B. pfeifferi* after 24 hours of exposure

IV. DISCUSSION

When adult snails were exposed to salt concentrations in the range 3200-6800 mg/l they retracted into their shells, attempted to crawl out, bleed and released a mucous substance. This is a distress response, and is often observed for freshwater snails when in distress as a result of exposure to chemicals or other unpleasant conditions e.g. extreme heat (Oliveira-Filho, Geraldino, Coelho, De-Carvalho, & Paumgarten, 2010). The behavioral responses observed might have physiological basis as has been observed for other snails (Van Gaest, Young, Young, Helms, & Arellano, 2007) However, at higher salt concentrations, the snails did not survive and they may have died due to dehydration (El-shazly,

2012). In some cases, snails retracted into their shells and remained motionless, but when the snails were removed from the salt solution, they recover, and began to move. Retraction into the shell is a strategy often used by snails in response to distress conditions for survival until favorable conditions are re-established, and is often accompanied by reduction in physiological activity (Van Gaest et al., 2007) (Miranda, Perissinotto, & Appleton, 2010)

One of the effects of chemical distress such as NaCl is inhibition of snail respiration, which could be temporary or permanent depending on the chemical concentrations. Other effects of NaCl on the snails included abnormal movements such as muscular and spiral twisting of the body and clamping on each other, and this suggests that NaCl may have acted as a neurotoxin, thus affected the neuromuscular system of the snails. (Deshmane, 2012) observed that a plant toxin from *Acacia sinuate* (Family *Mimosaceae*) affected the central nervous system of the snail *Viviparus bengalensis* (Family *Viviparidae*) causing abnormal behavior in the snail. (Yadav & Singh, 2013) also reported that exposure of Leaf extracts of the medicinal euphorbious plants *Lymnaea acuminata* (Family *Lymnaeidae*) and *Indoplanorbis excustus* (Family *Planorbidae*) to *Codiaeum variegatum* and *Croton tiglium* caused them to crawl on each other and also experience muscle twisting.

Snails in the experimental group, especially those exposed to 3200 and 4400 mg/L tried crawling out, so as to escape the molluscicidal effects of NaCl. This phenomenon enhances their survival chances, as well as explains the reduced mortalities in 3200 and 4400mg (Adetunji & Salawu, 2010). The neonate's snails were observed to float to top of the different NaCl concentrations, in an effort to obtain oxygen and to protrude the aperture through the aperture and escape the effects of NaCl (Jurberg, Barbosa, & Rotenberg, 1988).

Although low concentrations of NaCl (<1600 mg/L) did not seem to interfere with normal embryonic development, salt concentration at 5600 mg/L and above inhibited egg hatching completely. Death of embryo at higher concentrations could be attributed to the interference of the embryo physiology by the high concentrations of NaCl (Adenusi, 2009). The ability of egg masses to survive and hatch in some NaCl concentrations is due to presence of jelly layer of egg masses (Sukumaran, Parashar, Gupta, Jeevaratnam, & Prakash, 2004). In higher concentrations, the jelly layer could not fully prevent the effect of the NaCl concentrations leading to damage of the embryo, as result of dehydration (Mohamed A. Dagal, E. Suchart Upatham, 1986). The encapsulating structures are suggested to help reduce desiccation and rate of salinity change (H. Arthur Woods and Robert L. DeSilets, 1997).

Salinity did not only have ovicidal effect on the egg masses, it also, inhibited fecundity of the adult snails by reducing the number of egg masses laid by the adult snails relative to controls rather than the number of egg cells (Donnelly, Appleton, & Schut, 1983). Egg masses laid by the experimental group had egg masses ranging between 11-13 egg cells per egg mass. The number of egg masses laid differed greatly depending on concentration of salinities the snail was exposed to prior.

