EFFECT OF CORN OIL EMULSION FORMULATION OF *Cercospora piaropi* Tharp AND *Myrothecium roridum* Tode Fries IN THE CONTROL OF WATER HYACINTH UNDER GREENHOUSE CONDITIONS IN KIBOS, KENYA

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in Maseno University or any other university. All sources of information have been acknowledged by means of references and citation.

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DEDICATION

I dedicate this Thesis to my Uncle Prof. Wanjala, wife Irene Gathoni, children Lachelle-Sabina, Lynnpeace Wanja, Favour Lavender and Leon Blessing.

ABSTRACT

Accelerated settlement and industrialization around Lake Victoria have led to alarming pollution levels in the lake. Water hyacinth (*Eichhornia crassipes* [Mart.] Sol. Laub.) that thrives best in polluted waters has therefore found a favourable niche in the lake. Though the water hyacinth is a bio cleaning agent that would be necessary to have in such waters, its growth and biomass accumulation makes it have a propensity for blocking the economic use of the lake, hence the need for its control. Control effort has involved the development of vegetable oil formulations. These control methods have lacked standardized inoculums concentration. There is therefore a need for coming up with a pathogen formulation in locally available material that has known inoculum concentration for easy use by the stakeholders. There are unanswered questions regarding the efficacy of mycoherbicides with varied inoculum concentrations. There are limited studies that have been done to test the host reaction of water hyacinth, in terms of leaf spot intensity, under varying inoculum levels. The purpose of this study was to determine the effect of corn oil emulsion formulation of *Cercospora piaropi*Tharp and *Myrothecium roridum*Tode Fries in the control of water hyacinth under greenhouse conditions in Kibos, Kenya. The objectives of the study were to determine the effect of corn oil spore concentrations of *C. piaropi* and *M. roridum*on; disease severity, disease incidence, relative shoot length and relative biomass of water hyacinth. The study was carried out at KALRO Kibos greenhouse located at latitude 0^0 37' S and longitude 37^0 20' E. The greenhouse conditions were25 to 30^0C and 22 to 27^0C temperature averages during the day and night respectively and 60 to 69% relative humidity. Plants symptomatic to *C. piaropi* and *M. roridum* leaf spots were sampled from Dunga Beach of Lake Victoria. The pathogens were aseptically cultured on PDA until sporulation, harvested and formulated in corn oil. Through serial dilution, corn oil formulations of $1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$ and $1x10^9$spores/ml of each of the pathogens were attained to form 6 treatments. Healthy water hyacinth plants with 50 to100 cm^2leaf area were selected for the trial. The plants were separately misted with100ml of the corn oil formulations using hand sprayers held at 45^0 and 30cm from the plants. Control plants were not inoculated but misted with 100mlcorn oil emulsion without the pathogens. Both inoculated and control plants were placed in3 foot diameter and 1.5 cm depth basins filled with 20 liters of aged tap water. The experiment was laid out in completely randomized design with three replications. Disease severity (DS) score, disease incidence (DI),relative shoot length (*R*)and relative biomass (*I*) were determined at 2, 4 and 6 weeks after inoculation.DS scores were converted to area under disease progress stairs (AUDPS). Analysis of variance on mean DS, AUDPS, DI,*R* and *I* was done using PRO GLM in SAS (Institute, Inc.1999) computer software and LSD ($p \leq 0.05$) used for mean separation. Disease severity scores for both pathogens showed a significant ($p \leq 0.05$) increase as the concentration of spores in the formulations increased. The highest DS score was 5.00and 4.67 for *C. piaropi* and *M. roridum* respectively at week six at concentrations of $1x10^8$ spores /ml. The respective AUDPS values for the two pathogens were significantly different with 20.67 for *C. piaropi* and 18.50 for *M. roridum.* The highest DI values were 82.23% and 88.9% for *C. piaropi* and *M. Roridum* respectively at week six at$1x10^9$ spores/ml. Compared to the control, *Cercospora piaropi* had relative shoot length of 55.07% while *M. roridum* had 51.93% for $1x10^9$ spore/ml at week six. Relative biomass was significantly increased to a high of 73.53%for *C. piaropi*at $1x10^9$ spore/ml. In conclusion, that *C. piaropi* and *M.roridum*in corn oil formulation were deemed feasible mycoherbicides in the greenhouse with spore concentrations of $1x10^8$and $1x10^9$spores/ml being most effective. Therefore any of the two bio pathogens can be formulated in corn oil at spore concentrations of $1x10^8$or $1x10^9$spores/ml for adoption in the control of water hyacinth in Lake Victoria.

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LIST OF ABBREVIATIONS AND ACRONYMS

2, 4-D	2, 4-Dichlorophenoxyacetic acid
Anon.	Anonymous
APS	Associated Press Services
ARC	American River College
AUDPS	Area under Disease Progress Stairs
CAB	Commonwealth Agricultural Bureaux
Cd	Cadmium
Co	Cobalt
Cr	Chromium
CRD	Completely Randomized design
DAP	Di-ammonium Phosphate
FAO	Food and Agricultural Organization
Hg	Mercury
IOBC	International Organization for Biological Control
KALRO	Kenya Agriculture and Livestock Research Organization
L	Liter
LSD	Least Significant Difference
LVEMP	Lake Victoria Environmental Program
N	Nitrogen
OCWCD	Orange County Water Conservation Department.
Ni	Nickel

P	Phosphorus
PC	Personal computer
Pb	Lead
PDA	Potato Dextrose Agar
SB	Short bulbous
TN	Tall non-bulbous
UK	United Kingdom
UNEP	United Nations Environmental Program
USA	United States of America

