BIOACTIVITY OF *SYNADENIUM GLAUCESCENS* (PAX) EXTRACTS ON HELMINTH EGGS AND LARVAE FROM WASTEWATER STABILIZATION PONDS IN MOROGORO MUNICIPALITY, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULLFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN NATURAL PRODUCTS TECHNOLOGY AND VALUE ADDITION OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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ABSTRACT

The increased use of wastewater and sludge from wastewater stabilization ponds in agriculture predisposes the consumers to the health risks. The objective of this study was to evaluate the bioactivity of *Synadenium glaucescens* (Pax) extracts on helminth eggs and larvae from wastewater and sludge in Morogoro. Helminth eggs and larvae were recovered using Bailenger and Baerman methods, and then quantified using McMaster techniques. Extracts (*S. Glaucescens*) used during hatchability and larvicidal bioassays were obtained using hot and cold solvent extraction. The effect of *S. glaucescens* extracts on hatchability and larvicidal was tested using *in vitro* and *in vivo* methods. One litre of wastewater was collected from anaerobic, facultative and maturation ponds. One hundred grams of sludge were sampled from the ponds and piles. Lethal concentration fifty (LC_{50}) and inhibitory concentration fifty (IC_{50}) were used to determine larvicidal and hatchability effects. The identified helminth eggs were from nematodes including *Ascarid, Strongylid* and *Trichuris.* Minimum amount of ≤ 1 e.p.l. were found in wastewater sampled from maturation ponds, same amount recommended by WHO guideline of 2006, and a maximum of 700 e.p.l. from anaerobic ponds. It was found that sludge samples contained a minimum of ≤ 1 e.p.g. from maturation ponds and maximum of 100 e.p.g. in anaerobic pond. The ethanol extracts of root bark and leaves were the most effective with IC_{50} 19.34 and 39.56 $\mu g ml^{-1}$. The two extracts also demonstrated the highest LC_{50} of 19.41 and 30.19 $\mu g ml^{-1}$ respectively. The root bark extracts were more active than leaves extracts. This study demonstrated a high potential of using *S. glaucescens* extract in controlling helminths in wastewater and sludge.

DECLARATION

I, Salum Hassan, do hereby declare to the senate of Sokoine University of Agriculture that, this dissertation is my own work, done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

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(MSc. Candidate)

The above declaration is confirmed

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DEDICATION

This work is dedicated to my parents, my late mother Martha Lazaro Ghulla; my father Hassan Mkenga Msangi; my lovely wife Juliana and my sons Sele and Gift Mkenga; my sisters Fatuma and Hafsa and my brother Hussein Msangi.

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LIST OF ABREVIATION

BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DANIDA	Danish International Development Agency
DO	Dissolved Oxygen
IC_{50}	Inhibitory Concentration Fifty
LC_{50}	Lethal Concentration Fifty
LCE	Leaves extract- Complete extraction - Ethanol
LCW	Leaves extract - Complete extraction - Water
LSD	Leaves extract - Sequential extraction - Dichloromethane
LSE	Leaves extract - Sequential extraction - Ethanol
RCE	Root barks extract - Complete extraction - Ethanol
RCW	Root barks extract - Complete extraction - Water
RSD	Root barks extract - Sequential extraction - Dichloromethane
RSE	Root barks extract - Sequential extraction - Ethanol
SUA	Sokoine University of Agriculture
WSP	Wastewater Stabilization Pond

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Wastewater and sludge from Wastewater Stabilization Ponds (WSPs) are used for agricultural related activities around the world. The major reason that propagates the continued use of wastewater from WSPs is the increased human population and rapid urbanization which results in increased pressure on fresh water resources (Jiménez-Cisneros, 2007). Lack of quality water and high level of water demand in urban and peri urban areas leads to water scarcity and consequently drives the use of non-conventional water such as urban wastewater.

Urban and peri urban irrigation agriculture sector is one of the major wastewater users. Wastewater serves as a cheap source of water for irrigation. It has some of the essential nutrients required by plants thus can represent a reduction on the fertilizer requirement and hence associated costs. However, wastewater has to be properly managed to minimize environmental and health risks (FAO/UNW-DP/UNU-INWEH, 2011).

Wastewater from WSPs is a combination of one or more of the following: domestic effluent consisting of black water (excreta, urine, and faecal sludge) and gray water (kitchen and bathing wastewater); water from commercial establishment and institutions, including hospitals; industrial effluent; storm water and other urban run-off (Raschid-Sally and Jayakody, 2008). Sludge on the other hand is the general term for the undigested or partially digested slurry or solids that results from the storage or

treatment of the black water in so called on site sanitation systems such as septic tanks, latrines, toilet pits, dry toilets, non sewered public toilets and aqua privies.

Wastewater stabilization ponds (WSPs) are shallow basins that use natural factors such as biodegradation, sunlight, temperature, sedimentation, predation and adsorption to treat wastewater (Mara, 2004). Conventionally, they consist of anaerobic, facultative and maturation ponds arranged in series. Wastewater stabilization ponds function mainly as an alternative for treating wastewater. In the process, pathogenic organisms such faecal coliform bacteria and helminths are removed (Curtis *et al.*, 1992). Helminth eggs are removed by sedimentation and thus remain in the pond sludge. However, when a series of ponds are used most helminth eggs are retained in the first pond and remain viable for several years in the pond sludge. Studies by Nelson *et al*. (2004) from a survey of several WSPs in Mexico reported a content of 14 viable eggs per gram in sludge stored at least for nine years. Direct use of wastewater from sewage outlet occurs when it is directly disposed off on land from WSPs where it is used for crop production. Indirect use of untreated urban wastewater occurs when water from rivers receiving urban wastewater is abstracted by farmers downstream of the urban centres for agriculture (Raschid-Sally and Jayakody, 2008; Jiménez and Asano, 2008).

The use of wastewater from WSPs in agriculture may have negative impacts to human health due to contaminants such as heavy metal, organic pollutants and pathogenic microorganisms including helminths (Mokhtari *et al.*, 2012).

Helminths are parasitic worms and some are zoonotic pathogens (i.e. transmitted naturally between species) of humans and livestock. Generally, helminthiasis is one

of the major problems in developing countries, particularly in areas with poor sanitation due to inadequate management and disposal of effluent, polluted crops, water and food that when ingested serve as media for transmitting helminths (Jiménez and Maya, 2007). In 1989, the World Health Organization (WHO) declared that presence of helminth eggs in wastewater and sludge as a major cause of diarrhoeic diseases. This is because; over a third of the world's population is infected with helminths (de Silva *et al*., 2003; Bethony *et al*., 2006). Helminthiasis in the human population represents a strain to the country's limited financial resources. Attempts to develop effective methods for control of helminthiasis in developing countries have yielded unsatisfactory results (Jiménez-Cisneros, 2007). Although helminths are not microscopic organisms, their eggs are microscopic and serve as a source for infective agents for helminthiasis, when discharged to the environment in excreta. The oral faecal route is the main dissemination pathway of the disease (Jiménez and Maya, 2007). According to WHO (2006), helminths causing waterborne diseases can be grouped into nematodes (round worms), trematodes (flukes) and cestodes (tape worms).

Several studies have been done to document traditional uses and treatments of medicinal plants in Tanzania (Maine *et al.,* 2012; Kitula 2007; Maregesi *et al.,* 2007; Augustino *et al.,* 2012; Amri and Kisangau, 2012). Their compounds have shown interesting anticancer, antimalarial, antileishmanial, antimicrobial and Anti-HIV bioactivity (Magadula and Erasto 2009). *Synadenium glaucescens* (Pax) which belongs to euphobiaceae family is used in the preparation of various traditional medicines, besides being poisonous and perceived as of no therapeutic value

(Mabiki, *et al.* (2013a), ethnic communities in Tanzania have used it effectively in the treatment and control of human and animal diseases (including helminthiasis) with no reported adverse effects (Mosha *et al.,* 2002). Cytoxicity studies on *Synadenium glaucescens* (Pax) using brine shrimp lethality test, tested that all the extracts from the plant have lethal concentration (LC_{50}) of less than 30 μgml^{-1} which could indicate the pesticidal capacity of the plant (Mabiki *et al.*, 2013a) the fact of which is potential to environmental hygiene.

1.2 Problem Statement and Justification

1.2.1 Problem statement

Plants are an indispensible source of chemicals useful for prevention and control of both human and animal diseases (Mosha *et al.,* 2002) including helmithiasis. However, due to their unknown potentials and lack of scientific evidence for their effectiveness against helminths in water and sludge in Tanzania, uses of such botanicals are limited. Natural products extracted from *S. glaucescens* (Pax) have effectively been used to control diseases in humans and domestic animals in Tanzania (Mabiki *et al.*, 2013a). However, *S. glaucescens* (Pax) bioactivity has not been scientifically validated for its potential for controlling helminths in wastewater and sludge.

1.2.2 Justification of the study

Lack of control methods including use of botanicals in helminth eggs has led to increased magnitude of helminths infection to both producers and consumers of vegetables grown using wastewater and sludge. This study therefore will provide

empirical information on ovicidal and larvicidal efficacy of *S. glaucescens* (Pax) and; quantities and characteristics of helminths eggs and larvae in wastewater and sludge in Mafisa wastewater stabilization ponds. Findings from this study will be useful to different stakeholders (vegetable producers and consumers, health officials, agricultural extension staffs and, water and sewerage Authority in Morogoro Municipality) to control health risks that may result from use of untreated wastewater and sludge.

