THE BIOACTIVITY OF *COMMIPHORA SWYNNERTONII* RESIN EXTRACTS AGAINST HELMINTHS EGGS/LARVAE FROM LOW QUALITY WATER AND VEGETABLES IN MOROGORO MUNICIPALITY

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ABSTRACT

This study was conducted on urban vegetable farming sites at Mzumbe, Mafisa and Fungafunga in Morogoro municipality-Tanzania. The objective of this study was to investigate the effect of *Commiphora swynnertonii* resin extract against helminths eggs/larvae isolated from low quality water and vegetables at Mzumbe, Mafisa and Fungafunga in Morogoro municipality. A total of 90 vegetable samples and 70 water samples (30 treated, 30 river water and 10 untreated) were collected from the study areas to assess their contamination level with helminth eggs using Baelinger techniques. Isolation, identification and quantification of helminths eggs present in low quality water and irrigated vegetables were carried out and the effect of *C. swynnertonii* resin on hatchability of helminth eggs and survival of helminth larvae was assessed. The study showed that untreated water samples (from anaerobic ponds of both study sites) were positive of *Ascaris* and Strongyle eggs. Treated water samples (effluents), river water and vegetable samples were all negative of helminth eggs. Results for the in vitro test of *C.swynnertonii* resin extract against helminths eggs/larvae indicated that the resin extract was very effective in higher concentrations (25, 50, 70 and 100 %V/V) in inhibiting the hatching of Strongyle eggs, *Ascaridia galli* eggs and caused death of larvae of *Haemonchus* spp, *Trichostrongylus* spp, *Oesophagostomum* spp and *Strongyloides* spp. It is recommended that further studies be done on the effect of resin on other pathogenic microorganisms such as bacteria and protozoa in effluents used for irrigation in the study areas. Studies on the possibility of making products from the plant extracts to be used for controlling wide range of water pathogens are also recommended.

DECLARATION

I, Florence Felician Basiga, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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The declaration above is confirmed by

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Prof. A.A. Kassuku **Date**

(Co -Supervisor)

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DEDICATION

This dissertation is dedicated to my beloved parents Mr. and Mrs. Felician Kalokora Baserwa and my relatives who laid down the foundation for my education. Last I dedicate my dissertation to my lovely wife Elizabeth Yotham for her love, care and prayer.

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LIST OF ABBREVIATION AND SYMBOLS

µL	micro litre
µm	micro mitre
^{0}C	Degree centigrade
ANOVA	Analysis of Variance
DBPs	Disinfection by-products
g	grams
MAFF	Ministry of Agriculture Food and Fisheries
rpm	revolutions per minute
UK	United Kingdom
V/V	Volume by volume
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

Clean water resources at present have become scarce, no longer reliable, more expensive and highly competed by the agricultural and domestic users in rural settings due to rapid population growth and climate change (Shannon and Grieve, 2000; Andoh *et al*., 2009). As a result farmers are forced to use low quality water for vegetables production (Ackerson *et al.*, 2012). Currently about 20 million hectares of agricultural land worldwide is irrigated with treated and untreated low quality water (Mokhtari *et al.*, 2012). Use of low quality water in agriculture has negative impacts to human health (Mokhtari *et al.*, 2012). Those negative human health problems include: health problems due to heavy metal contaminants, organic pollutants and diseases from pathogenic microorganisms. Epidemiological studies show the actual risk of infection for people exposed to low quality water is the highest for the round worms; *Ascaris lumbricoides*, the whip worm; *Trichuris trichiura* and the hookworms; (*Ancyloma duodenale* and *Necator americanus*) (WHO,1989, 2006; Cifuentes *et al.,* 1993). The risk of acquiring a bacterial, protozoan or fungal infection due to exposure to low quality water has been reported to be very low because of their low survival rate in the environment (Sabiena *et al*., 2000). A study done by Andoh *et al*., (2009) reported a number of helminths from low quality water that was able to contaminate vegetables and thus affecting human health. These included *Ascaris lumbricoides*, Schistosoma, Strongyl*e* eggs, *Trichuris trichura*, *Taenia*, *Clonorchi*s and Strongyloides larvae. Eating raw or lightly cooked vegetables (in order to preserve heat labile nutrients) and fruits have been reported to

increase the possibility/likelihood of getting food bone infections in human (Jimenez, 2006; Erdogrul and Sener, 2005).

Several hygienic practices and techniques used to decontaminate vegetables have been reported to be effective against bacteria and some protozoa with less effect to helminths. Washing is amongst the commonly reported technique used to decontaminate vegetables but reported to be not sufficient to completely remove helminths eggs from vegetables (Fallah *et al.*, 2011 and Raicevic *et al.*, 2010). Another technique is by use of chemicals, such as chlorine, UV-disinfection, ozone, washing with organic acid and H_2O_2 (Raicevic *et al.*, 2010). The uses of chemicals as decontaminant have shown some limitation on their utilization. These limitations include resistance of some helminths to chemical decontaminants (such as chlorine) (Melo *et al.,* 2003 and Jimenez *et al.,* 2006). Also issues of safety over the use of the chemical decontaminants; costs, accessibility and availability to ordinary farmers (Fallah *et al.,* 2011; Amoah *et al.*, 2007 and Somi *et al*, 2011).

Furthermore, some of chemical disinfectants used to decontaminate vegetables and low quality water do produce chemical by - products in water which are associated with harmful health effects to humans (Somi *et al.*, 2011). Due the above mentioned challenges some researchers have been struggling to come up with alternative agent(s) for treating low quality water and hence vegetable decontamination using natural plants (Chun-Yang Yin, 2010). Several natural remedies such as plants have been explored and reported to be used as water decontaminants. Such natural plants include *Ocimum sanctum* and *Azadrachta indica* reported to be effective against

bacteria (Somi *et al.,* 2011), lemon juice reported to be effective against bacteria including *Vibrio cholerae* (Aquino and Teves, 1994) and *Moringa oleifera* observed to be effective against bacteria and have water conditioning properties (Ghebremichael and Kebrea, 2004). Other studies on Nirmali seed ((*Strychnos potatorum*) (Chun-Yang Yin, 2010), *Molinga oleifera*, Tannin based cationic polymer (Graham *et al*., 2008) and Cactus (*Opuntia cactaceae*) were also reported. These plants are efficient in treatment of waters with low to medium turbidity and they are technically promising as coagulant for dyeing effluents (Chun-Yang Yin, 2010). Though several plants have been reported to control bacteria, fungi and protozoa due to low quality water, but little has been scientifically documented to be effective on addressing the problem of helminth in low quality water and vegetables. *Commiphora swynnertonii* plant is highly distributed in Tanzania, Asia and other parts of the world and is reported to have several effects on ecto and endo parasites, (Bakari *et al*., 2013) against bacteria, virus and protozoa in humans and animals (Bakari *et al.*, 2012). Following the scientifically reported effect of *C. swynnertonii* against several microbes and parasites there is a need to assess its effectiveness on controlling the microbes and parasites in low quality water and vegetables. Therefore this study was aimed at establishing the activity of *C. swynnertonii* resin extract against helminths eggs/larvae isolated from low quality water and vegetables.

From present study the presence or absence of helminths eggs in low quality water and vegetables of Morogoro Municipality was determined and the activity of *C. swynnertonii* resin extract on eggs of helminths hatching and larvae survival was also determined.