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CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Accelerated settlement around Lake Victoria and industrialization have led to alarming pollution levels in the lake waters (Otieno, 2016).The invasive water hyacinth (*Eichhornia crassipes* [Mart.] Sol. Laub.) has been rapidly induced by high soil erosion and nutrient runoff, urban and industrial pollution and atmospheric deposition (Nasution *et al*., 2016).The weed growth in terms of stem elongation and biomass accumulation makes it have a propensity for compromising the economic use of the waterways(Tobias *et al*., 2019). Though the weed is a bio cleaning agent that would be necessary to have in such waters according to Ambastha *et al*. (2017),its growth and biomass accumulation have given it the ability of blocking the economic use of the lake, hence the need for its control. It has however continued to defy control methods as reported by Mengist and Moges (2019) so that the spate of conferences on the blue economy will remain just that, if the country continues ignoring the devastating ecological and economic challenges the water hyacinth poses for the people around Lake Victoria (Bwire, 2018). In spite of the application of control methods, Ongore *et al*. (2018)and Segbefia *et al*. (2019) have reported that water hyacinth has remained resurgent and difficult to manage hence affecting livelihoods. High proliferation coupled with high seed production rate, ability for both sexual and asexual reproduction, high expenses have made physical control and herbicide application to be non-sustainable (Worku and Sahile, 2018).

Much research on water hyacinth bio control has been devoted to the development of new mycoherbicide formulations using vegetable oil as the carrier material (Berestetskiy and

Sokornova, 2018). These efforts have been given impetus by the fact that water hyacinth has been ranked as the worst water weed in the world and the main invasive weed in the local water bodies especially in Lake Victoria (Asmare, 2017). The bio control efforts have however hit a snag due to among other reasons; poor efficiency of application, low efficacy and lack of optimized product quantity and quality (Su *et al*., 2018).The efforts, particularly with fungal pathogens have not generated data on standardization of inoculum concentration for improved disease severity, incidence and general adverse effect on water hyacinth growth. Piyaboon *et al.* (2016) for example in pathogen city tests that involved disease severity scoring and the extent of disease incidence identified *M. roridum* as being effective against water hyacinth. The shortcoming in this study was that only $1x10^9$ spores /ml was used. From the results of the study, it could not be ascertained if this spore concentration was the optimum level for maximum disease severity, disease incidence, and suppressed biomass and shoot elongation.

Proliferation of biomass, luxuriant shoot elongation and ecological adaptability are the properties of water hyacinth that make it pose a threat to the economical use of the lake waters (Worku and Sahile, 2018). Putting a check on these properties would go a long way in opening up the lake waters for economic use (Eid and Shaltout, 2017). Bolton *et al.* (2013) made applications of *C. piaropi* to water hyacinth plants and the results indicated that treatment with this pathogen applied in the surfactant Silwet L-77 provided the best levels of biomass reduction and disease severity. However, this study did not involve the variation of spore concentration and therefore the optimal level of *C. piaropi* inoculum for recommendation could not be ascertained.

As a way of addressing this shortcoming, studies have been carried out on using various vegetable oils from plants (Boyette and Hoagland,2013). In the studies corn, peanut,

rapeseed, sunflower, soybean and safflower oils were used as formulations for pathogens. Results with *Alternaria* spp and *Colletotrichum truncatum* (Schw.)showed that when it came to disease incidence (DI) and disease severity (DS), corn oil, peanut, soybean and sunflower had both DI and DS values that showed an increase but were not significantly different from each other. However, these studies did not include variation of spore concentration for the pathogens that were tested.

Many surveys for pathogens as likely bio control agents for water hyacinth have brought to light the need for using strategies that enhance infectivity of the pathogens upon the weed (Firehun *et al*., 2015). Fungi are the dominant causal agents of plant diseases (Doehlemann *et al*., 2017). The potential for fungal bio pathogens for water hyacinth control has received a lot of attention (Admas *et al*., 2017). Fungal pathogens such as *C. piaropi* and *M. roridum* that are widely distributed, most virulent as a result of their propensity for formation of toxins and with reported characteristics of host specificity have increasingly been the subject of focus (Okunowo *et al*., 2019). *Cercospora piaropi* and *M. roridum* fungi have been successfully isolated from infected water hyacinth plants and tested for efficacy (Tegene *et al*., 2014). Biological control using these fungi has proved to be less labour and equipment intensive with a potential of being self-sustaining (Sharma *et al*., 2016).

In the past, there have been research activities on water hyacinth control using arthropod pests (Dutta and Puja, 2017). Management of water hyacinth with *Neochetina* weevils has hit a snag due to the difficulties in breeding the insects (Venter *et al*., 2013).In an attempt to address this shortcoming, the use of virulent toxin producing fungal bio pathogens has gained prominence as a way of complimenting the control methods that involve weevils (Firehun *et al*., 2015).Among the bio pathogens, controlled experimental studies have

confirmed that *Acremonium zonatum*, *Alternaria eichhorniae*, *A. alternata, Bipolaris* spp., *Cercospora piaropi* (*Cercospora rodmanii*), *Drechslera hawaiiensis*, *Fusarium equiseti*, *F. pallidoroseum, Rhizoctonia solani, Myrothecium advena, Sclerotinia sclerotiorum,* and *Ulocladium atrum* significantly reduce water hyacinth biomass (Ambastha *et al*., 2017; Safeena and Queene, 2017). However, information on the optimum level of concentration for these pathogens is lacking. Further, effort at biological control with pathogens has faced a number of challenges including technological, economic, and biotic and a biotic factors (Charudattan, 2014). The fungus *Cercospora* has been extensively studied and was patented by the University of Florida. It has been released in South Africa as classical bio control agent for water hyacinth (Firehun *et al*., 2015). Evaluation of this pathogen locally in suitable formulation materials that are locally available has not been tried in spite of this being a matter of urgency in the face of the current water hyacinth menace. While basically all pathogens interfere with primary plant defense, necrotrophs such as *Cercospora* and *Myrothecium* secrete toxins to kill plant tissue. Hence, *C. piaropi*and *M. roridum* isolates have potential for use in water hyacinth control they produce a toxin; cercosporin and roridin respectively. Cercosporin is able to lower the growth rate of water hyacinth (To-Anun *et al*., 2011). It is a photosensitizing metabolite with host non-specific toxicity that acts during pathogenesis. Its toxic effects are the result of the production of light activated oxygen forms (Daub and Ehrenshaft, 2000). Cercosporin toxin incites a debilitating leaf spot disease causing the infected leaves to die back from the tip. Severely infected plants become chlorotic and stressed. In advanced stages of the disease, root deterioration occurs. With the spread of the disease, the plant population begins to decline, and open water appears where previously there had been dense stands of water hyacinth (Tegene *et al*., 2014).