1.3 Objectives

1.3.1 Main objective

To evaluate the bioactivity of *S. glaucescens* (Pax) for control of helminth eggs and larvae in wastewater and sludge in Mafisa WSPs, Morogoro Municipality.

1.3.2 Specific objectives

i. To characterize helminth eggs in wastewater and sludge from Mafisa wastewater stabilization ponds.
ii. To quantify helminth eggs in wastewater and sludge from Mafisa wastewater stabilization ponds.
iii. To determine the bioactivity of *S. glaucescens* (Pax) on helminth eggs and larvae.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Wastewater/Sludge Use and Helminthiasis

The most common diseases associated with wastewater and excreta/sludge are diarrhoea and helminth infections. Helminthiasis is common where poverty and poor sanitary conditions prevail; and under these conditions they can affect up to 90% of the population (Bratton and Nesse, 1993). Helminthiasis is frequently linked to use of wastewater and sludge or excreta in Agriculture (Navarro *et al*., 2009) and may be transmited through the ingestion of helminth eggs (from contaminated crops) of a wide variety of pathogenic worms (Jiménez, 2009). Some helminth eggs are considered to be the most resistant biological particles such that they cannot be inactivated with chlorine, UV light or ozone (Jiménez, 2007a). Farmers and irrigation workers can acquire helminth infections and parasitic diseases due to direct contact with polluted sludge, faeces, untreated wastewater and contaminated soils, especially if exposed for long duration of time (Ensink, 2006). Ascariasis is the most common disease and is endemic in Africa, Latin America and Far East. Even though helminthiasis has a low mortality rate, most of the people affected are children under 15 years old with problems of faltering growth and impaired fitness (Jiménez *et al*., 2010). It is estimated that approximately 1.5 million of these children never attain expected growth, even after treatment (Silva *et al.*, 1997).

Use of wastewater and dried sludge as fertilizers is associated with contamination of vegetables with helminths eggs (Gupta *et al.*, 2009; and Amin, 1988). The market survey by International Water Management Institute (IWMI) in Kumasi, Accra and

Tamale showed that it is very difficult to find any irrigated vegetables by wastewater (e.g lettuce, spring onions, cabbage) without contamination with helminth eggs and faecal coliforms (Keraita and Drechsel, 2004). Studies conducted by Pescod (1992) and Ensink *et al.* (2008) on farmers' exposure to untreated wastewater, revealed high incidence of helminths infections while the use of partially treated wastewater was associated with high incidences of *Ascaris. lumbricoides.*

2.2 Helminth Eggs from Wastewater and Sludge

Ayres and Mara (1996) conducted a study with wastewater and were able to identify *Ascaris lumbricoides* (the human roundworm), *Trichuris trichiura* (the human whipworm), and *Ancylostoma duodenale* and *Necator americanus* (the human hookworms). Also Mahvi and Kia (2006) identified Tapeworm (*Taenia spp)* when assessing the type and load of helminth in wastewater in Tehran. Studies done in France, both *Ascaris* and *Trichuris* eggs were recovered from sludge and in Barcelona – Spain, helminth eggs concentrations varied from 20 to 340 eggs per kg sludge were also reported (Schwartzbrod *et al*., 1989). Generally, *Ascaris* eggs are more common in wastewater and sludge than eggs of *Trichuris* and hookworm, probably because the female worm of *Ascaris* can produce up to 200,000 eggs per day (Sengupta, 2012; Soulsby, 1986). The concentration of *Ascaris* eggs in raw wastewater varies from 10-100 eggs per litre in endemic areas to 100 – 1000 eggs per litre in hyper endemic areas (Mara and Sleigh, 2009).

2.3 Control of Helminth in Wastewater and Sludge Using Medicinal Plants

The use of medicinal plants for decontaminating wastewater is reported in literature. Plants such as *Ocimum sanctum*, *Azadrachta indica*, and *Moringa oleifera*, were reported to be used in different ways to control helminths from wastewater (Somani *et al.*, 2011; Ghebremichael, 2004; Aquino and Teves, 1994). Sengupta (2012), reports on the use of *Moringa oleifera* seeds extract to assess the potential for reducing helminth eggs and turbidity in irrigation water, turbid water, tape water and wastewater. It was concluded that *Moringa oleifera* seed extracts were effective in reducing the number of helminth eggs by 94-99.5% to 1-2 eggs per litre. Ukwubile (2012), studied anthelmintic activity of some plants including *Allium sativa, Zingiber officinale, Annona senegalensis* concluded that all showed good anthelmintic activities in dose dependent manner.

The use of medicinal plant to control helmithiasis in human and animal is a common practice in Tanzania. *Albizia anthelmintica* is a popular medicinal plant among the Maasai that use it extensively for the control of worms, not only in man but also in livestock. *Albizia anthelmintica* is among the priority medicinal trees in Shinyanga Region and Simanjiro District (Minja and Allport, 2001) in Manyara Region – Tanzania for control of helminths infection. Dhar *et al.* (1965) established anthelmintic property of aqueous extract of seeds of *Carica papaya* against *Ascaris lumbricoides* and *Ascaridia galli*. In another study, Srivastava *et al.* (1967) found that aqueous and ethanoic extracts of *Cucurbita mexicana* seeds exhibited significant anthelmintic activity against *Ascaris lumbricoides, Moniezia expensa* and *Hymenolepsis duminata*. In that study aqueous extract was found to possess most

significant efficacy as compared to ethanoic extract. In an assey of inhibition of transformation of eggs to filariaform larvae of *Haemonchus contortus,* Prakash *et al.* (1980) established the dose dependent anthelmintic activity of alcoholic extract of *Punica granatum*.

Synadenium glaucescens (Pax) which belongs to euphobiaceae family is used in the preparation of various traditional medicines, besides being poisonous and perceived as of no therapeutic value (Mabiki, *et al.* (2013a), ethnic communities in Tanzania have used it effectively in the treatment and control of human and animal diseases with no reported adverse effects (Mosha *et al.,* 2002). It is a bush or shrubby tree, up to 9 m which grows in sandy stony and rocky slopes with dry deciduous woodland, altitude 300-1800 m above sea level (Bruyn *et al*., 2006). It is known as "*Mvunjakongwa"* in Swahili. It is endemic and grows in several regions in Tanzania (Mosha *et al*., 2002). In Tanga region it is used for control of poultry diseases mainly Newcastle disease (Wickaman *et al.,* 2006). The latex is also used as a fish poison (Neuwinger, 2004). Excessive use of its concoctions for purgative purposes, cause poisoning and even death. Its latex cause irritation and can causes blindness (Mosha *et al.,* 2002). Previous cytoxicity studies using brine shrimp lethality test, established that extracts from the plant have lethal concentration (LC_{50}) of less than 30 μgml^{-1} which could indicate the pesticide capacity of the plant (Mabiki *et al.*, 2013a). Furthermore Mabiki *et al.* (2013b, c) reported that *S. glaucescens* (Pax) extracts demonstrated potential and feasibility for treatment and control of viral diseases such as infectious bursal disease virus (IBDV), fowl pox (FPDV) and Newcastle disease (ND) in chicken. Despite the facts that *S. glaucescens* (Pax) have been used for

various purposes little is known on the use of the plant in controlling pathogens (helminth) in wastewater and sludge for agricultural use which was the focus of this study.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of Wastewater and Sludge Sample Collection Area

The field data were collected at Mafisa in Morogoro Municipality. Morogoro Municipality has the sewerage system connected to WSPs at Mafisa (shown in Fig. 1a). The total length of sewer is approximately 30 km of which 9.7 km is the main sewer and the rest (20.51 km) are laterals. Seven hundred customers have been connected to the Morogoro sewer system (MORUWASA, 2012). It is the largest well kept waste stabilization system with seven ponds that collect sewage from various sources. The amount of wastewater generated at Mafisa is 28693.60 litres and the capacity of WSPs is 57908 litres (MORUWASA, 2012). Quality parameters of influent and effluent of wastewater at Mafisa are shown in Table 1:

Table 1: Quality parameters for influent and effluent of wastewater at Mafisa, Morogoro Municipality

	pH	DO(mg/l)	BODs $20^{o}C$ (mg/l)	COD (mg/l)	Colour (mgpt/l)
Influent	7.30	1.60	44.10	88.13	70.00
Effluent	7.90	0.30	60.40	127.00	190.00

Source: MORUWASA (2012)

3.2 Description of Plant Sample Collection Area

Fresh plant samples i.e leaves and root barks of *Synadenium glaucescens* (Pax) were collected from Njombe region Wangin'gombe district Mahongole ward in Mtulingala village. The village is located within altitude of 1650 m and 1950 m above sea level in Southern Highlands of Tanzania.