1.1 Problem Statement

Although low quality water has been used for production of various crops and vegetables worldwide, the benefits of its use may be limited by potential health hazards due to heavy metal contamination, organic pollutants and pathogenic organisms. Helminths are among pathogenic organisms associated with use of low quality water affecting health of vegetables consumers. Like in other parts of the world, farmers in the urban and peri-urban areas of Morogoro municipality use low quality water for crops and vegetable production despite the fact that microbiological status (especially helminths) of the water being used is not clearly known. Use of chemical disinfectants as the means to decontaminate low quality water and agricultural produce is rarely and /or not practiced. People rely on washing vegetables which is not an effective means for removing helminth eggs. Moreover, chemicals are expensive to most farmers and their safety is not well established and hence calls for the need of cheap, safe and effective decontaminants for vegetables and low quality water. Medicinal plants for example *Moringa oleifera*, Lemon juice, *Ocimum sanctum* and *Azadrachta indica* have been an alternative approach for managing some pathogenic organisms in water such as bacteria, fungi and some viruses as the result of challenges posed by modern medicine in developing countries. Similarly, studies on medicinal plants such as *C. swynnertonii* can help to come up with alternative, safe, effective and cheap helminths decontaminating agent to be used in low quality water and vegetables. This study will increase knowledge by assessing the presence of helminths eggs in low quality water and vegetables of Morogoro Municipality and the potential of

using *C. swynnertonii* resin extract as an agent against helminths eggs/larva from low quality water and vegetables.

1.2 Justification

Since untreated/partially treated low quality water is an important source of helminths when used in agricultural irrigation schemes, this may present high risk to farm workers and to consumers. The problem of helminths infection becomes more serious with vegetables, because many of them are being eaten raw or lightly cooked in order to preserve the heat labile nutrients (Erdogrul and Senertt, 2005). It is important therefore to assess the quality of water used for vegetable production and other purposes as part of expanding the protection of the health of the population depending on low quality water and its produces by using laboratory analyses in order to obtain information such as the concentration of pathogenic helminths by establishing their presence or absence. Assessing the presence of helminths in low quality water and vegetables is important in order to establish the potential health risks that farm workers and consumers of the produces are exposed to. This in turn will help to plan for the appropriate interventions where necessary. Moreover understanding the activity of *C. swynnertonii* resin extracts against helminth eggs may pave way to formulation of products to be used in waterborne helminthiasis

1.3 Objectives

1.3.1 General objectives

To investigate the effect of *C. swynnertonii* resin extract against helminths eggs/larvae isolated from low quality water and vegetables.

1.3.2 Specific Objectives

i. To isolate and identify the types of helminths eggs present in low quality water and low quality water irrigated vegetables.

ii. To quantify each type of helminth egg in low quality water and low quality water irrigated vegetables.

iii. To assess the effect of *C. swynnertonii* resin extract on the helminth egg hatching and larvae survival *in vitro.*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Low Quality Water Usage

Low quality water is defined as water that possesses certain characteristics which have the potential to cause health problems when it is used for an intended purpose (Pescod, 1992). For example, brackish water is low quality water for agricultural use because of its high dissolved salt content; municipal wastewater is low quality water because of the associated health hazards (Pescod, 1992). From the viewpoint of irrigation, use of low quality water requires more complex management practices and more stringent monitoring procedures than when good quality water is used (Pescod, 1992). The use of low quality water is a historical practice which dates back to 5000 years in ancient Greece and has mainly been due to overcoming shortages of freshwater that existed in Minoan civilization (Vigneswaran and Sundaravadivel, 2004). Since then, the practice of using low quality water has been recorded in German (in 16th Century) and in UK (in the 18th Century). Because of continuing decrease in fresh water resources due to many reasons including climatic change and population growth, the use of low quality water is increasingly becoming a common practice to many countries in the world (Andoh *et al.*, 2009). For some decades now, the benefits of promoting reuse of low quality water as an alternative water resource for irrigation practices have been recognized by national governments. Its value is increasingly understood in arid and semi-arid areas and many countries are struggling to find ways to improve and expand the use of low quality water. The benefits and hazards of using low quality water are known to

water researchers and are continuously evaluating low quality water as one of the option for future water demands (Vigneswaran and Sundaravadivel, 2004).

2.2 Benefits Associated with use of Low Quality Water for Irrigation

There are a lot of benefits associated with use of low quality water for agriculture. These include reduced cost of artificial fertilizers to most farmers due to presence of nutrients in low quality water that can increase crop production (Andoh *et al.*, 2009). Use of low quality water is linked to creation of employment opportunities, source of income and significantly contributes to food security in urban and peri-urban areas (Trang *et al.*, 2007). According to Mougeot (1994) approximately 40% of African urban dwellers were involved in urban farming in 1994 with the number expected to have increased up to date. Moreover, Stevenson *et al*., (1996) reported that in Tanzania about 90% of leafy vegetables coming to the market in Dar-es-Salaam had their origin within the city, the production of which involved use of low quality water in one way or another.

2.3 Pathogens in Low Quality Water used for Irrigation

The health hazards that may result from the reuse of low quality water for irrigation farming need to be understood despite its potential benefits (Korentajery, 1991). This is because its safety in vegetable production has been questioned by many researchers due to presence of high contaminants including pathogenic microorganisms such as protozoa, bacteria and helminths which can contaminate vegetables and fruits if used untreated (Gupta *et al*., 2009, Esma *et al*., 2004 and Daryani *et al*., 2008). Although vegetables can be contaminated along the way from

the field to the market and then during preparation for consumption, irrigation using low quality water has been emphasized to contribute more to vegetable contamination. Study done by Andoh *et al.*(2007) reported that irrigation using low quality water resulted in high faecal contamination in spinach cultivated in China. Moreover, Okafo *et al.* (2003) reported much higher concentration of coli form in amaranths irrigated with low quality water. Furthermore, contamination with salmonella to crops irrigated with low quality water whose edible parts develop on the ground surface such as lettuce has also been reported (Melloul *et al.*, 2001).

Health problems due to consumption of vegetables and fruits irrigated using low quality water have been reported, for example in Ghana the annual loss of 12000 Disability-Adjusted Life years due to waste water contaminated vegetables (Amoah, *et al.,* 2011), food borne diseases in Lome -Togo (Karou *et al*., 2011), helminth infection (mainly *Ascaris lumbricoides*), bacterial infection (typhoid, cholera, and *Helicobacter pylori*), symptomatic diarrhea diseases and other enteric infections (Blumenthal and Peasey, 2002, and Trang *et al.*, 2007).

2.3.1 Helminths as one of pathogens associated with use of low quality water

Several pathogenic organisms were reported to be associated with use of low quality water. Helminths are among them and are mentioned be among threatening pathogenic organisms associated with use of low quality water affecting health of consumers of vegetables, farm workers and their families including nearby community (WHO, 2006, Mokhtari *et al.*, 2012, and Hussain *et al*., 2002). According to WHO (2004), the use of low quality water had significant contribution

to about 160 million people who were infected with helminths worldwide. Study by Andoh *et al*. (2009) reported a number of helminths which originated from low quality water and were able to contaminate vegetables and thus affecting human health. These helminths included *Ascaris lumbricoides*, *Hookworms*, *Trichuris trichura*, *Taenia*, *Chlonorchi*s and *Strongyloides* larvae and cercaria. Moreover, the use of low quality water and dried sludge as fertilizers has been reported by a number of studies to be associated with contamination of vegetables with helminths eggs in which *Ascaris lumbricoides* is frequently reported to have higher prevalence when compared to other helminths (Gupta 2009; Andoh *et al.,* 2009 and Amin, 1988). Amahmid *et al.,* (1999) reported a direct association between use of low quality water and contamination of crops designated for food consumption with *Ascaris* eggs and *Giardia* cyst. In the same study variation in the contamination level between different varieties of crops were reported, with turnip and mint having high contamination level. Furthermore, a study by Pescod 1992, and Ensink *et al.* (2008) on exposure to untreated low quality water to farmers revealed high incidence of helminths infections while use of partially treated low quality water was associated with high incidence of *A. lumbricoides* in the same study. Farming families occupationally exposed to untreated and partially treated low quality water had increased risk of hookworms, *Ascaris lumbricoides* and *Trichuris trichura* infection in the study by Jeroen *et al*. (2007). High prevalence of helminthiasis in areas using low quality water for irrigation farming is due to the high survival rate of helminths eggs in the environment given their ability to survive in hash environmental condition for prolonged period of time when compared to other

pathogens (Sabiena *et al.*, 2000). WHO (2006) summarized the health risks associated with use of low quality water to the exposed group (Table 1).