Myrothecium roridum produces phytotoxin roridin A and roridin E that was reported to be similar to paraquat used for water hyacinth control (Okunowo *et al*., 2019). It is a facultative parasite that co-migrates with its water hyacinth host. This pathogen causes zonate necrotic leaf spots that have a yellow center zone. Studies by Charudattan (2014) indicate that in severe infection, the spots coalesce to cover the entire lamina, hence, killing the entire plant. In plate culture, colonies are zonate with a yellow center and a whitish edge. The culture turns black upon sporulation.

It has been reported that foliar pathogens working under natural disease pressure generally do not have the capacity to kill water hyacinth plants completely and quickly unless they can be used in conjunction with efficacy-enhancing formulations and adjuvants (Mutebi *et al*., 2013; Charudattan, 2014). A formulation is the form of a specific product that is used to control a pest (Libs and Salim, 2017).Another method of improving pathogen efficacy is the use of selected adjuvants that include surfactants, stickers, sun screen agents, humectants, anti-evaporation agents and micro-nutrients that may improve bio herbicidal efficacy through various mechanisms (Boyette and Hoagland, 2013).Recent trends in the implementation of bioherbicide use in the control of water hyacinth have depended primarily on several strategies. The use of bio herbicides has been stimulated as part of the search for alternatives to chemical control, as the use of environmentally friendly formulations minimizes hazards resulting from herbicide residues (Dagno *et al.,* 2012). Inert solid carriers, alginate granules, invert emulsions and oil-in-water emulsions have been considered as vehicles for mycoherbicides as they reduce or eliminate the dew requirement for fungal colonization (Berestetskiy and Sokornova, 2018).

A commonly used formulation material has been corn oil, a vegetable oil that is gotten mostly by aqueous extraction methods from maize germ (Shende and Sidhu, 2014). It is

generally less expensive than most other types of vegetable oils, harmless to the environment, highly biodegradable and used domestically in foods (Kaltragadda*et al*., 2010). A quality that qualifies corn oil as a formulation agent is its low viscosity that makes dispersal of spores within the oileasy during spore harvesting and formulation(Boyette and Hoagland, 2013). Rugalema and Mugwe (2018) reported that Kenya consumes about 300,000 metric tons of maize per month. This is equivalent to roughly 3.3 million 90kg bags.

In the past studies of *C. piaropi* and *M. roridum*, there have remained exigent unanswered questions regarding the effect of varying spore concentration within formulations on the efficacy of the bio pathogens on water hyacinth. For instant Boyette and Hoagland (2013) in studies involving *Colletotrichum truncatum,* a novel bio pesticide formulated as a corn oil based mycoherbicide did not include the effect of variation of spore suspension on disease infection. Limited information is available on the effect of *C.piaropi* and *M. roridum* on water hyacinth leaf spot disease intensity and physical stress in terms of biomass and length of shoot. Disease intensity encompasses disease severity and disease incidence. Disease severity is the percent of the relevant host tissue or organ covered by symptoms or lesions of the disease (Chiang *et al*., 2017). Disease incidence on the other hand is the percentage of diseased plants or plant parts in the sample. Disease incidence values can be used to better understand the relationship between spore inoculum and subsequent disease development on plants (Ghanbarnia *et al.*, 2011). The measurement of disease intensity plays a key role in plant pathology in that without quantification of disease, no studies in epidemiology, plant disease survey and their application would be possible (Nutter, 2007). Disease assessment expresses the effects of various treatments or factors on disease in experiments. The severity of the damage caused by fungal pathogens

against water hyacinth has been evaluated in the greenhouse and in the field (Mutebi *et al.*, 2015). A common way of evaluation for diseases that attack the leaves of the plant is to determine the relative amount of leaf area that is showing symptoms usually expressed as a percentage (Chiang *et al.*, 2017).Past studies have not addressed the role of varying inocula concentration on disease severity, incidence and general growth of water hyacinth.

The purpose of this study was to determine concentration specific use of refined corn oil mycoherbicides of *C. piaropi* and *M. roridum* for the enhanced leaf spot disease infection on water hyacinth weed that deters the growth and biomass of the weed that opens up the water bodies for economic use.

1.2 Statement of the Problem

There has currently been a lack of information on the effect of adjusting concentration of the bio pathogens,*C. piaropi* and *M. roridum* included, on the growth of water hyacinth. It has therefore not been possible to recommend a corn oil based mycoherbicide for the management of water hyacinth as salient information on appropriate spore concentration has been lacking. Past studies with corn oil as formulation material have not made clear the relationship between spore concentration and disease severity. Boyette and Hoagland (2013) working with corn oil for formulation of *C. truncatum*, a novel bio pesticide did not include the effect of variation of spore suspension on the resultant disease intensity.

The relationship between both *C. piaropi* and *M. roridum* spore inocula and subsequent disease development on water hyacinth has not been understood due to lack of disease incidence values obtainable from different inoculum concentrations. Therefore the spore concentration level for both *C. piaropi* and *M. roridum* that can stimulate disease

incidence that would be required to incite an epidemic within the water hyacinth mats is not known.

Slowing down water hyacinth growth in order to check its spread to uninfected areas has not been possible because knowledge on the necessary spore inoculum concentration to curtail the spread is lacking. It is therefore currently difficult to recommend a concentration specific corn oil based mycohercide that can address the resurgent nature of the water hyacinth weed.

The effectiveness of control measures based on biomass estimation has not been assessed even though it has been reported by Robles *et al*. (2015) that biomass estimation is useful in determining the effectiveness of such measures. Moreover, knowledge on the inoculum threshold that can have a reducing effect on the weed biomass that can allow for the economic use of the waters is not available at the moment.