The present study has demonstrated that NaCl has molluscicidal properties against the snail *B. pfeifferi*, the snail

intermediate host of the parasite *Schistosoma mansoni* which causes human intestinal Schistosomiasis, a major public health problem in sub-Saharan Africa, and is potentially, a suitable candidate for further exploitation for Schistosomiasis control in Kenya. NaCl is commonly and readily available in Kenya, it is inexpensive, and a suitable alternative to the synthetic molluscicides. Further studies are certainly needed to further investigate the value of this chemical as a molluscicide.

ACKNOWLEDGEMENTS

I express my deepest gratitude to my principal supervisor, Dr Gerald Mkoji of KEMRI for his support as I undertook my research. He facilitated collection of snails used in the study, provided laboratory space and the material I needed for my research, and further more gave guidance, suggestion and valuable comments that help shape this work. I also sincerely thank my supervisor at JKUAT, Prof Zipporah Ng'ang'a for her guidance, encouragement and for her valuable comments during the entire study. I'm truly grateful to her for support. My sincere gratitude's to staff member of the Schistosomiasis section in the Centre for Biotechnology Research and Development (CBRD) for their support as I did this project and especially Mr. Martin Muku for guidance on snail breeding procedure and his valuable suggestion

REFERENCES

- [1] Ademusi, A. A. A. B. O. (2009). Effects of varying concentrations of the Crude aqueous and Ethanolic. *African Journal of Traditional, Complementary and Alternative Medicines*, 6(2), 139-149.
- [2] Adetunji, V., & Salawu, O. (2010). Efficacy of ethanolic leaf extracts of *Carica papaya* and *Terminalia catappa* as molluscicides against the snail intermediate hosts of schistosomiasis. *Journal of Medicinal Plants Research*, 4(22), 2348-2352. <http://doi.org/10.5897/JMPR10.468>
- [3] Colley, D. G., Bustinduy, A. L., Secor, W. E., & King, C. H. (2014). Human schistosomiasis. *Lancet*, 383(9936), 2253-64. [http://doi.org/10.1016/S0140-6736\(13\)61949-2](http://doi.org/10.1016/S0140-6736(13)61949-2)
- [4] Deshmane, J. (2012). Behavioral Responses of Freshwater Snail, *Viviparus bengalensis* to Plant Toxin in Fruits of *Acacia simuate*. *Biological Forum — An International Journal*, 4(1), 18-22.
- [5] Donnelly, F. A., Appleton, C. C., & Schut, H. J. (1983). The influence of salinity on certain aspects of the biology of *Bulinus* (Physopsis) *Africanus* species. *International Journal for Parasitology*, 13(6), 539-545.
- [6] El-shazly, M. M. Y. (2012). Lethal Effect of Salts on the Terrestrial Snail *Monacha Cantiana*. *The Online Journal of Science and Technology*, 2(2), 44-50.
- [7] Gryseels, B. (2012). Schistosomiasis. *Infectious Disease Clinics of North America*. <http://doi.org/10.1016/j.idc.2012.03.004>
- [8] H. Arthur Woods and Robert L. DeSilets, J. (1997). Egg-Mass Gel of *Melanochlamys diomedea* (Bergh) Protects Embryos from Low Salinity. *Biological Bulletin*, 193(3), 341-349.
- [9] Jenkins-Holick, D. S., & Kaul, T. L. (2013). Schistosomiasis. *Urologic Nursing*, 33, 163-170. <http://doi.org/10.7257/1053-816X.2013.33.4.163>
- [10] Jurberg, P., Barbosa, J. V., & Rotenberg, L. (1988). The role of behavior in the survival of *Biomphalaria glabrata* in bioassays with the plant molluscicide *Phytolacca dodecandra*. *Memorias Do Instituto Oswaldo Cruz*, 83(1), 41-46. <http://doi.org/10.1590/S0074-02761988000100006>