Table 1: Summary of health risks associated with the use of low quality water for irrigation

Exposed group	Health threat		
	Helminths	Bacteria/Viruses	Protozoa
Consumer	Significant risks of helminth infection for both adults and children with untreated waste water.	Cholera, Typhoid and Shingellosis outbreaks reported from use of untreatedwastewater, seropositiveresponses for *Helicobacter pyrori*; increase in non-specific diarrhea when water exceeds 10^4thermotorerant coli forms per 100ml.	Evidence of parasitic protozoan found on waste water-irrigated vegetable surfaces but not direct evidence of disease transmission.
Farm workers and their families	Significant risk of helminth infection for both adults and children in contact with untreated wastewater increased risk of hookworm infection to workers who do not wear shoes; risks for helminth infection remain especially children even when wastewater is treated to< 1 helminth egg per litre; adults are not at increased risk at this helminth infection.	Increased risk of diarrheal disease in young children with waste water contact if water quality exceeds 10^4 thermo tolerant coli forms per 100ml; elevated risk of *Salmonella* infection in children exposed to untreated wastewater, elevated sero response to non-virus in adults exposed to partially treated wastewater.	Risk of *Giardia intestinalis* infection insignificant for contact with both untreated and treated waste water. In Pakistan estimated to a threefold increase in risk of Giardia infection for farmers using raw wastewater compared with fresh water; increased risk of Amoebosis observed from contact with untreated wastewater.
Nearby communities	Transmission of helminths infections not studied for sprinkler irrigation, but same as above for flood or furrow irrigation with heavy contact.	Sprinkler irrigation with poor water quality (10^6—10 total coli forms/100 ml) and high aerosol exposure associated with increased rates of infection.	No data for transmission of protozoan infections during sprinkler irrigation with wastewater.

Source: (WHO, 2006)

2.4 Methods used for Management of low Quality Water

2.4.1 Use of chemical treatment

Due to negative health effects as the result of pathogenic organisms present in low quality water used for irrigation, several techniques have been employed to decontaminate low quality water produces and to treat low quality water itself. Among the commonly used techniques for decontamination of vegetables and treatment of low quality water as reported by Somi *et al.* (2011) include filtration of various impurities, use of chemicals such as chlorine, chlorine dioxide, ultraviolet treatment, use of vinegar, hydrogen peroxide, citrox, Aussan, Citro Fresh, Nylate, iodine and ozonation (Raicevic *et al.*, 2010 and Robert 2013). They are the current techniques used to decontaminate low quality water and vegetables. However, these mentioned various chemicals employed as disinfectants produce various chemicals known as disinfection by-products in water (DBPs) (Somi *et al.*, 2011). These produced chemicals are associated with harmful health effects to humans such as cancer, nervous system effects, hemolytic anemia and liver effect (Somi *et al.*, 2011). Chlorine for example has the tendency to combine with naturally occurring organic matter to generate DBPs such as halogenated DBPs. In addition, the mentioned chemicals are rarely/not practiced by most farmers and consumers of vegetables in developing countries because of being expensive to purchase making farmers and consumers to rely on washing (with tape water/sometimes untreated water) vegetables with water as the means of decontamination which does not remove helminths eggs and other pathogens completely (Fallah *et al.,* 2011and Amoah *et al*., 2007).

2.4.2 Use of waste water stabilization pond

In developing countries, treatment of low quality water is mainly through the use of waste water stabilization ponds (Quadir, *et al*., 2008). Waste water stabilization ponds are considered the effective means to remove pathogens in low quality water including helminths eggs (WHO, 2006). It is a commonly used means to treat low quality water in most of the developing countries (WHO, 2006). According to Konate *et al*. (2011), waste water stabilization ponds are the effective means to treat municipal waste water. In the same study, no detectable cyst or helminth eggs in the effluent of maturation pond were recorded after treatment of raw waste water in a series of three ponds (anaerobic, facultative and maturation). Moreover, although waste water stabilization ponds are the cost effective waste water treatment technology for removal of pathogenic microorganism, sometimes the ponds may not achieve this function as evidenced by failure of Egerton and Nakuru-kaloleni waste stabilization ponds to achieve the WHO recommendations of pathogenic removal by stabilization ponds in the study by Kimani *et al.* (2009). The failure of waste water stabilization pond to achieve the recommended removal rate of pathogens by WHO, may be attributed to poor design of the pond itself and the environmental surroundings which may comprise of wildlife and other animals hence leading to constant contamination of water with pathogens. However, the use of waste water stabilization pond require enough land and their capacity and effectiveness is challenged by poor settlement plan (causing failure in collecting sewages from households) and rapidly increasing population (leading to generating more wastewater in respect to the capacity of ponds themselves) in most of developing

countries (Msoka and Rajabu, 2009). These challenges require alternative methods/ways of treating the generated low quality water.

2.5 Medicinal Plants Used for Treatment of Low Quality Water

Rural or underdeveloped communities have been depending on simple and relatively cheap technologies such as coagulation for treatment of water as the result of lack of proper water treatment system (Chun-Yang Yin, 2010). Natural plant-based coagulants have been used in different ways for water treatment. The most reported plant based coagulants are from Nirmali seed (*Strychnos potatorum*) (Chun-Yang Yin, 2010), *Molinga oleifera* (Pritchard *et al.,* 2010), Tannin based cationic polymer (Graham *et al*., 2008) Copra (*Cocus nucifera*) (Fatombi *et al*., 2013) and Cactus (*Opuntia cactaceae*). These plants are used for treatment of waters with low to medium turbidity. They are also technically promising as coagulant for dyeing effluents (Chun-Yang Yin, 2010). The increasing usage of the mentioned plants in these communities is attributed to being cheap when compared to chemical coagulants, can be easily processed into usable form and are biodegradable (Chun-Yang Yin, 2010). Compelled by similar challenges posed by modern water treatment systems, researchers in developing countries are striving to come up with alternative to chemical water treatment technologies by using natural plants for treating water pathogens (Melo *et al.,* 2003 and Waller, 1997).

Such natural plants that have been researched include; *Ocimum sanctum, Azadrachta indica* and wheat grass which are effective in removing *E. coli* from water (Somi *et al.*, 2011), lemon juice which is effective against most water bacteria including *Vibrio cholerae* (Aquino and Teves, 1994) and *Moringa oleifera* which is effective

against bacteria some of which are resistant against antibiotic treatment (Ghebremichael and Kebrea, 2004). Moreover, *Moringa oleifera* has been proven to have good water conditioning properties. Though several plants have been used to control bacteria, protozoa and viruses in water, there are limited information on the natural plants used to control helminth in low quality water and vegetables. *Commiphora swynnertonii* is among plants used for several purposes and widely distributed in Tanzania. The plant has been used as medicinal to control ecto and endo parasites, against bacteria, virus and protozoa in humans and animals (Bakari *et al.*, 2012). The plant is safe and having very low residual effect (Bakari *et al.*, 2012). Similar approach of using medicinal plants for controlling pathogenic organisms in water can be adopted to come up with formulations that can be used to control helminths due to low quality water and vegetables in humans by studying activity of *C. swynnertonii* resin extracts against helminths eggs/larvae.