1.3 Justification

Current control measures for water hyacinth have not been effective (Borokini and Babalola, 2012). There is therefore a need for more effective methods that are not only environmentally friendly but sustainable too. Contemporary biocontrol efforts have not been successful due to among other reasons; lack of optimized product quantity and quality (Su *et al*., 2018).It is necessary to formulate *C. piaropi* and *M. roridum*pathogens for use by stakeholders for the management of water hyacinth (Berestetskiy and Sokornova, 2018) since formulation has the potential of being effective, environmentally friendly and sustainable control means for water hyacinthas reported by Hoagland (2013).It also increases efficiency of application The bio control efforts have however hit a snag due to among other reasons; poor efficiency of application, low efficacy and lack of

optimized product quantity and quality (Su *et al.*, 2018).Formulation of *C. piaropi* and *M. roridum* in corn oil is expected to offer a more effective and sustainable solution to the problem of water hyacinth in the local water bodies that will be easily available to stakeholders in the control of water hyacinth. Formulation will help to solve a major problem inherent in using leaf fungi for weed control; the fact that such fungi require optimal relative humidity for germination in order to infect the weed (Admas *et al.*, 2017). The effect of the two mycoherbicide on water hyacinth would be determined on the basis of their ability to reduce development of the weed, fewer living leaves and more dead leaves on individual plants and no production of new clones.

The study was meant to generate information on the possibility of using corn oil as efficacy enhancing formulation material for *C. piaropi* and *M. roridum* bio agents that could be used in not only killing the weed but also slowing down its fast growth that was found to be responsible for the weed's resurgence. The research findings will be very useful to local communities living around water bodies such as Lake Victoria that are facing water hyacinth problems.

1.4 Objectives

1.4.1 General Objective

To assess the effect of corn oil spore concentration of *C. piaropi* and *M. roridum*on the severity, incidence, relative shoot length and relative biomass of water hyacinth plants under greenhouse conditions in Kibos, Kenya.

1.4.2 Specific Objectives

(1) To determine the effect of different corn oil spore concentrations of *C. piaropi* and *M. roridum* on the severity of leaf spot disease of water hyacinth plants.

(2) To determine the effect of different corn oil spore concentrations of *C. piaropi* and *M. roridum*on the incidence of leaf spot disease of water hyacinth plants.

(3) To determine the effect of different corn oil spore concentrations of *C. piaropi* and *M. roridum*on the relative shoot length of water hyacinth plants.

(4) To determine the effect of different corn oil spore concentrations of *C. piaropi* and *M. roridum* on the relative biomass of water hyacinth plants.

1.4.3 Hypotheses

(1) Increase incorn oil spore concentrations of *C. piaropi* and *M. roridum*do not significantly reduce leaf spot disease severity in water hyacinth.

(2) Increase incorn oilspore concentrations of *C. piaropi* and *M. roridum*do not significantly reduce leaf spot disease incidence in water hyacinth.

(3) Increase incorn oil spore concentrations of *C. piaropi* and *M. roridum*do not significantly affect relative shoot length of water hyacinth.

(4) Increase incorn oilspore concentrations of *C. piaropi* and *M. roridum*do not significantly affect relative biomass of water hyacinth.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin of water hyacinth

According to Bhattacharya *et al*. (2015), water hyacinth has established a niche in diverse geographical locations as a result of it being introduced for its beneficial characteristics. Human activity is responsible for its spread into Lake Victoria from River Kagera in Rwanada (Waithaka, 2013). The plant is native to South America (Higuti and Martens, 2016) though It has been present in Africa, particularly in the River Nile, since the 1870s, being only reported as a major problem in the 1980s (Koutika and Rainey, 2015). The problem has been made worse by the lack of natural enemies, abundance of space, suitable temperature conditions, and abundant nutrients (Higuti and Martens, 2016).

2.2 Botany of Water Hyacinth

Water hyacinth is an invasive fresh water weed that belongs to the Kingdom Plantae, Order Commelinales, Family Pontederiaceae (Bhattacharya*et al*., 2015). The weed is found in different morphological growth forms, different leaf architecture with high plasticity in clonal groups (Higuti and Martens, 2016). Its scientific name synonyms are: *Piaropus crassipes* (Mart.) Britton (Acevedo and Strong, 2012)and *Eichhornia speciosa*Kunt (Bhattacharya*et al*., 2015).The weed has a capacity to grow to a shoot length of a meter within one week (Villamagna and Murphy, 2010). It is this rapid growth that usually renders many control measures ineffective. Its foliage consists of wide 5 cm broad leaves and swollen spongy leaf stalks on a fibrous root system. These are adaptations that further make the weed resilient to control measures (Mujere, 2015).

Water hyacinth sexual reproduction structure consist of light purple flowers that have a darker blue/purple and yellow center(Eid and Shaltout, 2017). These are borne in dense spikes projecting above the plant. Each of the flowers on a stalk remains open for one to two days before beginning to wither. When all the flowers on a plant have withered, the stalk gradually bends into the water. Seeds are released from capsules at the base of each dead flower in three to four weeks (Koutika and Rainey, 2015). Asexual reproduction involves shoots produced vegetative that remain attached to the parent plant until broken off by the wind or other physical forces. In warm climates, vegetative reproduction is rapid and enables the formation of large, dense rafts of plants within a short time. Attempts at physical control have hit a snag due to shredded pieces re-growing rapidly via vegetative means (Borokoni and Babalola, 2012). Management studies aimed at incorporating disrupting the growth potential of water hyacinth plants as a way of slowing down its spread have not been adequately carried out.