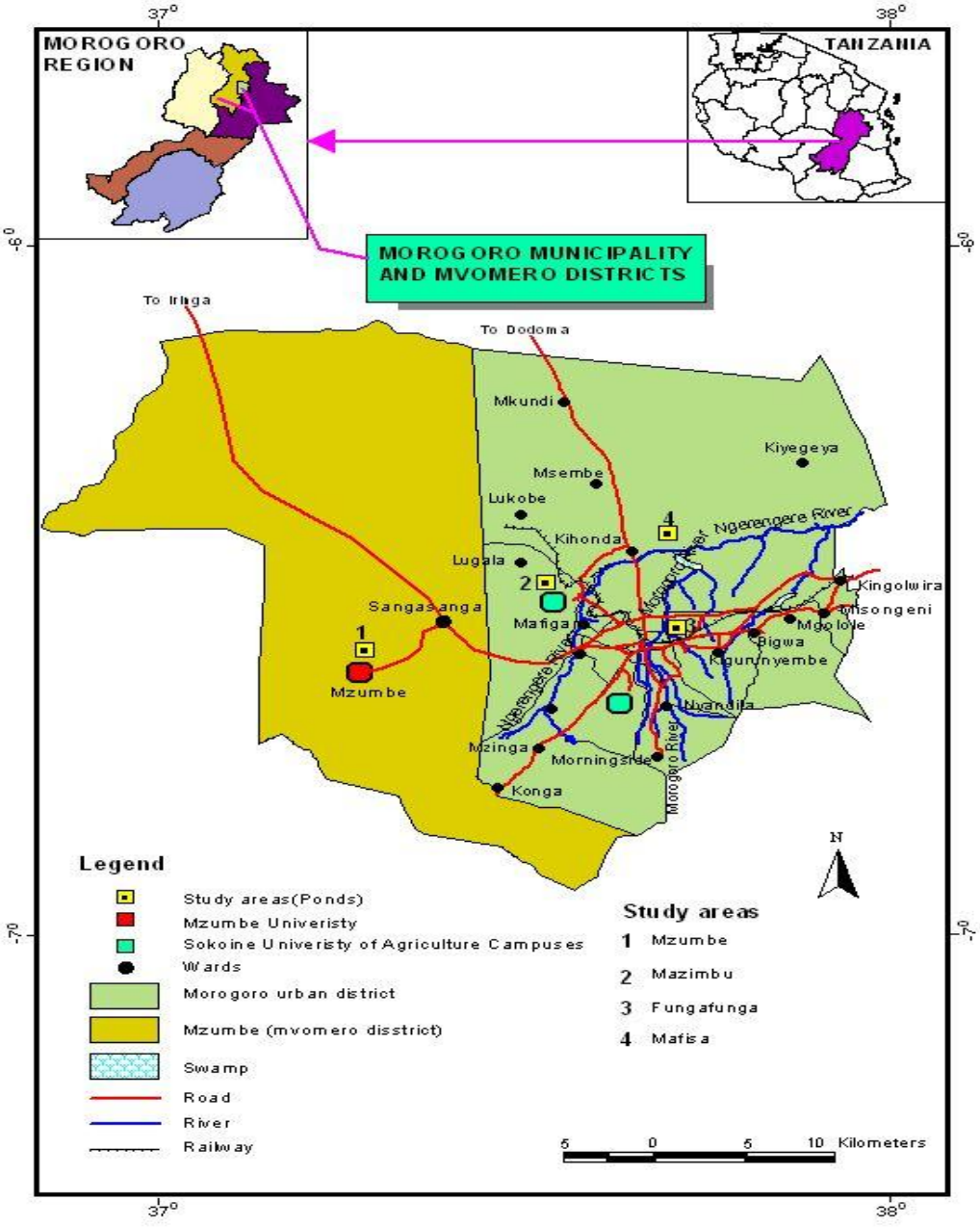

Figure 2a: Morogoro urban district describing study area (Mafisa SSP)

3.3 Sample Coding

Samples and extracts were coded as shown in the Tables 2 and 3.

Table 2: Codes of samples and collection points used during the study

Ponds Codes		**Sample source codes**		**Eggs/larvae codes**	
Ponds	**Codes**	**Sample source**	**Codes**	**Eggs/larvae**	**Codes**
Receiving pond by gravity	**Rpg**	Wastewater	**wastewater**	Ascarid eggs	**As**
Receiving pond by trucks	**Rpt**	Sludge	**sl**	Strogylid eggs	**St**
Anaerobic pond	**Ap**	Goats	**gt**		
Facultative pond	**FAp**	Chickens	**ch**		
1st Maturation pond	**Mat_1**				
2nd Maturation pond	**Mat_2**				
3rd Maturation pond	**Mat_3**				
4th Maturation pond	**Mat_4**				
Effluent	**Efl**				

Table 3: Codes of extracts used during the study

Plant part codes		**Extraction codes**		**Solvent codes**	
Plant part	**Code**	**Extraction**	**Code**	**solvent**	**Code**
Leaves	**L**	Total/Complete	**C**	Water	**W**
Root bark	**R**	Sequential	**S**	Ethanol	**E**
				Dichloromethane	**D**

3.4 Study Design

This study adopted experimental study design in the form of both observation (for objectives 1&2) and manipulation (for objective 3). Samples (collection elaborated from section 3.6.1 to 3.6.6) were taken to the laboratory for: preparation and observation under microscope; and extraction of *S. glaucescens* (Pax) crude extract. Collection was done purposively based on availability of plant species and Mafisa-WSPs being the only WSP system available in Morogoro Municipality.

3.5 Materials

3.5.1 Equipments

The equipments used in this study included screw cap bottles, Disposable plastic sleeves/gloves, Plastic bags, Grab sampler, Tea strainer, Sieves of different size (25-150 micrometers), Microscope, microscope slides, cover slips, Test tubes, beakers, Spoon, measuring cylinder, Mc Master slides, Pasteur pipette, Micropipette, Micro plates, small Petri dishes, Motor and Pestle, Sedimentation flasks, Bench centrifuge, Incubator, Soxhlet apparatus, Rotary evaporator, Weighing balance, Oven, Grinding machine and Working gears.

3.5.2 Chemicals and reagents

All chemicals used including solvents were analytical grade and purchased from university suppliers. These include Ethanol, Dichloromethane, Dimethyl sulphoxide, Floatation fluid, Tween 20, Distilled water and Iodine.

3.6 Methods

The methodology of this study followed the flow chart as shown in Figure 1b.

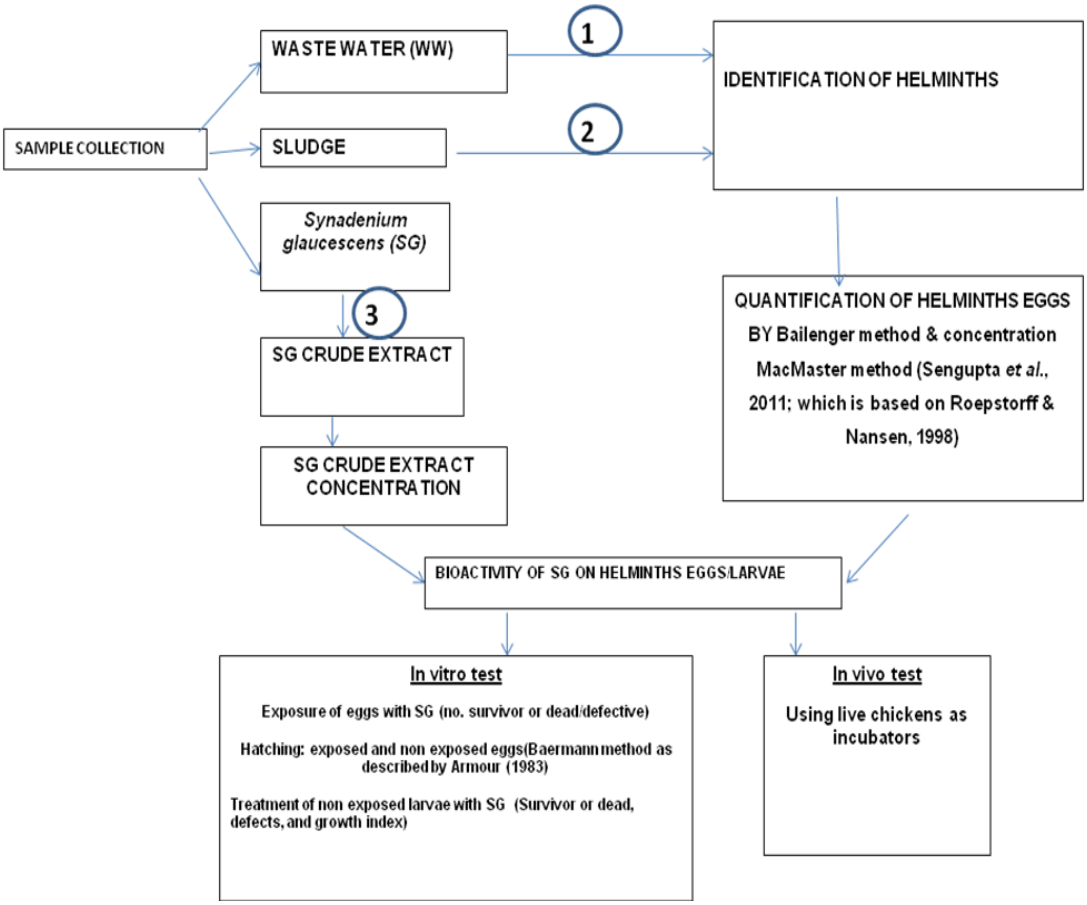

Figure 1b: Methodology flow chart

Key

1: Modified Bailenger method by Ayres and Mara (1996) and Sengupta *et al*. (2011)

2: Hansen and Perry method (1994)

3: Solvent extraction (Water, DCM and Ethanol).

3.6.1 Collection of wastewater and sludge samples

Figure 2 describes the set up of ponds at Mafisa WSP, movement of influent through the ponds to end pipe outlet and sites where wastewater and sludge samples were collected.