2.6 Description of *Commiphora Swynnertonii*

Commiphora plants are members of family Burseraceae. The generic name '*Commiphora*' is based on the Greek words 'kommi' (gum) and 'phero' (Goji *et al.,* 2009). *Commiphora* species range from small to medium-sized trees or shrubs while some are dwarf size species. The plants can have swollen stems or thick branches which are often thorny. They have increased water storage in their stems (even in species not considered succulent), an adaptation to living in dry environment. Their leaves are generally compound, alternate and normally come into leaves at or before the beginning of wet season and most lose leaves at the beginning of the dry seasons. Most of *Commiphora* species are dioeciously, flowers are very small and

usually yellow or white, in most species flowers are produced in the first half of the dry season and are followed by leaves and fruits if any. Fruiting and flowering are irregular and do not occur every year. Most of seeds produced by *Commiphora* species are hard and dispersed by various means mainly animals, birds and winds. The colors of the stem vary from grayish, green, yellowish pale to pinkish.

2.6.1 Geographic distribution of Commiphora plants

Most of *Commiphora* plants grow on sandy, clay type of soil prefer semi-arid and arid climates and thus can withstand drought or hot seasons for long period. Worldwide distributed in Africa (Native Angola, Botswana, Burkina Faso, Chad, Eritrea, Ethiopia, Kenya, Mali, Mauritania, Mozambique, Namibia, Niger, Senegal, Somalia, South Africa, Sudan, Swaziland, Tanzania, Uganda, Zambia, Zimbabwe), in Asia, for example *C. opobalsamum* (found in India) and *C. caudata* (found in Sirlanka and India) (Paraskeva *et al.*, 2008). In Tanzania, the plants can be found in bush land normally in dry savannah regions such as Dodoma and Manyara (Simangiro District) and known by different names: mbambara, mponda, mturturi, mtwitwi (Swahili), oltememwai (Maasai), mguta (Sukuma), dumbechanda (Taturu) Mzilanzi (Gogo) (Minja, 1999).

2.6.2 Phytochemical content of Commiphora plants

Different species of *Commiphora* studied have been identified to contain different active principles in different morphological parts (Al-Harbi *et al.*, 1993). It has been reported that *Commiphora* resins contain terpenes, sesquiterpenes, esters cumunic aldehyde, eugenol, steroids, resin acids and proteins. Aliyu *et al*. (2007) reported

that ethanolic extract of *C. africana* contained phenolic compounds and tannins, whereas the hexane fraction contained alkaloids triterpenes and sterol. Musa, (2008) found that *Commiphora kerstingii* methanol extract contains alkaloids, tannins, flavonoids, saponins, anthraquiniones and cardiac glycosides.

2.6.3 Studies on antimicrobial activities of Commiphora species

Different studies on the antimicrobial activities of *Commiphora* species have been reported. Bakari *et al*., (2011; 2012) reported *Commiphora swynnertonii* resin extracts to have antibacterial, antifungal, antprotozoal and antiviral activities in chicken and rats as model animals. In those studies the plant crude extracts were effective against *E. coli, Staphylococcus pyogens, Bacillus subtilis, Candida albicans, Staphylococcus aureus, Aspeligilusniger, P. aeruginosa, Salmonella typhimurium.* Furthermore, Aliyu *et al*., (2007) reported that toluene methanol-extracts of the leaves and the roots of *Commiphora quadricincta* plant had antibacterial activity against *Yersinia enterocolitica Staphyloccoccus aureus, E. coli* and *Staphyloccocus epidermidis*. Akor and Anjorin, (2009) reported that ethanolic extract of *Commiphora africana* root had antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. *Commiphora africana* has been used against *Salmonella typhi* (typhoid) (Paraskeva *et al.,* 2008). Musa, (2008) reported that *C. kerstingii* stem bark contains antimicrobial activities against *Staphylococcus aureus, Streptococcus pyogenes*, *Pseudomonas aeruginosa, Pseudomonas fluorescens* and *Bacillus subtilis. C. gileadensia* has also been reported to have antimicrobial activity against *Bacillus cereus* and *Pseudomonas euroginosa* (Iluz *et al.,* 2010).

2.6.4 Other uses of Commiphora species

Uses of *Commiphora* vary from one species to another and the part of the plant itself being used.*C. swynnertonii* has been reported to be used control ticks (Minja, 1999). *C. mukul* as an anti-arthritic agent (Quereshi *et al.*, 1993), *C. molmol* for treatments of inflammatory conditions, as antipyretic, antiseptic, stimulant, mouth wash and to cure tumors and in perfumes (Al-Harbi *et al.,* 1993). Stem bark of *C. kerstingii* used as an antioxidant (Musa, 2008). Stem bark extracts of *C. africana* as an insecticidal and enhancement of liver and kidney functions at lower dose (Aliyu *et al*., 2007; Haffor, 2010). Leaf extracts of *C. africana* for the management of cardiovascular disorders (Adebayo, 2006) and resinous sap extracted from *C. mukul* lowering cholesterol.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Areas

This study was done in Morogoro Municipality in three sites namely; Mafisa, Mzumbe waste water stabilization ponds, and Fungafunga (Morogoro River). Mafisa and Mzumbe were selected purposively because of existence of waste water stabilization ponds which are releasing effluents which are utilized by the farmers for irrigation of vegetables and crops. Fungafunga (Morogoro River) is highly utilized for vegetables production. In this study, therefore, the river was used as the reference study site.

3.2 Study Design

Experimental study design was used and involved observation of the collected and treated samples using microscope to collect data.

3.3 Sample Size

3.3.1 Sample size for low quality water

A total of seventy (70) water samples (one litre for each sample) were collected from the three study sites by simple random sampling. Five (5) samples from the receiving pond of Mafisa waste water stabilization, five samples (5) from the receiving pond of Mzumbe waste water stabilization, fifteen (15) samples of effluents from each of waste water stabilization pond of Mafisa and Mzumbe respectively. For the case of Fungafunga river, a total of thirty (30) water samples were collected. The determination and identification of helminths eggs in the collected water samples was done according to a method described previously by

Bailenger (1979). The water samples were left to sediment for about two hours. The supernant was discarded. The sediment water samples were centrifuged at 1000 rpm for 15minutes. The resulting supernant was discarded and floatation fluid (Sodium Chloride) was added to the pellet followed by vigorous shaking. Examination for the presence of helminth eggs was done according to the method described previously by Baelinger (1979) and microscopic observation of helminth eggs was performed in the Mc Master counting cell at × 10 objective.

3.3.2 Sample size for vegetables

Simple random sampling was conducted to collect 90 samples of vegetables from the field plots, (8 plots at Mzumbe and 10 plots at Fungafunga river) of 100g per each sample of vegetable. Forty vegetable samples were collected from Mzumbe and fifty vegetable samples from Fungafunga. No vegetable samples were collected from Mafisa because farmers did not grow vegetables instead they grew crops such as paddy and maize. Spinach, amaranth, pumpkin leaves, potato leaves and night shade, the commonly grown vegetables in the study area were selected. Each vegetable head was washed in two litres of distilled water by using tween 20 detergent (to reduce the surface tension between helminth eggs and vegetable leaves). The washing water was left to settle in a sedimentation flask for about six hours (Plate 1). The supernant was discarded. The remaining washing water was centrifuged at 1000 rpm for 15 minutes. The resulting supernant was discarded and floatation fluid (sodium chloride) was added to the pellet and then shaken vigorously. Examination for the presence of helminth eggs and larvae was done according to a method described by Baelinger (1979) and microscopic observation

of helminth eggs and larvae was performed in the Mc Master counting cell at × 10 objective lens.

Plate 1: Washing water (after removing vegetables) sedimenting in a sedimentation flask

3.3.3 Collection and preparation of Helminths eggs (Strongyle egg and *Ascaridia galli* egg) for in-vitro experiment

Strongyle eggs and *Ascaridia galli* were selected for the purpose of the study to test the efficacy of extract (C. *swynnertonii* resin) as anthelmintic agent. These worms are readily available (could provide enough eggs for testing the extract) and has been recommended as the suitable models for screening of anthelmintic drug (Mali and Anita, 2007). *Ascaris lumbricoides* and hookworms were isolated in fewer numbers and could not be sufficient for experimental testing of the plant extract.