2.3Ecology of Water Hyacinth

Water hyacinth can grow over a wide range of conditions with both tropical and temperate conditions being suitable for its growth (Kyser, 2017). Optimum growth temperature for its growth is 28^0 to 30^0C (Burton *et al*., 2010). Growth is hindered when temperatures rise above 30^0C and fall below 10^0C. Suitable pH range for its growth is 4.0 to 8.0. Large amounts of nitrogen and phosphorous are required for its growth. Polluted slow flowing with high amounts of nitrogen, phosphorous and potassium or stagnant fresh water has been found to be most suitable for infestation (Burton *et al*., 2010).There has been a lack of manipulation of pathogenic natural enemies such as fungal microorganisms that can be adopted in an augmentative approach. This has made the proliferation of the weed to spiral out of control as reported by Higuti and Martens (2016).

The fast growth of the weed in terms of biomass allows it to build huge populations in its ranges of introduction, developing dense mats on the surface of the water to become a major problem (Asmare, 2017). It also creates anoxic (total depletion of oxygen levels) conditions, inhibiting decomposing plant material, raising toxicity and disease levels to both fish and people (Gichuki *et al.*, 2012). It has highly efficient survival strategies and can easily overcome most control strategies. Sharma *et al.* (2016) reported that the weed's biomass accumulation, shoot: root ratio and plant height increase in accordance with increase in concentrations of nitrogen and phosphorous. Since there is a lot of inflow of these elements into the local water systems from agricultural land (Admas *et al.*, 2017), controlling the weed has become difficult, hence, the need for sustainable control strategies. Ability to store nutrients which can be used during later stages of the life cycle is another adaptation of water hyacinth that constraints its control. The weed can even survive on damp soil for many days and displays both sexual and vegetative reproduction characteristics. The need for implementation of control measures that attempt to lower the weed's biomass in order to allow for the economic use of the waters in the face of diminished biomass has not been addressed as yet.

2.4 Economic importance of Water hyacinth

Water hyacinth, the worst freshwater invasive weed in the world is known, by its accelerated growth and biomass accumulation, for impeding navigation, water harvesting and fouling fishing nets and traps (Lata and Dubey, 2010; Su *et al*., 2018).

Water hyacinth has demonstrated abilities to be used as raw material in various useful applications (Shah *et al.*, 2019). Large scale utilization of the weed can therefore be an attractive and efficient method which can replace the relatively ineffective conventional

management methods. The weed hinders growth of native plants leading to altered biodiversity (Shanab *et al*., 2010).

A positive aspect of water hyacinth is that it can be used in water purification (phytoremediation) by removal of heavy metals and toxins such as cyanide (Ambastha *et al*., 2017).Various studies have recorded the other important uses of water hyacinth as; industrial biofuel, biogas, farm animal feed, fish feed vermin composting and medicinal uses (Patel, 2012; Dhal *et al*., 2012; Lalitha and Jayanthi, 2014). However, harvesting the weed for these important economic undertakings has not been able to lower the volume of the weed to the level that can address its negative impact of the weed (Sharma *et al*., 2016), hence the need for this study to stop gap this shortcoming.

2.5 Contemporary water hyacinth control methods

In the management of water hyacinth, there has been a lack of knowledge to for unchecking its growth that has been reported to cause significant harm, including covering the water surface, depletion of oxygen, clogging channels and promotion of the breeding of flies and mosquitos (Su *et al*., 2018). In most cases, eradication of water hyacinth from a site is not feasible (Tewabe, 2015). Hence, continuous suppression and long-term reduction may be the most likely goal for control programmes (Sullivan and Wood, 2012). The nature and use of the waterway, climate, and size of the infestation and time of the year will all affect which control methods, or combination of methods to use (Dersseh and Dessalegn, 2019).All the control methods; physical, chemical, biological control and integrated control (Munjeri *et al*., 2016) have not been self-sustaining.

2.5.1 Physical Control

Physical control is the removal or containment of water hyacinth using mechanical methods such as machinery, containment booms or fences and manual removal (Güereña *et al.*, 2015). Physical control involves both manual and mechanical removal, a boring and prolonged use, connecting the use of enormous and extremely exclusive equipment and human being labour (Abera, 2018). There also occurs additional fees for disposal of plant material (Güereña *et al.*, 2015). The costs of water hyacinth management in China were estimated to about one billion Euros annually (Huan, 2011). Choosing which method or combination of methods to use will depend on the size of the infestation, resources available and the use of the waterway (Australian Government, 2012). It also involves the drainage of the water body, and/or pulling through nets (Patel, 2012). Physical removal however is associated with environmental and financial challenges (Sharma *et al.*, 2016).

2.5.2 Chemical Control

Herbicides are an effective method of control of water hyacinth that can be used in a variety of situations. Chemical control is generally a cost effective option that can be utilized in an integrated control programme for optimum results. It involves the use of Paraquat, Diquat, Glyphosate, Amitrole and 2, 4-D (Villamagna and Murphy, 2010).Study has suggested that use of glyphosate at low concentrations along with *A. alternata* in the integrated management of water hyacinth is effective (Ray and Martin, 2012). Chemical methods have an added disadvantage of being less selective and deleterious to non-target algae and macrophytes. Additionally, a major setback of chemical control is that the herbicides directly interfere with the biological control agents currently deployed against this weed (Villamagna and Murphy, 2010).Scientists and environmentalists have argued

that chemical control of aquatic plants treats the symptom rather than the problem (Agidie *et al*., 2018).

2.5.3 Biological Control with Arthropods

Arthropod biological control has majorly involved use of *Neochetina* beetles (Firehun *et al*., 2015). The beetles often suffer high levels of stress and show low growth rate potential so that they are unable to overcome the weed that can double its area in as little as five days.