- [11] Miranda, N. A. F., Perissinotto, R., & Appleton, C. C. (2010). Salinity and temperature tolerance of the invasive freshwater gastropod *Tarebia granifera*. *South African Journal of Science*, 106(3-4). <http://doi.org/10.4102/sajs.v106i3/4.156>
- [12] Mohamed A. Dagal, E. Suchart Upatham, M. K. and V. V. (1986). Effect of Some Physio-Chemical Factors on Hatching of Egg Masses And on The Survival of Juvenile and adult Snails of *Bulinus* (Physopsis) *Abyssinicus*.pdf. *Journal of Applied Sciences and Environmental Management*, 12, 23-30.
- [13] Oliveira-Filho, E. C., Geraldino, B. R., Coelho, D. R., De-Carvalho, R. R., & Paumgarten, F. J. R. (2010). Comparative toxicity of *Euphorbia milii* latex and synthetic molluscicides to *Biomphalaria glabrata* embryos. *Chemosphere*, 81(2), 218-227. <http://doi.org/10.1016/j.chemosphere.2010.06.038>
- [14] Pattanagul, W., & Thitisaksakul, M. (2008). Effect of salinity stress on growth and carbohydrate metabolism in three Rice (*Oryza sativa* L.) cultivars differing in salinity tolerance. *Indian Journal of Experimental Biology*, 46(10), 736-742.
- [15] Rollinson, D., Knopp, S., Levitz, S., Stothard, J. R., Tchuem Tchuenté, L. A., Garba, A., ... Utzinger, J. (2013). Time to set the agenda for schistosomiasis elimination. *Acta Tropica*, 128(2), 423-440. <http://doi.org/10.1016/j.actatropica.2012.04.013>
- [16] Santos, M. J. G., Ferreira, N. G. C., Soares, A. M. V. M., & Loureiro, S. (2010). Toxic effects of molluscicidal baits to the terrestrial isopod *Porcellionides pruinosus* (Brandt, 1833). *Journal of Soils and Sediments*, 10(7), 1335-1343. <http://doi.org/10.1007/s11368-010-0246-y>
- [17] Seto, E. Y. W., Wong, B. K., Lu, D., & Zhong, B. (2011). Human schistosomiasis resistance to praziquantel in China: Should we be worried? *American Journal of Tropical Medicine and Hygiene*, 85(1), 74-82. <http://doi.org/10.4269/ajtmh.2011.10-0542>
- [18] Soukhathammavong, P., Odermatt, P., Sayasone, S., Vonghachack, Y., Voumatsou, P., Hatz, C., ... Keiser, J. (2011). Efficacy and safety of mefloquine, artesunate, mefloquine-artesunate, tribendimidine, and praziquantel in patients with *Opisthorchis viverrini*: A randomised, exploratory, open-label, phase 2 trial. *The Lancet Infectious Diseases*, 11(2), 110-118. [http://doi.org/10.1016/S1473-3099\(10\)70250-4](http://doi.org/10.1016/S1473-3099(10)70250-4)
- [19] Sukumaran, D., Parashar, B. D., Gupta, a. K., Jeevaratnam, K., & Prakash, S. (2004). Molluscicidal effect of nicotinamide and its intermediate compounds against a freshwater snail *Lymnaea luteola*, the vector of animal schistosomiasis. *Memorias Do Instituto Oswaldo Cruz*, 99(2), 205-210. <http://doi.org/10.1590/S0074-02762004000200016>

- [20] Van Gaest, A. L., Young, C. M., Young, J. J., Helms, A. R., & Arellano, S. M. (2007). Physiological and behavioral responses of *Bathynereis naticoidea* (Gastropoda: Neritidae) and *Methanorhynchus dendrobranchiata* (Polychaeta: Orbiniidae) to hypersaline conditions at a brine pool cold seep. *Marine Ecology*, 28(1), 199–207. <http://doi.org/10.1111/j.1439-0485.2006.00147.x>
- [21] Wang, W., Wang, L., & Liang, Y. S. (2012). Susceptibility or resistance of praziquantel in human schistosomiasis: A review. *Parasitology Research*. <http://doi.org/10.1007/s00436-012-3151-z>
- [22] Yadav, R. P., & Singh, A. (2013). Toxic Effect of two Common Euphorbiales against freshwater target snail. *New York Science Journal*, 6(6).

IJIRAS