Fresh faecal samples were collected from the rectum of goats with clinical signs of worm infection. McMaster technique was used to determine egg counts. A portion of collected samples was processed to examine for the presence of helminth eggs in saturated salt (sodium chloride) solution. The eggs were examined and counted in the McMaster slide as explained in MAFF (1986). The remained faecal samples were pooled together. A portion of the mixture was ground for culture that was used for identification of parasite larvae to genus level. The remaining portion of mixture of faecal samples were ground and mixed with water to make homogeneous suspension. The suspension was then sieved through 200, 75 and 25 μm aperture test sieves and the eggs were collected underneath on the 25 μm sieve. The sample in the 25 μm sieve was washed into the test tubes, which were then centrifuged at 3000 rpm for 5 minutes. The supernant was discarded, the deposit was resuspended in a floatation fluid, and the tubes were filled up with the solution to the brim on which the cover –slips were applied, touching the faecal suspension and then centrifuged at 1000 rpm for one minute. The cover slips were removed from the top of the tubes and washed into 25 μm sieve to wash NaCl off eggs. The eggs were then rinsed using a copius water to remove salt, and then collected in clean graduated test tube. The volume of egg suspension was adjusted to a range of about 46 eggs per 100 μL.

To get *Ascaridia galli,* both faecal samples and helminth (*Ascaridia galli*) were collected from slaughtered chickens at the market. The worms were placed in the physiological saline solution to induce laying of eggs. Isolation of helimnth eggs from the faecal samples was done following the same procedures as described above. The volume of *Ascaridia galli* egg suspension was adjusted to a range of about 50 eggs per 20 μL.

3.4 Preparation and Extraction of Plant Materials

3.4.1 Collection of *C. swynnertonii* resin

The plant material (Plate 2a) for the study was collected from Simanjiro District. Resinous material of the plant (Plate 3) was freshly collected from the plant (Plate 2b) and transported to the laboratory at Sokoine University of Agriculture Morogoro for preparation, extraction and testing for its anthelmintic activity.

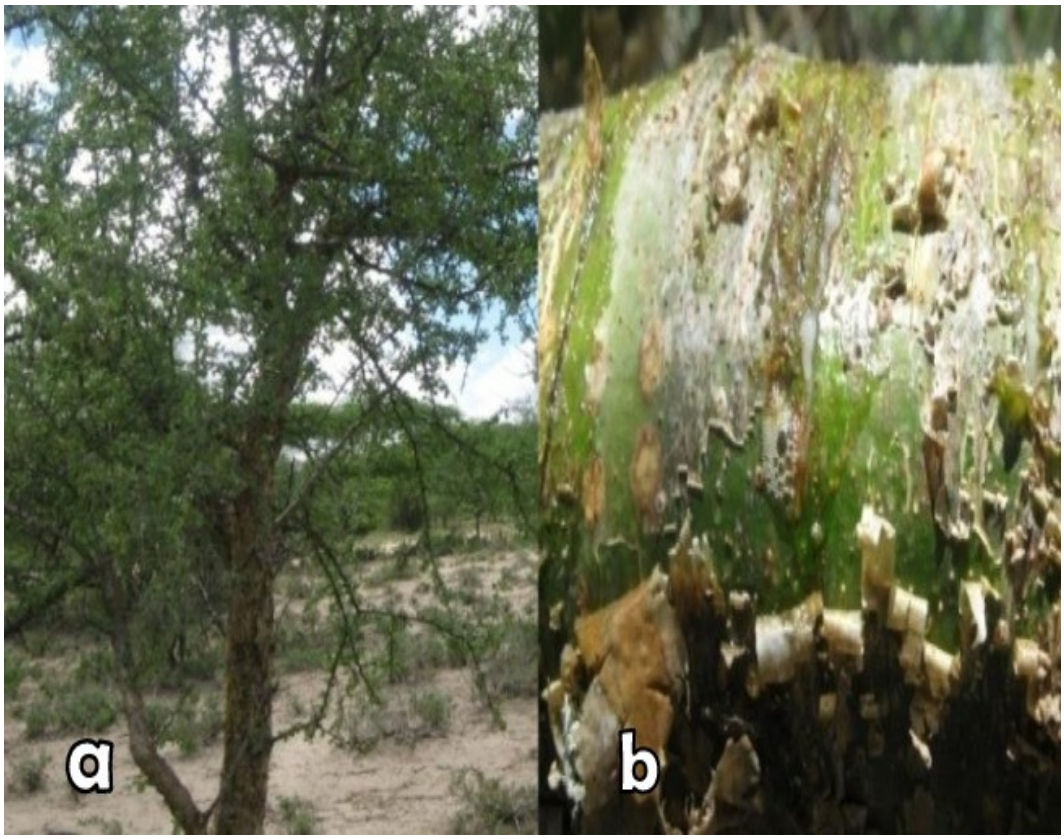

Plate 2: *Commiphora swynnertonii* plant (a); an incision on the stem of *C. swynnertoni*i showing resin harvesting (b)

3.4.2 Preparation and extraction of plant materials

Solvent extraction was carried out (at food science laboratory in the department of food science) according to a method described by Parekh and Chanda (2006) with some modifications by Bakari *et al.,* (2012). Briefly, 500 mL of the resin material

was measured and soaked in 1,000 mL of ethanol (99.8 % v/v) in a conical flask plugged with aluminum foil then vigorously shaken until all the material mixed in ethanol (Plate 4a). This was immediately concentrated on water bath at 50 °C using a rotary evaporator (Heidolph, Heizbad WB, Germany) until all the ethanol was cleared (Plate 4b). The resulting crude extract was stored at 4 °C in airtight bottles until used in an egg hatching and larvae survival assay.

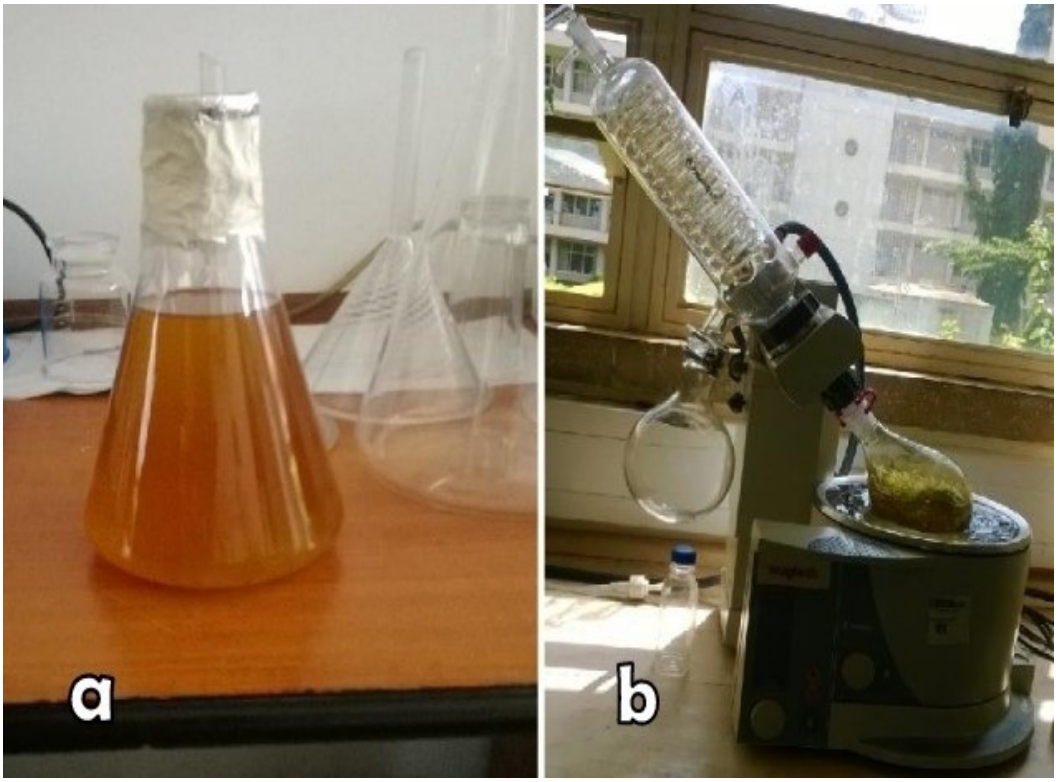

Plate 3: Resin soaked in ethanol (a); Resin being concentrated in water bath using a rotary evaporator (b)