Arthropods have been categorized into 3 priority groups (Firehun *et al*., 2015). The groups are; agents in use worldwide such as *N. eichhorniae*, *N. bruchi*, *Niphograpta albiguttalis*, and *O. terebrantis*, recently released candidates and/or under testing (*E. catarinensis*, *X. infusellus, C. aquaticum, B. densa, Paracles tenuis* and *Thrypticus spp*.) and candidates that are poorly known and/or of questionable specificity mostly with no recorded common names (bombardier beetle [*Brachinus* Weber sp. (Coleoptera: Carabidae)], water hyacinth moth [*Argyractis subornata* Hampson (Lepidoptera: Pyralidae)], root-feeding rice pest [*Macrocephala acuminata* Dallas (Heteroptera: Pentatomidae)], plant hopper [*Taosa inexacta* Walker (Homoptera: Dictyopharidae)], and several others). The first survey for natural enemies for water hyacinth conducted in Uruguay from 1962 to 1965 identified the water hyacinth stalk borer moth *Xubida infusellus* Walker and 2 weevil species, *N. eichhorniae* and *N. bruchi*; the water hyacinth mite (*Orthogalumna terebrantis* Wallwork) and the water hyacinth grasshopper (*Cornops aquaticum* Bruner). The petiole-tunneling moth, called the water hyacinth stem borer (*Niphograpta albiguttalis* Warren), 7 species of petiole-boring flies (*Thrypticus spp*. Gerstaecker and the water hyacinth mirid bug (*Eccri-totarsus catarinensis* Carvalho were added to the list of bio control agents in the 1968 surveys conducted in Guyana, Surinam, and Brazil(Pappas *et al*., 2017).

The biology, host-specificity, and potential for the management of water hyacinth for *M. scutellaris* have been better understood (Tipping *et al*., 2010). In addition, those findings indicated that *M. scutellaris* was highly specific to water hyacinth and provided 70% biomass and 73% leaf reduction (Tipping *et al*., 2010). Hence, that bio agent would be better categorized as belonging to the second-priority group. Other potential arthropod agents that have been targeted have been *C. aquaticum* (Franceschini *et al*., 2014) and *M. scutellaris* (Tipping *et al*., 2010). *Neochetina eichhorniae* and *N. bruchi* have been released on water hyacinth in 30 and 27 countries, respectively (Bownes *et al*., 2010). Both have been subjected to extensive screening. They have been tested against 274 plant species in 77 families worldwide. Overall however, these arthropod pests have not been able to completely control the water hyacinth menace mostly due to their low growth potential and high levels of stress in the water bodies due to pollution (Franceschini *et al*., 2014).

2.5.4 Biological control with fungal microbes

A summary of fungal microbes for water hyacinth control that have been identified and evaluated for host specificity, bio control efficacy and formulation efficiency are shown in Table 2.5.4 with those that have featured locally in biological control discussed.

Table 2.5 Microbes for water hyacinth control

Potential biocontrol agent	Country where developed	Stage of development
Alternaria eichhorniae	Egypt	Field tests
Alternaria alternata	India, Egypt	Field tests
Fusarium pallidoroseum	India	Field tests
Fusarium chlamydosporum	Egypt, India	Field tests
Myrothecium roridum	USA, India, Indonesia, Malaysia	Greenhouse tests
Uredo eichhorniae	Egypt, Brassil, Argentina, Uruguay	Greenhouse tests
Drechslera sp.	Egypt	Greenhouse tests
Phoma sp.	Egypt	Greenhouse tests
Alternaria jacinthicola	Mali	Field tests
Cadophora malorum	Mali	Field tests

Source: Dagno *et al*. (2012).

It is worth noting that field and greenhouse test of all the potential biocontrol agents listed in Table 2.5 have not involved variation of inoculum materials and therefore no standardized control agent for commercialization has emanated from those efforts. The occurrence of and severity of disease in each of the mentioned cases result from the impact of three factors: the host plant, the pathogen and environmental conditions represented as a disease triangle whereby in which if any of the three factors is missing, the triangle will be incomplete and no disease will occur (Tjosvold, 2018). The three factors along with time will make up the disease pyramid and in a disease intensity manifestation such as incidence these four factors of the pyramid must come together in a given point in time. As is the case of the disease triangle, biotic disease cannot occur if one of these pieces is

missing. A case in point is the studies by Aldars-Garcia *et al.* (2017) who showed that time of growth was important in inoculum production of *Aspergillus flavus* on pistachio extract agar.

2.5.4.1*Cercospora piaropi*Tharp

The host range of *Cercospora piaropi*fungus native to Mexico was evaluated using 31 plant species (some with several cultivars tested) representing 22 families of economic and ecological importance. The results showed that only water lettuce (*Pistia stratiotes*), another abundant weed in Mexico, was infected by *C. piaropi* (Groenewald *et al*., 2013). *Cerospora piaropi* is known to be virulent against water hyacinth, causing plant diseases in the form of leaf spots (To-Anun*et al.,* 2011). It is also known to be host specific (Montenegro-Calderon *et al.,* 2011).Its common symptoms are small (2–4 mm diameter) necrotic spots on leaf laminae and petioles (Ray and Martin, 2012). The spots are characterized by pale centers surrounded by darker necrotic regions. Occasionally, the spots may appear in the shape of 'teardrops' that coalesce into rectangular shapes as the leaf matures, causing the entire leaf to turn necrotic and senescent (Ray and Martin, 2012). Leaf senescence is accelerated by the *Cercospora* disease, and the disease can rapidly spread across water hyacinth infestations, causing large areas of the weed mat to turn brown and necrotic. Under severe infections, the plant may be physiologically stressed, lose its ability to regenerate, become water logged and sink or disintegrate (Charudattan, 2014). This is, however not likely to be noticed in waters where effluence flow is high due to the compensatory growth that the plant undertakes to replace the dead and dying tissue. Natural infection in the open waters are therefore unlikely to cause mortality.

The fungal isolates are believed to exhibit pathogenic variability depending on the growth and pigmentation in the culture (Pradeep *et al*., 2013). Hence, diffusible pigments in

culture and cercosporin production could be used as adjuncts to screen aggressiveness of the most effective isolates of *C. piaropi* for biological control (Tessmann*et al*., 2008). Isolates of *C. piaropi* are known to be variable in pigmentation in culture, spore morphology and virulence. However, molecular evidence points to a common phylogeny of *Cercospora* pathogenic to water hyacinth (Firehun*et al*., 2015).