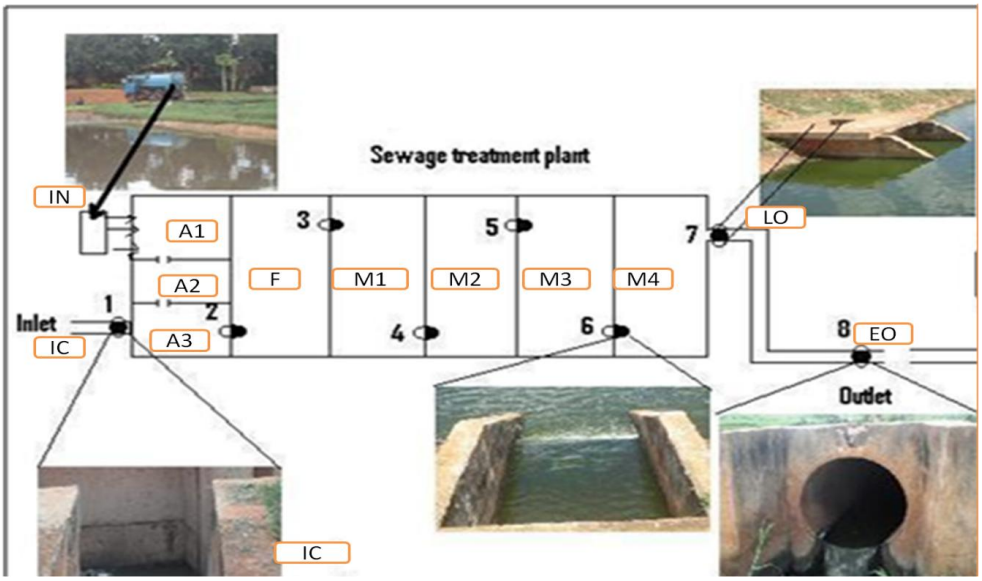

Figure 2: Mafisa WSP showing set up of ponds and sites of sample collection

(IN): inlet not connected to Municipal sewerage system; (**IC)**: inlet connected to Municipal sewrage system; (**A1-3)**: anaerobic ponds; (**F)**: facultative anaerobic pond; (**M1-4)**: maturation ponds; (**LO)**: Last ponds' immediate outlet; (**EO)**: end pipe outlet; (**1-6)**: inlets to the ponds; (**7&8)**: outlets

A total of 72 litres wastewater (One litre from each pond i.e. 2 anaerobic ponds, facultative anaerobic, maturation ponds 1-4, and effluent) were collected in three times a days for three days. In each day three samples were collected at an interval of five (5) hours (1L in Morning at 09.00 hrs, 1L in afternoon at 13.00 hrs and 1L in evening at 18.00 hrs). For sludge one hundred (100) grams of sludge were collected from each pond and from piles of sludge waiting to be taken to the farms. Figure 3 shows collection of wastewater and sludge at Mafisa WSPs.

Figure 3: Collection of wastewater and sludge

(A): anaerobic pond-truck inlet; (**B)**: anaerobic pond-gravity inlet; (**C)**: facultative pond-outlet; (**D)**: sludge-anaerobic pond outlet; (**E)**: sludge piles; (**F)**: sludge samples

3.6.2 Preparation, parasitological examination, identification and quantification of wastewater samples

Wastewater sample preparation for parasitological examination, identification and quantification of helminth eggs was based on protocols of Bailengers method (Ayres and Mara, 1996) and concentration McMaster method (Sengupta, 2012). In which wastewater samples were allowed to sediment for two (2) hours in the sedimentation flasks, then 90% of the supernatant were removed. Sediments were carefully transferred to a well rinsed (with 0.01% Tween 20 detergent) centrifuge (50ml) tubes and centrifuged at 168g (1000 rpm for 15 minutes). The supernatant for each individual sample was removed and all sediments were transferred to one tube and re-centrifuged at 168g. The supernatant was discarded and to the remaining pellet, floatation fluid (NaCl- glucose) was added to the tube up to 4ml. The sample was mixed, and then aliquot was quickly removed by pasture pipette and transferred to McMaster slide for examination. Both chambers of McMaster slide were filled and left to stand on a flat surface for five minutes to allow all eggs to float to the surface before examination. McMaster slides were then placed on microscope stage, each at a time, and examined under 10× and 40× magnification. Quantification was done by counting eggs within the grid lines of both chambers of McMaster slide, and then the means count were recorded. Calculation of the number of eggs per litre was done using equation 1:

$$N = AX/PV \quad \text{.....Equation 1}$$

Where:

N = number of eggs per litre of sample

A = number of eggs counted in the McMaster slide or the mean of counts from slides

X = volume of the final product (ml)

P = volume of the McMaster slide (0.3 ml)

V = original sample volume (litre).

3.6.3 Preparation, parasitological examination, identification and quantification of sludge samples

Sludge sample preparation method for parasitological examination, identification and quantification of helminth eggs was adopted from Hansen and Perry (1994) protocol with some few modifications.

Four grams of sludge sample were transferred into a beaker 1, and then 56 ml of floatation fluid was poured into it. The content was stirred thoroughly and the resultant sludge suspensions were poured through a sieve into second beaker and left to stand for10minutes. In order to extract sample of helminth identification, a test tube was pressed to the bottom of the filtrate then lifted some filtrate adhered to the surface of the test tube and was transferred to the micro slide as it dripped off the test tube surfaces. The cover slip was mounted on the micro slide for microscopic examination. Filtrate from the second beaker was stirred and a sample extracted with Pasteur pipette into both counting chambers of the McMaster slide, this was left to stand for 5minutes, and then examined under a microscope at 10×10 magnification for quantification. Eggs were counted within the engraved area of both chambers. The numbers of eggs per gram of sludge were then calculated using equation 2:

$$N = (A1 + A2)\ 50 \quad \text{.....Equation 2}$$

Where:

N = number of eggs per gram of sample

A1= number of eggs counted in first chamber of McMaster slide

A2= number of eggs counted in second chamber of McMaster slide

Figure 4 shows preparation of samples, from both wastewater and sludge, and examination of helminths eggs and larvae at helmintholoy laboratory at SUA.

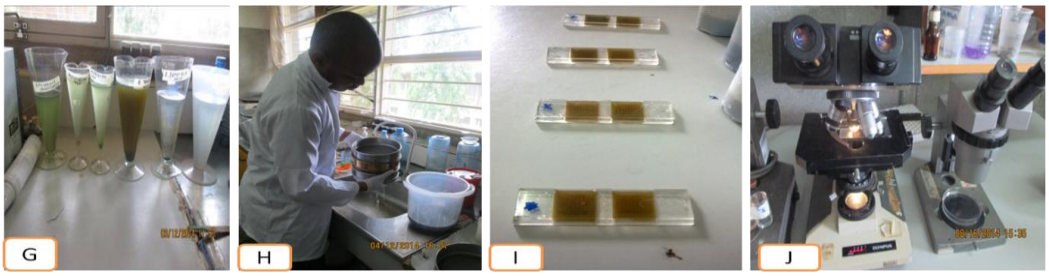

Figure 4: Preparation of samples and examination of helminths eggs and larvae (G):wastewatersamples; (**H**)sieving sludge samples; (**I)**:McMaster slides with samples; (**J)**:microscopic observation.

3.6.4 Collection, preparation and examination of faecal sample from goats

Helminth eggs obtained from wastewater and sludge were not sufficient to carry out the test for antihelminthic activity of *S. glaucescens* (Pax) plant. This challenge necessitated the use of alternative sources of strogylid eggs. In view of that, helminths were collected from goat for antihelminthic study.

Fresh faecal sample were collected from rectum of goats with clinical signs of worm infection. McMaster technique was used to determine egg counts. The samples were weighed to 3g each and processed for parasitological examination using Hansen and Perry (1994) protocol, and then worm eggs were counted. Faecal samples with high number egg count were pooled together and thoroughly mixed. A small portion of the mixture was grounded for culture that was used for identification of the parasite larvae to genus level. The remaining mixture of faeces was used to recover eggs that were then used for *in vitro* efficacy trials. Figure 5 shows preparation of faecal culture and recovery of larvae at helminthology laboratory at SUA.

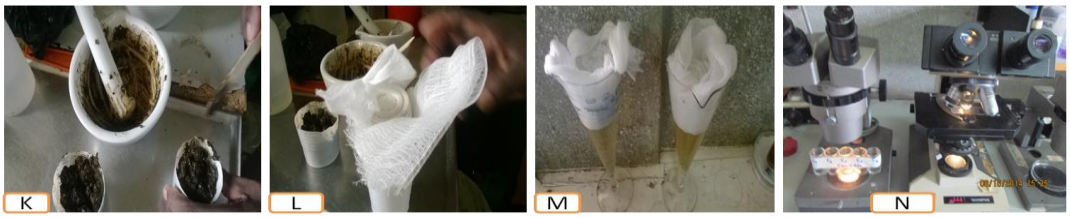

Figure 5: Preparation of faecal culture and recovery of larvae

(K):prep.of faecal sample; (**L)**: culturing faecal sample; (**M)**:recovering larvae; (**N)**:identification of larvae

3.6.5 Collection, preparation and examination of worms (*Ascaridia galli*) from local chickens

Helminth eggs obtained from wastewater and sludge were not sufficient to carry out studies for testing the antihelminthic activity of *S. glaucescens* (Pax) plant. This challenge necessitated use of alternative sources of ascarid eggs from chickens to carry out antihelminthic activity of *S. glaucescens* (Pax) extracts.