3.5 Determination of ovicidal and larvicidal activity of C. *swynnertonii* resin

3.5.1 Determination of activity of C. *swynnertonii* resin on Strongyle eggs

Flattened bottom micro plates were used to incubate the eggs for in- vitro test. For the test 100 µL of distilled water were distributed into each well from the second well of the plate to the 6 th well. In the first well, 100 µL (100 % V/V) of the test

extract was added, in the second well in which there was 100 µl of distilled water, 100 µL of the extract was added to make 200 µL of solution and was mixed well. From the mixture 100 µL of the solution was transferred to the third well where it was mixed again with distilled water to further dilute the test extract. This was conducted repeatedly to the 6^{th} well the last 100 µL were discarded. Following this serial dilution different concentrations of the test extract (100, 50, 25, 12.5, 6.25 and 3.125 % V/V) were obtained. 20 µL of egg suspension containing about 46 helminth eggs was added to each well with test extract (Plate 5).

For the controls, 100 µL of distilled water was added in six wells followed by addition of 20 µL of egg suspensions to each well. Both test and control were duplicated and then incubated at 27 ˚C for 48 hours. The test was then examined under the compound microscope at ×4 objective. The eggs and larvae were counted separately from each well and recorded.

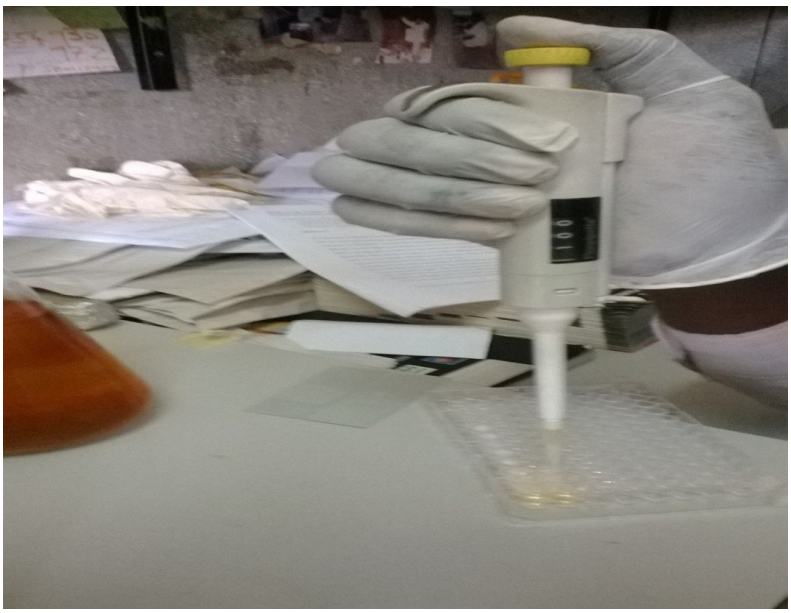

Plate 4: Serial dilution of different concentrations of resin extracts in micro plates

3.5.2 Determination of activity of C. *swynnertonii* resin in *Ascaridia galli* eggs

Following the same above procedure, the flattened bottom micro plates were used to incubate the eggs for in- vitro test. For the test 100 µL of distilled water were distributed into each well from the second well of the plate to the 5th well. In the first well, 100 µL (100 % V/V) of the test extract was added, in the second well in which there was 100 µL of distilled water, 100 µL of the extract was added to make 200 µL of solution and was mixed well. From the resulting mixture 100 µL of the solution was transferred to the third well where it was mixed again with distilled water to further dilute the test extract. This was conducted repeatedly to the 5th well, the last 100 µL were discarded. Following this serial dilution different concentrations of the test extract (100, 50, 25, 12.5 and 6.25 % V/V) were obtained. 20 µL of egg suspension containing about 50 helminth eggs was added to each well with test extract.

For the controls, 100 µL of distilled water was put in six wells followed by addition of 20 µL of egg suspensions to each well. Both test and control were duplicated and then incubated at 27 ˚C for 48 hours to allow development of *Ascaridia galli* eggs to infecyive stage. After 48 hours, both eggs from test and control experiment were transferred and fed to chickens. Seven groups of chickens, each group having five chickens were infected with the treated eggs for eight weeks (Plate 6). The first five groups of chickens were fed with eggs from the concentrations of 100, 50, 25, 12.5 and 6.25 % V/V respectively. The last two groups of chickens, one was fed with helminth eggs (not exposed to the extract) for the positive control and the other one did not receive helminth eggs (for negative control). Lastly the faecal samples from

the chickens were examined for the presence of eggs and the chickens were then slaughtered to examine for the presence of worms in their intestine as the predilection site for the worms (*Ascaridia galli*).

Plate 5: Chickens used in experimental infection with *Ascaridia galli* in their respective treatment groups

3.5.3 Determination of activity of *C. swynnertonii* resin extracts on larvae survival

In order to determine the effect of plant extract on larvae survival, strongyle eggs were hatched according to the procedures described by MAFF (1986). The eggs were placed in sterile vermiculate and wrapped in a gauze. Culturing was made by hanging the wrapped content into a container containing water (for supplying humidity) at room temperature. The culture was left for a week. Harvesting of larvae was made by hanging the wrapped content into sedimentation flask containing water where the larvae were found to sediment at the bottom of the flask (Glass Baermann

Method) (Plate 7). Then larvae were transferred by pippetting into a Petri dish and were identified on the microscope at x10 Objective lens. In order to test the effect of plant extracts, 1000 µL of distilled water were distributed into each of the four ungraduated glass tube beaker. In the first ungraduated glass tube beaker, 1000µL (100 % V/V) of the test extract was added, in the second in which there was 1000 µL of distilled water, 1000 µL of the extract was added to make 2000 µL of solution and was thoroughly mixed. From the mixture 1000 µL of the solution was transferred to the third ungraduated glass tube beaker where it was mixed again with distilled water to further dilute the test extract. This was conducted repeatedly to the 4^{th} ungraduated glass tube beaker, the last 1000 µL were discarded. Following this serial dilution five different concentrations of the test extract (100, 50, 25, 12.5, and 6.25 % V/V) were obtained. 40 µL of larvae suspension containing about 50 larvae was added to each ungraduated glass tube beaker with test extract. For the controls; 1000 µL of distilled water was put into each of five ungraduated glass tube beaker followed by addition of 40 µL larvae suspensions to each ungraduated glass tube beaker. Both test and control were duplicated and then incubated at room temperature for 24 hours. The test was then examined under the compound microscope at ×4 objective over different time interval to record death or survival of larvae.

Plate 6: Glass Baermann method for recovering the larvae from cultured eggs (a) and some of the larvae (3rd stage larvae) obtained from the culture (b).

3.6 Data Analysis

The Data were analyzed using Microsoft excel by simple descriptive statistics and the results reported in numbers and compared using percentages, range and means. Also, regression analysis was employed to compare the association of changing resin concentration and death of larvae/ inhibition of helminth egg hatching. The mortality of the larvae against logarithm of concentration was plotted using Kaleidagraph Synage Statistical software, the regression equations were used to calculate LC_{50} values.