In the USA the pathogen was tested individually and applied with a surfactant, an invert emulsion, and/or a humectant gel(Bolton *et al*., 2013). Applications were made to water hyacinth plants with and without natural populations of beetles (*Neochetina spp*). The results indicated that treatment with *C. piaropi* applied in the surfactant Silwet L-77 provided the best levels of biomass reduction and damage severity. This leaf spot disease was therefore considered promising, widespread and with its many virulent strains and was recommended for use as my coherbicides. The pathogen was also developed into a bio herbicide by Abbott Laboratories in Egypt for water hyacinth management. (Firehun *et al*., 2017). The formulation was a wettable powder that was applied with a humectant to preserve moisture and nutrients to sustain and stimulate propagule germination Although laboratory results appeared promising, high infectivity was not achieved in the field, and further development of the bio herbicide was curtailed. In an attempt to determine the possible reason for the low infectivity, this study was set out to determine how different concentrations of the pathogen in the locally available corn oil impact on disease infectivity and on shoot elongation and biomass as key growth parameters.

2.5.4.2*Myrothecium roridum*Tode Fries

Kwon*et al.,* (2014) investigated the pathogen city of *M. roridum* by inoculating it on PDA and incubating for 20 days at 25℃. They collected conidia from the PDA-grown *M. roridum* using sterile water. The conidial suspension was adjusted to 10^9 conidia/ml and

used as the inoculum. They used the wound/drop inoculation method to inoculate anthurium. Twenty-five days after inoculation, the applied *M. roridum* produced necrosis symptoms on the anthurium leaves. Sporodochia were produced on the edges of the necrotic portions of the anthurium leaves. When they observed sporodochia under a phase-contrast microscope, they observed that they contained hyphae and green conidial mass. They re-isolated conidia from the inoculated leaves with disease symptoms, and confirmed that the symptoms were indeed *M. roridum* leaf spot, confirming the pathogenicity of *M. roridum*.

This pathogen causes characteristic teardrop shaped spots on the leaves. The spots are rounded on the side towards the petiole and taper in the direction of the lamina tip. Older spots become brown and necrotic with a white conidial mass at the center (Doehlemann *et al*., 2017). This pathogen has been extensively studied in the greenhouse in USA, India, Malaysia and Indonesia by Dagno *et al.* (2012). Field tests have not been carried out to confirm the greenhouse findings. This pathogen shows high variability in pathogenicity that renders it less effective as a bio pathogen.

Studies by Piyaboon *et al*., (2016) reported that leaf blight disease of water hyacinth was observed and collected from different geographical areas of Thailand. The disease was caused by a fungal pathogen that was identified as *Myrothecium roridum* by using its morphological characteristics. The most effective fungal strains were evaluated for pathogenicity on water hyacinth under greenhouse and natural conditions. Host range tests showed that the fungus did not cause disease on 74 economically important plants but did produce disease signs on water hyacinth and two other aquatic weeds, duckweed and water lettuce. Leaf blight occurs on water hyacinth leaves after being treated with crude extracts of *M. roridum* and it was indicated that secondary metabolites were released from

the fungal mycelia. *Myrothecium roridum* that was grown on boiled paddy rice produced β-1, 4-exoglucanase, β-1, 4-endoglucanase, β-glucosidase, xylanase and pectinase more than *M. roridum* that was grown on potato dextrose agar. The results indicated that *M. roridum* is a pathogen of water hyacinth and the fungus is capable of producing different enzymatic activities on potato dextrose agar and boiled paddy rice, which might be important for infection. This study was set out to determine a standard *M. roridum* concentration that could be incorporated in the locally available corn oil as formulation for use in the control of water hyacinth.

2.5.4.3 *Alternaria* spp

Alternaria alternata (Fries) Keissler was described as a pathogen of water hyacinth in Australia (Zaferanloo *et al*., 2014). Recently, the pathogen was later evaluated intensively as a bio control agent in India and Egypt (Dagno *et al*., 2012). Test results indicated that the fungus was highly virulent on water hyacinth, leading to plant death. Its symptoms (spots and lesions) were mainly expressed on the leaves but not on the stolons. The host-range assessment result indicated that *P. stratiotes* (both in India and Egypt) and foxtail sedge (*Cyperus alopecuroides* Rottb.) (Egypt) were susceptible to the fungus. Host range tests therefore render it not suitable for use without accompanying host specificity tests data.

Alternaria eichhorniae Nag Raj & Ponnappa infection on water hyacinth shows dark necrotic spots that appear mostly on the older leaves (Dagno *et al*., 2012). The spots have a green to yellow appearance, finally becoming brown as the infection spreads to cover the entire leaf. This is host specific and damages and suppresses the weed. Formulation for this pathogen was deemed insufficient to overcome the weed's growth rate conditions without the benefit of prolonged dew periods. A good understanding of the biology and

pathology of the fungus is still being gained its development as a mycoherbicide for controlling water hyacinth in Egypt started last decade (Dagno *et al*., 2012) but so far conclusive results have not been forthcoming.

2.5.4.4*Rhizoctonia solani* Kuhn

A study by Losada *et al*. (2014) shows that *Rhizoctonia solani,* a soil inhabiting fungus is one of the most widely distributed fungi throughout the world. The symptoms of this fungus resemble the damage that is normally caused by a desiccant type of chemical herbicide such as diquat. The symptoms may consist of irregular, necrotic spots, and broad lesions. Unlike chemical damage, the brown necrotic areas are usually surrounded by noticeable, thin, water-soaked margins of darker brown colour than the rest of the lesion. *Rhizoctonia* disease has been reported on water hyacinth from the southeastern United States, Brazil, Mexico, Panama, Puerto Rico, India, Malaysia, and Indonesia Losada *et al*. (2014). This fungus is usually very aggressive and destructive, capable of rapidly killing water hyacinth plants (Opande *et al.,* 2017). The extent of variability in virulence of *R. solani* pathogenic to water hyacinth is not clear, but isolates collected in the USA, Panama, and Brazil have been found to be extremely virulent (Charudattan, 2014). Host specificity issues however make it not to be a fungal agent of choice.