Worms (*Ascaridia galli*), were collected from small intestine of local chicken brought from Dodoma and Morogoro Municipal local markets. After collection the intestine were cut open 'run' out into a tray. Worms were collected, identified, counted and placed into normal saline solution for 48 hours to lay eggs as in adopted techniques for post-mortem differential parasite counts by Hansen and Perry (1994). The sample preparation for parasitological examination was done based on protocols of modified Bailengers' method (Ayres and Mara, 1996) and modified concentration McMaster method (Sengupta, 2012) explained on section 3.6.2 above. The samples were coded as indicated in Table 2. Figure 6 shows collection of *Ascaridia galli* from intestine of local chicken at Dodoma local market.

Figure 6: Collection of *Ascaridia galli* from intestine of local chicken

(N):slaughtering chickens; (**O)**:collecting chickens'intestines; (**P)**:*Ascaridia galli* from chickens'intestine

3.6.6 *Synadenium glaucescens* (Pax) samples collection, processing and extraction

3.6.6.1 Plant sample processing

Fresh plant sample of *S. glaucescens* were collected from Mutulingala village and air-dried under the shade and grinded by a machine to 1mm diameter particle size for use during extraction. The samples were coded as indicated in Table 3 (page 12).

3.6.6.2 Cold complete extraction

Cold extraction was aimed at targeting heat labile compounds using ethanol and water. Samples of the root barks and leaves were soaked in water and ethanol for 48 hours at room temperature. Then filtration was done using whatman no.1 filter paper. Extracts were then concentrated using rotary evaporator at reduced pressure for ethanol and air dried for water samples then finally both samples were dried in air circulating oven for 24 hours. The concentration 500 μgml^{-1} was made as a stock solution using distilled water as solvent. From stock solution, seven concentrations were made following the dilution law 500 μgml^{-1} was the highest concentration and 7.8125 μgml^{-1} was the lowest concentration to be tasted for efficacy. The samples were coded as indicated in Table 3 (page 12).

3.6.6.3 Soxhlet Extraction

Ten grams (10 g) of dry ground sample (sieved through 1mm diameter mesh) was placed in thimbles (33 mm, 80 mm) in the extraction chamber and extracted using a common soxhlet apparatus consisting of a condenser, soxhlet chamber and an extraction flask. Sequential extraction with 250 ml dichloromethane (DCM) and

ethanol was done. Extraction time of 4 hours at a temperature of 30^{o}C for DCM and 70^{o}C for ethanol was employed. Liquid extracts obtained after filtering with whatman No.1 filter paper were concentrated using the rotary evaporator at reduced pressure and finally dried to obtain dry crude extracts. Root bark and leaf extracts were dissolved in distilled water to make a stock solution of which was further diluted to make concentration range from 500 µgml^{-1} (stock solution) to 7.8125 µgml^{-1} (lowest concentration). The samples were coded as indicated in Table 3. Figure 7 (a and b) show plant sample collection, drying, grinding and extraction.

Figure 7 (a) Plant sample collection, drying and grinding

(Q);*synadenium glaucescens* plant; (**R)**:*s.glaucescens* leaves; (**S)**:*s.glaucescens* rootbarks; (**T)**:harvested leaves; (**U)**:drying leaves; (**V):**dried leaves; (**W)**:grinding samples; (**Y)**:meshing samples; (**Z)**:weighing samples

Figure 7 (b) Extraction processes at natural products; mazimbu chemistry; and food science laboratories

(a): soaking samples; **(b)**: filtering samples; (**c)**: extraction (soxhlet apparatus); (**d)**: evaporating solvent (rotary evaporator); (**e)**:drying crude extract (air circulating oven); (**f)**: sample storage for use

3.6.7 Bioactivity assays

3.6.7.1 *In-vitro* hatchability test using strogylid eggs from goats

Bottom flattened 96 wells micro plates were used to incubate eggs for *in-vitro* test. A hundred micro litres (100 µl) of distilled water was added into each well of micro plate from the second well of plate to seventh well. A hundred micro litre of test extract at concentration of 500 $\mu g ml^{-1}$ was added in the first well to make 200 µl of solution. After mixing 100 µl of the resulting solution was transferred the next well where it was mixed again with distilled water to further dilute the test extract to a half. This was done repeatedly up to the 7^{th} well and the remaining 100 µl were discarded. Distilled water with no extracts served as a negative control. Twenty micro litres (20 µl) of egg suspension containing about 50 eggs per 20 µl was added

to each well with test extract and the control. Both tests and control were incubated at 27^{o}C for 48 hours. To determine the hatchability of the eggs and live hatched larvae were examined under the compound microscope after 48 hrs for each well. The hatched larvae were observed under the microscope for six (6) more days to study the effect of exposure to extract by recording the number of dead larvae after 24 hours interval. Figure 8 shows *in vitro* egg hatching assay using strogylid eggs from goats.

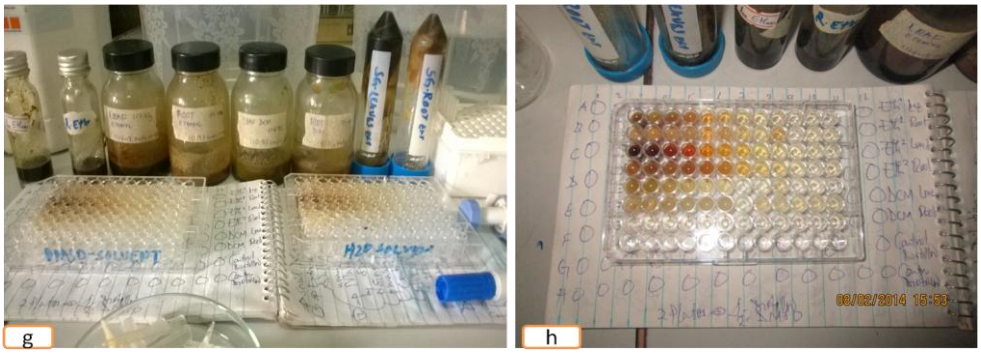

Figure 8: *In vitro* egg hatching assay

(g): making different concentrations; **(h)**: exposing eggs to extracts

3.6.7.2 *In-vitro* larvicidal test using 3 rd stage larvae from strogylid helminth eggs of goats

Fresh faecal sample was collected from rectum of goats based on initial surveys of investigation of clinical disease caused by intestinal nematodes. Preparation of faecal culture, identification and recovery of larvae for larvicidal test of extracts was done based on the protocol of Baermann method as described by Hansen and Perry (1994) with some modification. In this protocol, faecal sample were finely broken up and placed in sterile vermiculate mixed well and then wrapped in a gauze. Culturing was

made by hanging the wrapped content into a beaker containing water for supplying humidity at room temperature. The cultured eggs were left for seven days. Harvesting of larvae was done by hanging the wrapped content into sedimentation flask containing water where the larvae were recovered at the bottom of the sedimentation flask. Then larvae were transferred by pippetting into a beaker. Larvae were identified on the microscope at x100 followed by treatment with extracts. Larvae were then exposed to extracts at concentrations range from 500 μgml^{-1} to 7.8125 μgml^{-1}, and then the number of survivors and dead were recorded at an interval of six (6) hours for two days. Figure 9 shows *in vitro* larvicidal test using larvae from strogylid helminth eggs of goats.

Figure 9: *In-vitro* larvicidal test using larvae from strogylid helminth eggs

i: exposing larvae to extracts; **j**: identification of larvae

3.6.7.3 *In-vivo* test using *Ascaridia galli* eggs from local chicken

A total of 50 chickens (broilers) of the age of 4 weeks and approximately the same weight were de wormed. To make sure that the chickens are de wormed each chicken's faecal samples were screened for worm infections using McMaster technique. Bottomed flattened micro plates were used to expose *Ascaridia galli* eggs

(embryonated to infective stage for 10 days at 28 ^{0} C) to the prepared concentrations of *S. glaucescens* (Pax) extracts and left for 24 hours. The same procedure was done as in *in-vitro* test above. After 24 hours of eggs exposure to extract concentrations were used to inoculate chickens. Chickens were grouped into three main groups of which group 1 had five (5) sub groups for each root barks and leaves extracts. Group 2 was positive control (infected with untreated eggs) and group 3 was negative control (not infected with *Ascaridia galli* eggs). All groups (1-3) had four replicates each making up a total of 48 chickens. After introducing eggs, chickens were left under close supervision allowing for life cycle of *Ascaridia galli* to be completed, duration of which was eight (8) weeks before slaughtered for worm examination and recording. Figure 10 shows *in-vivo* test using *Ascaridia galli* eggs from local chicken.

Figure 10: *In-vivo* test using *Ascaridia galli* eggs

(k): chickens rearing; (**l)**: deworming chickens; (**m)**: tagging chickens; (**n)**:slaughtering chickens; (**o)**: collecting chickens'intestines; (**p)**: observation of *Ascaridia galli* eggs; (**q)**: preparation of faecal samples; **r**: examination of eggs/larvae

3.7 Data Analysis

The data were analyzed using Microsoft excel tool for descriptive statistics. The results were reported in numbers using logarithm transformation, percentages means and standard deviation.