CHAPTER FOUR

4.0 RESULTS

4.1 Identification and Quantification of helminth Eggs in Low Quality Water

A total of 10 wastewater samples (5 samples from each receiving pond at Mafisa and Mzumbe) were examined to determine presence and type of helminth eggs found in waste water stabilization ponds In the current study, it was observed that all samples examined were positive for helminth eggs. The commonly found helminth eggs that were observed were hookworm eggs and *Ascarid* eggs. In all samples examined, helminthes eggs ranged from 40—180 eggs/L with mean concentration of 174.2 ± 100.00 for samples from Mafisa (Table 2). The hookworm eggs ranged from 0 ± 240 with mean concentration of 159.75 ± 111.60 eggs / L for samples from Mzumbe. Samples from Mafisa, *Ascari*s eggs ranged from 134—267 with the mean concentration of 180.2±160 helminth eggs/L. Water samples collected from Mzumbe receiving pond were negative of *Ascaris* eggs

Table 2: **Occurrence of hookworms and *Ascaris* eggs in the receiving ponds of Mafisa and Mzumbe waste water stabilization pond**

Sample	**No of helminthes eggs (range and mean)/L**			
	Hookworm		**Ascarid**	
	Range	*Mean*	*Range*	*Mean*
Raw waste water (Mafisa)	40 – 180	174.2 ± 100.0	134 - 267	180.2 ± 160.0
Raw waste water (Mzumbe)	0-240	159.7 ± 111.6	NIL-	NIL

4.2 Identification and Quantification of Helminthes Eggs in Effluent and Vegetables

A total of 90 vegetable samples and 60 water samples (30 from effluent and 30 from river water) from irrigated areas of Mafisa, Mzumbe and Fungafunga were examined for the presence of parasitic helminth eggs. Results have shown that all the examined vegetable and water samples were negative of helminth eggs. Only eggs of free living nematodes could be observed in the collected water samples from Fungafunga.

4.3 Effect of *C. swynnertonii* Resin on Strongyle Eggs Hatchability

Table 4 shows the percentage inhibition of hatchability of Strongyl*e* eggs by ethanolic resin extracts of *C. swynnertonii*. The results showed that higher concentration of 100%V/V and 50%V/V inhibited hatching of eggs by 100%. The effect of ethanolic resin extract to the hatchability of Strongyle eggs were decreasing with decreasing concentrations in a dose dependent manner (R^2 = 0.62; P=0.013). This was shown by respective hatching of few larvae in the concentrations of 25%V/V, 12.5%V/V, and 6.25%V/V and 3.125%V/V which died few hours following exposure to different resin concentration.

Table 3: Percentage inhibition of helminth eggs hatchability by ethanolic extracts of *C. swynnertonii* resin at different concentrations

Concentration of Resin extract (%V/V)	Percentage (%) Inhibition
100	100
50	100
25	91.3
12.5	79.71
6.25	71.01
3.125	55.79

4.4 Effect of *C. swynnertonii* Resin on Larvae Survival

Table 5 and Fig 1 show the percentage inhibition of larvae survival by different concentrations of ethanolic resin extracts at different time intervals. After culturing of helminth eggs (obtained from goats), the larvae were identified to be of mixed types namely *Haemochus spp*,*Trichostrongylus spp*, *Oesophagostomum spp and Strongyloides spp.* Following exposure of the larvae to different concentration of resin extract, it was observed that higher concentration of 100 % V/V and 50 % V/V was able to inhibit the survival of larvae by 100 % within the first three hours of exposure of larvae to the resin extract. As the concentrations of resin extract decreased, the survival of larvae was prolonged, though after 24 hours of exposure all larvae died (as indicated in Table 5 below).

Table 4: **Larvae survival following exposure with different concentrations of *C. swynnertonii* resin extracts at different time interval**

Concentration (% V/V)	**Percentage larvae death/ time (in hours)**					
	3	**6**	**9**	**12**	**17**	**24**
6.25	50	90	97	97	99	100
12.5	53	96	98	98	99	100
25.0	58	98	100	100	100	100
50.0	100	100	100	100	100	100
100.0	100	100	100	100	100	100

The LC_{50} of larvae exposed in different treatment decreased significantly (R^2= 0.92 and P= 0.0021) with increased time of exposure (Figure 2). Larvae in a (distilled water) control group were able to survive to the end of this experiment.

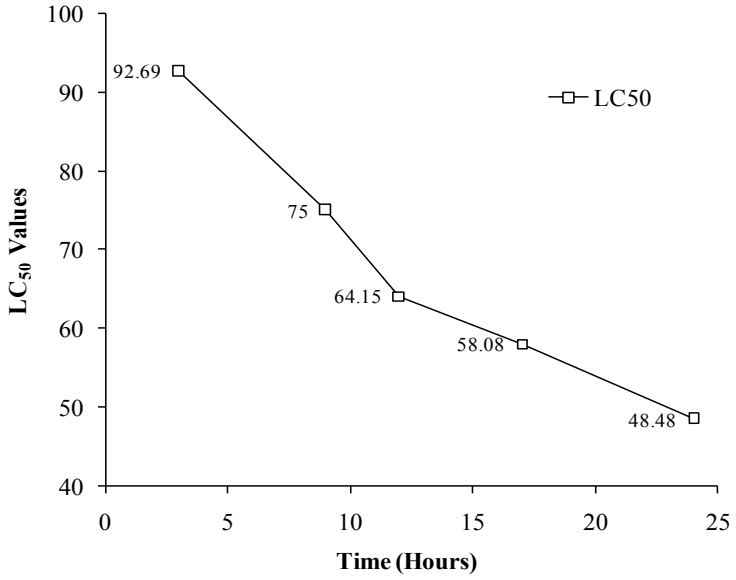

Figure 1: LC_{50} values of resin (on larvae survival) at different time intervals in 24 hours

4.5 Effect of *C. swynnertoni*i resin on *Ascaridia galli* Eggs Hatchability

Table 5 shows the results of the effect of *C. swynnertonii* resin extract on the hatchability of *Ascaridia galli.* Following exposure of *A. galli* to different concentration of the extract and incubation of the eggs at room temperature for 48 hours, the eggs were then transferred and fed to chickens (in a group of five chickens for each concentration). The chickens were used to stimulate hatching as eggs require the host to hatch. After eight weeks, the chickens were slaughtered to examine for the presence of worms. The results show that chickens fed eggs from concentrations of 100, 50 and 25%V/V had no worms while few worms were observed in the chickens fed eggs from concentration of 12.5%V/V. In the experimental group used as positive control, four chickens out of five had larvae while no larvae were observed in five chickens used as negative control.

Table 5: Effect of *C. swynnertonii* resin extract on *Ascaridia galli* eggs hatchability

Concentration (%V/V)		**100**	**50**	**25**	**12.5**
Larvae observed	Test group	-	-	-	+
	Positive Control	+	+	+	-
	Negative Control	-	-	-	-

Note:

- indicates absence of helminth larvae

\+ indicates presence of helminth larvae

Positive control group is the group that was fed with eggs not subjected to resin extract. At the end of the experiment, chickens fed with helminth eggs in this group were found to have worms except for one group (of five) chicken.

In a negative control group, chickens were not given helminth eggs and at the end of the experiment no worms were found in the chicken after being slaughtered.

The test group is the group of chickens that was fed with helminth eggs that initially was treated with different concentration of resin extract (Table 5). In some of concentrations not worms were found in the slaughtered chickens (negative sign) and in the remaining concentrations worms were found to be present.