CHAPTER FOUR

4.0 RESULTS

4.1 Identified Helminth Eggs from Wastewater/Sludge

The wastewater and sludge samples were prepared for parasitological examination as explained in section 3.6.2 and 3.6.3. Helminth eggs and larvae were identified to genus level (Fig. 11) with the help of technical personnel in the laboratory. The identified helminth eggs were mostly nematodes including *Ascaris spp*, Strongylid eggs (e.g. Hookworm), and *Trichuris spp.*

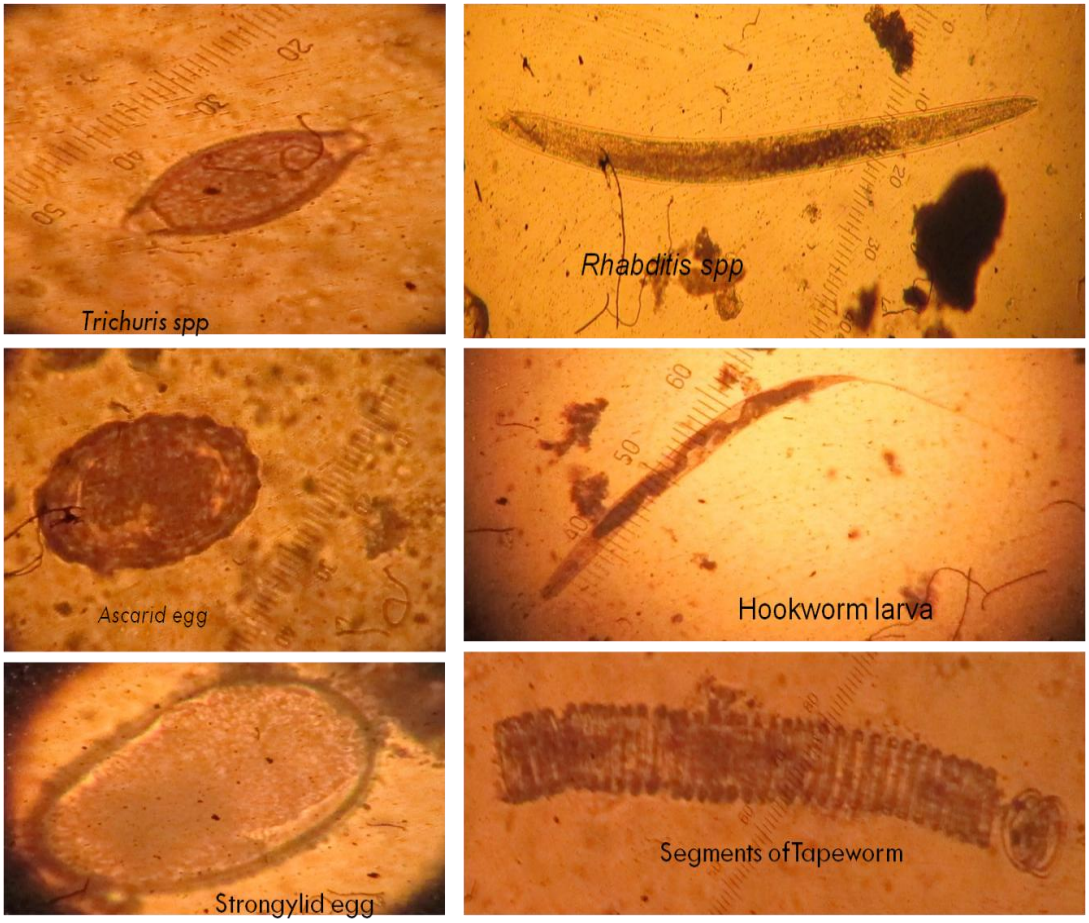

Figure 11: Helminth eggs and larvae identified from wastewater and sludge

4.2 Quantification of Helminth Eggs from Wastewater/Sludge Samples

The number of eggs per litre (e.p.l.) from wastewater samples and eggs per gram (e.p.g.) from sludge sample are as indicated in Table 4. The percentage of helminth eggs and their types are shown in Table 4. Minimum amount of ≤ 1 e.p.l. and e.p.g. were found in maturation ponds and the maximum amounts of 700 e.p.l. of helminth eggs were found in anaerobic pond, receiving wastewater by gravity and by trucks. As indicated from Table 4, most of the eggs were found in the anaerobic ponds, few from facultative pond and non from maturation ponds. Also sludge from anaerobic and facultative ponds contained helminth eggs compared to the rest of the ponds in the series. Generally the trend shows that the number and types of helminth eggs decreased from anaerobic ponds to effluent.

Table 4: Helminth eggs content in wastewater and sludge

Sample type	**Types of eggs**	**Percentage composition of types of helminth eggs in e.p.l/e.p.g**								
		AG	**AT**	**AN**	**FP**	**MP1**	**MP 2**	**MP3**	**MP 4**	**E/S piles**
Wastewater	Strogylid	40	50	50	100	0	0	0	0	0
	Ascarid	40	50	50	0	0	0	0	0	0
	Trichuris	20	0	0	0	0	0	0	0	0
Total eggs (e.p.l.)		583	700	466	117	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1
Sludge	Strogylid	NA	50	75	10	0	0	0	0	0
	Ascarid	NA	50	25	0	0	0	0	0	100
	Trichuris	NA	0	0	0	0	0	0	0	0
Total eggs (e.p.g.)		NA	30	20	50	≤ 1	≤ 1	≤ 1	≤ 1	50

(NA): not applicable; (**AG)**: Anaerobic pond receiving wastewater by gravity; (**AT)**: anaerobic pond receiving wastwater by trucks; (**AN)**: anaerobic main pond; (**FP)**: facultative pond; (**MP)**: 1, 2, 3, 4: maturation pond 1, 2, 3, 4; (**E)**: effluent; (**S)**: sludge

4.3 *In vitro* Hatchability Inhibition of *S. glaucescens* on Strongylid Eggs

Hatchability inhibition test of *S. glaucescens* extracts on strongylid helminth eggs was done at concentrations ranging from 500 µgml^{-1} to 7.8 µgml^{-1} following dilution law sequence. Extract concentrations were the same for both root bark and leaves samples extracted with water, ethanol and dichloromethane. The results from hatchability inhibition assay of *S. glaucescens* extracts are shown in Fig. 12. At concentrations of 500, 250 and 125 µgml^{-1} no eggs hatched for all extracts. From concentration of 62.5, 31.25, 15.625 and 7.8125 µgml^{-1} larvae were observed and number of eggs hatched increased as the concentration decreased. Figure 12 shows the hatchability inhibitory concentration IC_{50} which shows that ethanoic root bark extract (RSE) had highest inhibitory effect on hatchability with the lowest IC_{50} of 19.34 µgml^{-1}. Dichloromethane root bark extract (RSD) had lowest inhibitory effect on hatchability of helminth eggs with highest IC_{50} of 66.83 µgml^{-1}.

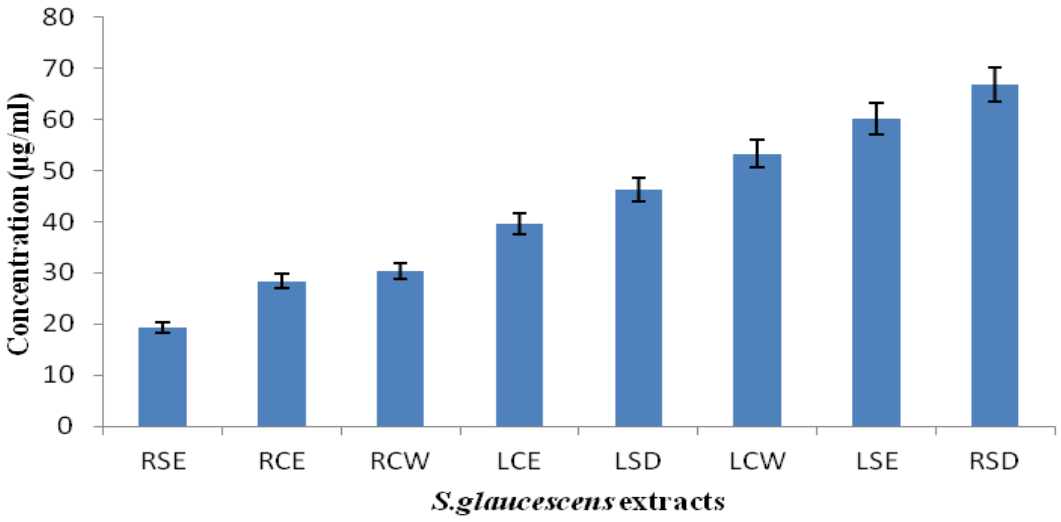

Figure 12: Hatchability inhibitory concentration IC_{50} of *S. glaucescens* extracts

(R):rootbark; (**L**):leaves; (**S**):sequentilly extracted; (**C**):completelly extracted; (**E**):ethanol solvent; (**D**):dichloromethane solvent; (**W**):water solvent

4.4 *In vitro* larvicidal efficacy of *S. glaucescens* on strongyle eggs

From the results, the treated group of larvae showed gradual decline in number of survivors when compared to untreated control group. This shows that the extracts had some negative effects on the survival of larvae. Also for all extracts as time of exposure increases the number of survivors decreased as shown in Fig. 13. From concentrations of 500 μgml^{-1} to 125 μgml^{-1} no larvae survived. Few larvae survived in extract with concentration of 62.5 μgml^{-1} and the number of survivors increased at lower concentration of 31.25 μgml^{-1}. Figure 13 shows lethal concentration (LC_{50}) of *S. glaucescens* extracts which was able to kill 50% of exposed larvae to it. Leaf extract completely extracted by water (LCW) and Root bark sequentially extracted by ethanol (RSE) had higher efficacy on larvae within 6 hours of exposure to extract as they have lower LC_{50} of 63.21 and 69.24 μgml^{-1} respectively. Root bark extract extracted sequentially using dichloromethane (RSD) had lowest efficacy on larvae as it had highest LC_{50} of 86.02 μgml^{-1}. The trend shows that efficacy of extracts increased with increasing time of exposure. After 48 hours of exposure root bark extract sequentially extracted by ethanol (RSE) had higher efficacy with LC_{50} 19.41 μgml^{-1} on larvae compared to 69.24 μgml^{-1} when exposed for 6 hours.

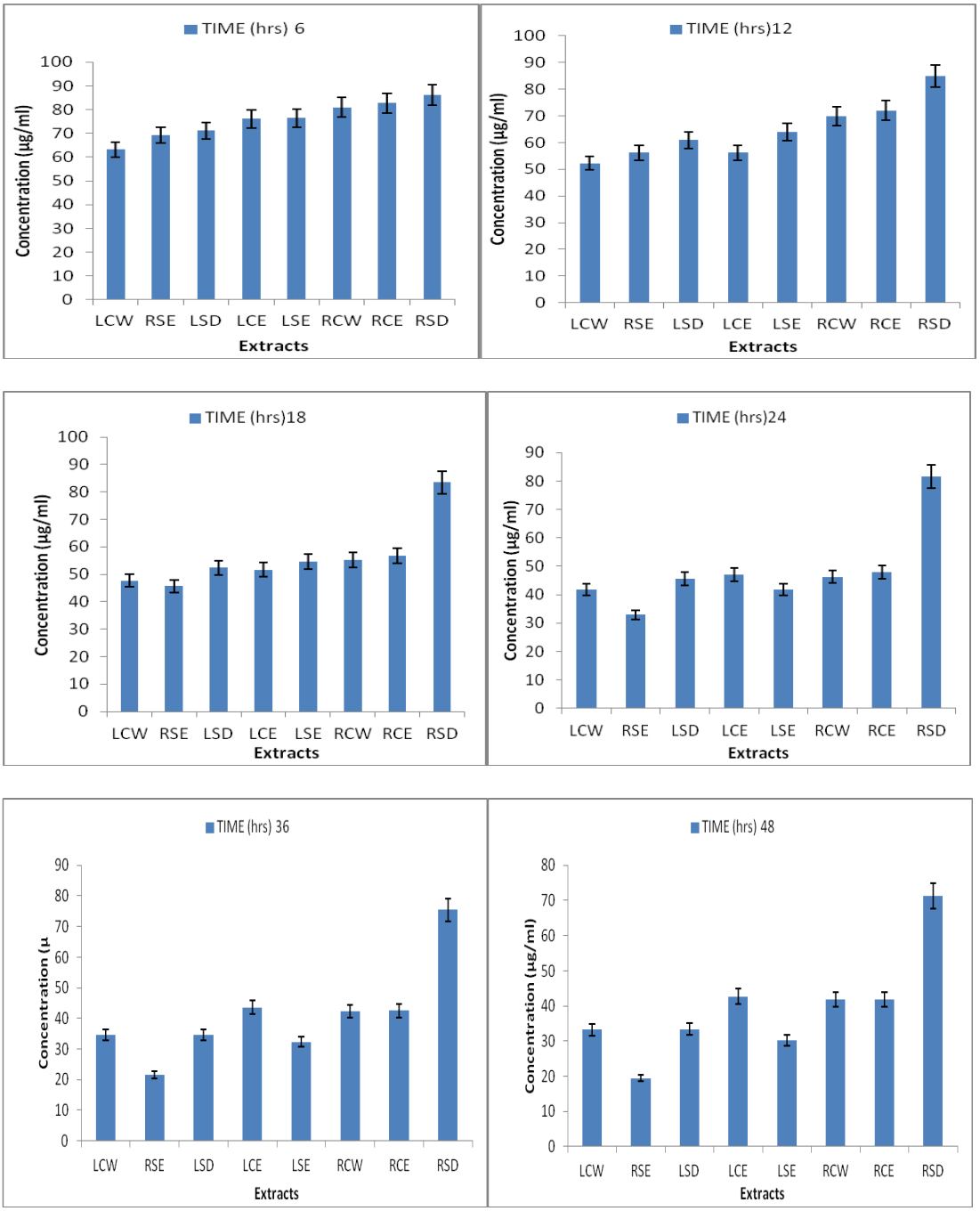

Figure 13: Lethal concentration (LC_{50}) of *S. glaucescens* extracts against larvae

(R): rootbark; (**L)**: leaves; (**S)**: sequentilly extracted; (**C)**: completelly extracted; (**E)**: ethanol solvent; (**D)**: dichloromethane solvent; (**W)**: water solvent

4.5 Hatchability Effect of *S. glaucescens* (Pax) on *Ascaridia galli* eggs

Ascaridia galli eggs embryonated to infective stage for 10 days at 28 ^{0}C were exposed to concentrations range of 500 µgml^{-1} to 7.8125 µgml^{-1} of *S. glaucescens* (Pax) extracts of both root barks and leaves for 24 hours before inoculated to chickens as explained in section 3.6.7.3. After eight (8) weeks, the results on hatchability effects of *S. glaucescens* (Pax) on helminth eggs are indicated in Fig. 14. The hatchability decreased as the concentrations were increased from 31.25 µgml^{-1} to 62.5 µgml^{-1} (i.e. from 17-4% for root bark and 14-6% for leaf). From concentrations of 125 µgml^{-1} to 500 µgml^{-1} no hatchability was observed. From the figure 14 at control (0 µgml^{-1}) hatchability was 24% for both root bark and leaf extract.

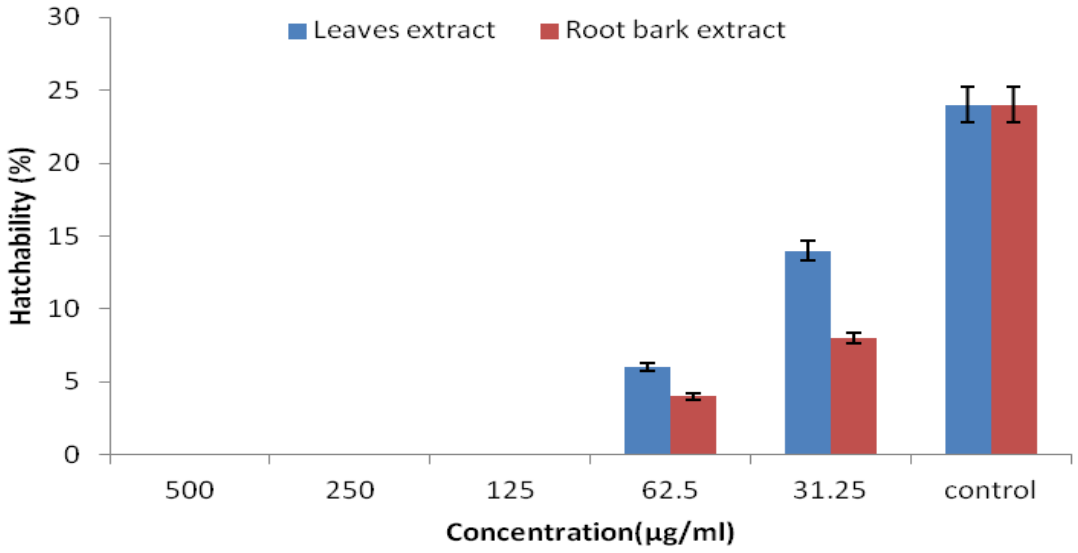

Figure 14: Hatchability inhibition of *S. glaucescens* on *Ascaridia galli* eggs

CHAPTER FIVE

5.0 DISCUSSION

5.1 Identification and Quantification of Helminth Eggs

Different helminth eggs genera were identified either in wastewater and sludge from Mafisa WSPs as seen in Figure 11. The identified helminths eggs and larvae reflect local level of contamination of an area in terms of helminth infection. Other studies have shown that due to differences in environmental factors, helminth eggs content in wastewater and sludge may vary from country to country (Jiménez-Cisneros, 2007). Jiménez and Mara (2007) reported *Ascaris lumbricoides, Strongyloides stercoralis, Trichuris trichiura* in sludge and wastewater from North Africa, Far East and some parts of Asia. Thus, findings from this study are in agreement to what was previously reported (Jiménez, 2007a), of which resemble with that of Mafisa WSPs with slight difference in number and types of helminth eggs. Based on epidemiological studies, World Health Organization (WHO) has set guideline and recommended that, if the final effluent from WSPs is to be used for irrigation, it is necessary to ensure that it contains not more than one egg per litre of effluent (WHO, 2006) a situation of which is observed in effluent from Mafisa WSPs (Table 4).

In the present study, the quantity of helminth eggs decreased downstream from receiving ponds to effluent indicated that treatment was efficient. Helminth eggs from WSPs are removed by sedimentation process thus remain in the pond sludge (Curtis *et al*., 1992). When a series of ponds are used, most of helminth eggs are retained in the first pond. The precise value of the removal of helminth eggs depends on number of ponds in the series and their retention times (Grimason *et al*., 1996;

Mara, 2004). To remove helminth eggs, the minimum required total retention time in a series of WSP is 5-20 days. The difference depends on their number in the raw wastewater (Mara, 2004). The Mafisa WSPs where there is seven ponds series the calculated retention time during data collection was 19 days, the trend that falls within the required treatment time.