CHAPTER FIVE

5.0 DISCUSION

The present study has shown that the examined samples of raw wastewater (from the receiving ponds) were positive for *Ascaris* eggs and Hookworms (Table 2). The concentration of the two identified species of helminth varied slightly with *Ascaris* eggs having the mean concentration per litre of 180.2 ± 160 and hookworms 174.2 ± 100.0 for Mafisa and 159.7 ± 111.6 (for Mzumbe). High number of *Ascaris, Ancylostoma, Trichuris* in the receiving ponds has been documented in the study by Konate *et al*., (2011). Kara *et al*. (2004) reported almost similar results in which eggs of *Ascaris lumbricoide*s, *Hymenolepis*, *Trichuris trichura* and *Toxocara spp* were detected in the receiving ponds of waste water stabilization ponds. Furthermore, Shanthala *et al.,* (2007) encountered *Ascaris lumbricoides*, *Hymenolepis diminuta, Hymenolepis nana* and *Enterobius vermicularis* from the receiving pond in the study on removal of helminth parasitic eggs from waste stabilization ponds. It has been reported that the concentrations and varieties of eggs found in wastewater are based on various climatic, socio-economic and demographic factors and are closely linked to their origins (domestic water, industrial water, slaughterhouses, storm water) (Hajjami *et al.,* 2012). WHO (2006) reported that helminth eggs become more abundant in hot weather due to high temperature, humidity, oxygen and sunlight that favor the maturation of these helminths.

From the examined samples of effluents and river water, all were found negative of helminth eggs. This reflects on the effectiveness of stabilization pond in removing helminths eggs. Similar results has been reported by Konate *et al*. (2011) who did not found helminth eggs in the effluent (of maturation pond) after a series of

treatment of wastewater containing *Ascaris, Ancylostoma, Trichuris* and *Trichostrongylus* eggs in the stabilization pond. Amahmid *et al*. (2002) did not find helminth eggs in the outlet (effluents) from wastewater stabilization pond. Hybrid stabilization pond has been proposed by Mara (1998) to produce microbiologically safe effluents to be used for both restricted and unrestricted crop irrigation. The results from the current study also supports the findings of Kouraa *et al.* (2002) who found that combined stabilization ponds have excellent performance in removing helminth eggs by 100 % and that they produces high quality effluents which can be used for unrestricted irrigation.

The collected, processed and examined vegetable samples (from the study sites) were negative for helminth eggs. This was directly linked to the quality of the effluents that was being generated from the stabilization pond and being used for irrigation of vegetables in the area. The quality of effluent in terms of microbiolocal safety has been emphasized for unrestricted irrigation (Mara and Pearson, 1998). Moreover, the results in this study support the findings of Kouraa *et al.* (2002) who found combined stabilization ponds to have excellent performance in removing helminth eggs by 100 % and that produces high quality effluents which can be used for unrestricted irrigation. It is therefore evident that irrigation water quality influences greatly the extent to which vegetables can be contaminated with helminth eggs and other pathogenic microorganism (Okafo *et al.*, 2003).

The anthelmintic activity of *C. swynnertonii* resin extract is probably being reported for the first time. Higher concentrations of *C. synnertonii* resin showed high activity on Strongyl*e* eggs based on *in vitro* hatchability study. This showed that change in

the dosage concentration had influence on the hatchability of eggs. At a concentration of third dilution (12.5 % V/V), few eggs hatched and the larvae died within an hour of exposure to the extract. In the fourth and fifth dilutions (6.25 and 3.125 % V/V) also few eggs managed to hatch and the larvae survived for about 6 hours before they died. This showed that, at lower concentrations of 12.5, 6.25 and 3.125 % V/V , the extract was able to inhibit hatching of eggs at a lower rate compared to concentrations higher than 25 % V/V. This indicated that at higher concentrations (> 25 % v/v) the extract was able to either kill the eggs or suppressed further development of eggs. In the control experiment, most of the eggs managed to hatch, very few did not hatch and this could be attributed to eggs either being not fully developed or to being dead before exposure to the extract. Similar results were reported on other *Commiphora spp.* and strong activity against earthworms and *Ascaridia galli* were observed at higher concentrations for all aqueous extract (leaf, stem and bark) from different plant parts of *Commiphora africana* (Gbolade and Adeyemi, 2008). Another study done by (Feven *et al*., 2010) showed that aqueous extract of *Commiphora myrrha* was effective against earthworms as compared to the standard mebendazole.

In the present study it was observed that at concentrations higher (over 25 % v/v) managed to inhibit the development of eggs of *Ascaridia galli* as it was revealed by absence of worms in slaughtered chickens after the period of eight weeks of hatching stimulation in chickens. Chickens fed with eggs exposed to lower concentration of resin extract had worms in their intestinal contents assuming that at lower concentration of resin, eggs were able to develop into larvae stage and finally

to adult worms in chicken. Worms could be observed with naked eye during slaughtering of chickens. Chicken which were used as control groups had a number of larvae (that ranged from 8-12) after slaughter. This is an indication that the *A. galli* eggs used in this experiment were viable as the hatched and developed into worms.

Exposure of those larvae to higher concentrations of the resin (100, and 50 %V/V) resulted into death of all larvae within 3 hours. This could be due to effect of resin extract that resulted into the death/killing of larvae. The survival rate of larvae increased with decreased resin concentration. The LC50 of larvae exposed in different concentration decreased significantly (R^2 = 0.092 and P = 0.0021) with increased time of exposure, although after 24 hours all larvae in their respective treatment concentration died. This shows that at lower concentrations the extract worked slowly that maximum exposure time was required to result into death of the exposed larvae. Overall, this showed that resin extract had strong activity in causing death of exposed larvae and inhibits egg hatching at higher concentrations.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The results of this study have shown the presence of helminth eggs in the receiving ponds from both study sites (Mafisa and Mzumbe). Moreover, the effluents from wastewater stabilization ponds had no helminth eggs. This indicated that the waste water stabilization ponds were effective in removing helminths eggs by 100 %. It was also observed that the river water used for vegetable irrigation at Fungafunga was free from infectious helminth eggs. The vegetables irrigated with effluents from Mzumbe waste water stabilization pond and river water from Mafisa were not contaminated with helminths eggs. This shows that the effluents and river water being used for irrigation of vegetables were free from helminths eggs and hence the reflection on the excellent performance of stabilization pond in removing helminth eggs (for Mzumbe wastewater stabilization pond).

The absence of helminth eggs in vegetables irrigated with river water indicated that the water being used was either not contaminated with helminth eggs or given the large volume of running river water, there was more dilution for small contaminations of helminths eggs (if at all could be there) to be detected in vegetables and the water itself.

The bioactivity of *Commiphora swynnertonii* resin extract against helminth eggs (Strongyle eggs and *Ascaridia galli*) and larvae (*Haemonchus spp, Trichostrongylus spp, Oesophagostomum*, and *Strongyloides spp*) has been demonstrated. Ethanolic

extract of *Commiphora swynnertonii* resin has been shown to be effective in inhibiting helminth eggs hatching and larvicidal activity (in higher concentration). With this study, it can be concluded that *Commiphora swynnertonii* resin has anthelmintic activity.

6.2 Recommendations

Although the study did not detect helminth eggs from the examined effluents and vegetables, studies on other pathogenic microorganisms such as bacteria, virus and protozoa and chemical contaminants are recommended before declaring the effluent being generated to be safe for vegetable production.

Since the effluents and vegetables were free of helminth eggs, farmers need to be made aware of other possible sources (for example, fertilizers, general individual hygiene and domesticated animals) which can result to vegetable contamination apart from water which have been shown to be free of helminth eggs.

Good management of waste stabilization ponds (for example continuous desluging and maintenance of storm water drainage canals) has contributed to the production of high quality effluents with no helminth eggs. For future good performance of the stabilization ponds such management practices are emphasized by the study.

From the results of bioactivity of *Commiphora swynnertonii* resin it is recommended that more studies should be carried out to ascertain the active constituents responsible for the demonstrated activity. This will help in formulation and

packaging of products to be used as anthelmintics. Moreover, the study recommends *in vivo* studies on the anthelmintic activity of *Commiphora swynnertonii* resin in order to establish its effect in animals when used for controlling helminths.

Since *Commiphora swynnertonii* has been reported to be effective against bacteria, virus, protozoans and now helminths, efforts to study the possibility of generating a product to be used in controlling wide range of pathogens commonly found in low quality water is recommended.

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