In the sludge, there was a decreasing trend of concentration of helminth eggs from receiving pond to effluent. Sludge produced at Mafisa WSPs contain viable helminth eggs though are few in numbers compared to those from wastewater due to sedimentation which takes place in the anaerobic and facultative ponds. Helminth eggs can remain viable for several years in the sludge. For instance a survey of several WSP in Mexico, viable eggs were found in sludge stored for at least nine (9) years (Nelson *et al*., 2004). Eggs contained in wastewater are not infective. To be infective they need to develop larva (infective 3^{rd} stage larva) for which a certain temperature and moisture are required (for strongylid eggs 26^{o} C and 7 days in laboratory conditions). Conditions usually found in the soil or crops are suitable for the development of larvae in 10 days, hence the risk of using wastewater or sludge in agricultural fields (Jiménez, 2007b). The fact that sludge produced at Mafisa WSPs contains viable helminth eggs means they requires further treatment before any application to agricultural field to reduce risk of helminth infection to pond and farm workers, local communities in close proximity to activities of WSPs and product consumers.

5.2 Inhibition of hatchability of Strongylid eggs exposed to *S. glaucescens*

Findings from this study revealed that the concentration of the plant extract was an important factor to effect treatment. There is a negative concentration effect on hatchability due to the fact that, as concentration increased there was a reduction in number of eggs hatched. Therefore, the percentage hatchability inhibition increases by increasing the concentration of extract. Hatchability inhibitory concentration IC_{50} of all *S. glaucescens* extracts were determined as indicated in Fig. 12. Root bark extract extracted sequentially using ethanol (RSE) had highest inhibitory effect on hatchability with IC_{50} of 19.34 μgml^{-1}. Root bark extract extracted sequentially using dichloromethane (RSD) had lowest inhibitory effect on hatchability of helminth eggs with IC_{50} of 66.83 μgml^{-1}. There is an observed difference between root bark extract sequentially extracted from dichloromethane (RSD graph) from the rest of the graphs (Fig 12). This could be due to the fact that the extract could not mix with distilled water during concentration preparation.

5.3 *In vitro* Larvicidal effectiveness of *S. glaucescens* on Strongylid Eggs

It was revealed that the dosage was an important factor to effect treatment. The effectiveness of extracts was higher at high concentration and lower at low concentrations. Also there is survival-time relationship where as time of exposure increases the number of death increases. At concentration of 62.5 μgml^{-1} and 31.25 μgml^{-1} effectiveness of extract increased with increasing time of exposure. Lethal concentration LC_{50} of all *S. glaucescens* extracts were determined as shown in Fig. 13. Leaves extract completely extracted by water (LCW) and Root bark sequentially extracted by ethanol (RSE) had higher effect on larvae within 6 hours of exposure to

the extract with LC_{50} of 63.21 and 69.24 μgml^{-1} respectively. Root bark extract extracted sequentially using dichloromethane (RSD) had lowest efficacy on larvae as it has highest LC_{50} of 86.02 μgml^{-1}. The trend shows that efficacy of extracts increased with increasing time of exposure. After 48 hours of exposure root bark extract sequentially extracted by ethanol (RSE) had higher effect with LC_{50} 19.41 μgml^{-1} on larvae compared with 69.24 μgml^{-1} when exposed for 6 hours. Thus the numbers of survived larvae were reduced as the time of exposure increased. *Synadenium glaucescens* (Pax) has been deployed by communities in Tanzania for ethno veterinary purposes (Wickaman *et al*., 2006; Mabiki *et al*., 2013a). Also extract from *S. glaucescens* (Pax) have demonstrated anti molluscicidal activity (Kloos *et al*., 1987).

5.4 Effect of *S. glaucescens* (Pax) on *Ascaridia galli* hatchability

On in vivo trial, the treated *Ascaridia galli* eggs showed gradual decline in their hatchability compared with untreated control group (Fig. 14). This shows that the extract had effect on eggs hatchability when administered at a dose not less than 125 μgml^{-1}. Although the number of viable eggs in control was small (24%) compared to number of eggs incubated (50 eggs) in a chicken model, the groups with concentrations of 31.25, 62.5 and 125 μgml^{-1} have shown steady gradual decrease on viable eggs output, indicating that hatchability of eggs has been affected by extracts. Mabiki *et al.*, (2013) investigated the effects of *S. glaucescens* (Pax) crude extracts against infectious bursal disease virus and fowlpox and found potential and feasibility of using the plant for treatment of viral diseases. There was no significant difference between leaf and root bark extracts on effects of hatchability of *Ascaridia*

galli eggs. From control group the hatchability of *Ascaridia galli* eggs went below expectation as it was only 24% maximum. This could be due to chickens body immunity against infections; traces of antihelmintic drug administered to de worm chickens before experiment and; viability of *Ascaridia galli* eggs.

5.5 Limitation of the Study

5.5.1 Helminth eggs obtained from wastewater and sludge were insufficient in number to support studies on antihelminthic activity of *S. glaucescens* (Pax) plant.

5.5.2 Limitation of time for the research work necessitated alternative sources of strongylid eggs from goats and ascarid eggs from local chicken to carry out antihelminthic activity of *S. glaucescens* (Pax) extracts. Strongylid eggs from goats and *Ascaridia galli* eggs from local chicken have been recommended as suitable model for screening of antihelminthic drugs (Mali and Mehta, 2008).

5.5.3 Helminth eggs (strongylid eggs) obtained from specified worms such as *Haemonchus, Oesophagostomum* could not hatch as most of them were still in their development stages (Strongylid eggs from faecal samples were the only option to get fully developed eggs).

5.5.4 Faecal samples had mixed helminth species which could not be identified as they needed more advanced and sophisticated equipments which were not readily available.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The aim of this study was to evaluate the bioactivity of *S. glaucescens* (Pax) for control of helminth eggs and larvae from wastewater and sludge from WSPs at Mafisa, Morogoro Municipality.

The identification of helminth eggs in wastewater and sludge from Mafisa WSP was done using methods adopted (with modification) from Hansen and Perry (1994) for sludge and Bailenger method by Ayres and Mara (1996); Sengupta (2012) for wastewater. Three types of helminth eggs were identified namely Ascarid, Strogylid, and *Trichuris*. Also larvae of *Rhabditis spp* and hookworm and segments of tapeworm were observed.

Enumaration of helminth eggs from wastewater and sludge was conducted using Bailenger method and concentration McMaster method as adopted by Sengupta (2012). Enumaration was done to all ponds from influent to effluent and to the sludge piles waiting to be taken to agricultural field to serve as manure in crop production.

It was observed that the number of eggs showed to decrease in sequence from influent to effluent where the effluent had ≤ 1 egg per litre making wastewater from Mafisa WSPs suitable for irrigation as by following recommended WHO guideline of 2006. Sludge from first to fourth maturation ponds had ≤ 1 egg per gram making it suitable to be used as manure in crop production. However, sludge from anaerobic

ponds had more than 100 eggs per gram, a result of which indicates the need for treatment before being taken to agricultural field for crop production.

The bioactivity of *Synadenium glaucescens* (Pax) against helminth eggs and larvae were determined. Eggs were exposed to difference concentrations of leaves and root bark extracts extracted from different solvents. The results showed that eggs exposed to higher concentrations (500, 250 and 125 μgml^{-1}) did not hatch. The hatchability inhibition was 100%. However with concentrations of 62.5 to 7.8125 μgml^{-1} helminths eggs hatched, and as the concentration decreased the number of eggs hatched increased.

Hatchability inhibitory concentration IC_{50} of *S. glaucescens* extract with the lowest IC_{50} of 19.34 μgml^{-1} showed that root bark extract had the highest inhibitory effect on hatchability.

A group of untreated eggs were also incubated, and hatched larvae were exposed to different concentrations of *S. glaucescens* extract. It was observed that larvae exposed to concentrations of 500, 250 and 125 μgml^{-1} died within 6 hours. Some of the larvae exposed to 62.5 and 31.25 μgml^{-1} survived and it was noted that the number of survivors increased as the concentration of *S. glaucescens* extract decreased. Furthermore the efficacy of *S. glaucescens* extracts on helminths larvae increased with increasing time of exposure. For example, after 48 hours of exposure root bark extract had higher efficacy with LC_{50} 19.41 μgml^{-1} on larvae compared to 69.24 μgml^{-1} when exposed for 6 hours.

The effect of *S. glaucescens* extract on hatchability of helminths eggs indicated that with concentrations of 500, 250 and 125 μgml^{-1} there was no helminth worms observed. However, for the concentrations of 62.5 and 31.25 μgml^{-1} few larvae were observed and their number increased with decreasing concentration of *S. glaucescens* extract.

Generally extracts had effects on hatchability of helminth eggs and to the survival of larvae. However the hatchability inhibition of helminth eggs and efficacy of extracts on larvae depends on concentration of dosage and time of exposure to extracts.

Therefore, results from this study provide data and meaningful information on types of helminth eggs and larvae and the extent of contamination in wastewater and sludge from Mafisa WSP. The study also has showed that *S. glaucescens* extracts from root barks and leaves have bioactivity effect in combating helminths.

6.2 Recommendations

Based on the findings from this study, future studies should focus on the isolation of responsible active compound(s) that kills helminth larvae and inhibit hatchability of helminth eggs; Effect of *S. glaucescens* extracts on morphological conditions of helmith eggs and larvae; Utilization of *S. glaucescens* in controlling helminth in vegetable production; Existence of resistant stages of helmith eggs as they cannot be inactivated with chlorine, UV light or Ozone; Assessment of viability of helminth eggs in wastewater and sludge; Developing a guideline to control the unplanned reuse of wastewater and faecal sludge to avoid contamination of soil and water

bodies and endanger human health; Helminth in vegetables irrigated by wastewater and fertilized with sludge manure from WSPs.

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