2.5.4.5*Acremonium zonatum* Saw. Gams

According to Park *et al*. (2017), anamorph *Acremonium* Link is the largest genus within the order Hypocreales. The genus *Acremonium*, formerly called *Cephalosporium*, includes approximately 100 species; these organisms are known to be saprobic on dead plants or soil dwellers. Many *Acremonium* species are recognized as opportunistic pathogens of both man and animals, causing eumycetoma, onychomycosis and hyalophomycosis

manifesting as arthritis pneumonia and other sub cutaneous infections. *Acremonium zonatum* can also be referred to as *Cephalosporium zonatum* Sawada. It is a facultative parasite that co-migrates with its water hyacinth host. This pathogen causes zonate necrotic leaf spots that are easy to identify (Devika *et al*., 2017). The lesions are more noticeable on the upper leaf surface. On the lower surface, white mycelia growth may be seen (Dagno *et al.,* 2012). Studies by Charudattan (2014) indicate that in severe infection, the spots coalesce to cover the entire lamina, hence, killing the entire plant. Each spot may be small (2 mm diameter) to large (> 3 cm diameter). In plate culture, colonies form milky white fluffy material that turns brown upon sporulation. Low power microscopy reveals hyaline, fine septate and branched mycelia with unicellular conidia either scattered or in chains (Dagno *et al.,* 2012). It is a pathogen that is easy to culture in the laboratory. This pathogen is reported to be of native origin (Tegene *et al*., 2012). It infects water hyacinth with marked host specificity and no cases of non-target attack have been reported so far reported *A. zonatum* as one of the potential fungal pathogens that could be used as a bio herbicide agent in areas where the strains are pathogenic to the water hyacinth (Tegene *et al*., 2012). Opande *et al*., 2013) also indicated that *A. zonatum* has a favourable characteristic for consideration as a mycoherbicide, especially if formulated in appropriate oil emulsion. As an added advantage to the use of this pathogen, reports of attacks on crop plants by this genus are rare. Charudattan (2014) reported *A. zonatum* as one of the potential fungal pathogens that could be used as a bio herbicide agent in areas where the strains are pathogenic to the water hyacinth but not to plants having economic and ecological importance (e.g., Mexico).

2.6 Formulation of Fungal bioherbicides.

Zvonko (2015) termed bioherbicides as biologically based control agents useful for biological control. They have advantages such as clearly defined for target weeds, no side effect on beneficial plants or human health, lack of pesticide residue build-up in the environment and effectiveness for control of some herbicide resistant weed biotypes. Among the factors that have constrained the progress of bioherbicdes is technical restrictions arising from lack of wholesale production and formulation development of reliable and effective bioherbicide (Bo *et al*., 2019).

Under both greenhouse and field conditions, the bio control efficacy of fungal isolates was improved with unrefined *Carapa procera* (L.) oil and refined palm oil, supplemented with soybean lecithin and Tween 20 (Libs and Salim, 2017). When such a formulation was used, the incubation time was 4 to 5 days in the greenhouse and 7 to 9 days on the field, and the damage severity (DS) recorded 6 weeks after treatment varied from 87.02 to 93.13% in the greenhouse and from 59.11 to 63.00% in the field. For unformulated *C. malorum* and *A. jacinthicola* respectively, the incubation times were longer and the DS values were only 22.11 and 29.05% in the greenhouse and 12.05 and 15.15% on the field (Dagno *et al*., 2012). The results highlighted good substrates for mass production of these mycoherbicides and demonstrated the ability of vegetable oil formulations to improve their efficacy (Karim*et al*. (2011).

Different fungal formulations have been evaluated by Piyaboon *et al*. (2016) for their level of control of water hyacinth. The results showed that spore suspensions with 10% palm oil or 1% Tween 20 caused a higher level of disease severity, compared to spores applied in water alone. Formulation is recognized as a way of increasing both efficiency of application and efficacy (Bo *et al*., 2019). Maximizing the potential for successfully

developing and deploying a bio control product begins with a carefully crafted microbial screening procedure, proceeds with developing mass production protocols that optimize product quantity and quality, and ends with devising a product formulation that preserves shelf-life, aids product delivery, and enhances bioactivity (Bo *et al*., 2019). It is this bioactivity that is the bedrock of sustained disease severity when using bio pathogens for the control of noxious weeds such as water hyacinth.

Microbial selection procedures that require prospective bio control agents to possess both efficacy and amenability to production in liquid culture increase the likelihood of selecting agents with enhanced commercial development potential (Tegene, 2012). Oil emulsions in particular may reduce the dew requirement of fungi and the number of spores required to ensure bio control agent efficacy. Formulations need to be used to improve product stability, bioactivity and delivery or the ability to mix and spray the product (Mola and Afkari, 2012). In addition, it helps in the integration of the bio pesticide into a pest management system. Other important characteristics of a successful formulation are convenience of use, compatibility with end user equipment and practices, and effectiveness at rates consistent with agricultural practices.

Dagno *et al*. (2012) reported on the number of challenges encountered in the formulation of promising bio control agents isolated from water hyacinth. It is necessary to include good market potential, ease of production and application, adequate product stability and shelf life during transportation and storage in order to ensure propagule viability and efficacy over the long term. The material to be used for formulation needs to be readily available too. Some reasons why bio control agents have not been adopted by stakeholders are: difficulty of production, sensitivity to UV light, desiccation, a requirement for high humidity for infection insufficient performance over a wide range of environmental

conditions and lack of appropriate formulation with standardized inoculum concentrations (Boyette and Hoagland, 2013). For foliar bio control agents such as *Alternariasp.* Some environmental factors that influence plant infection and disease development are: temperature, free moisture, protection against UV irradiation and desiccation (Boyette and Hoagland, 2013).Vegetable oil formulations have been found to improve the efficacy of pathogens by concentrating the inoculum within the formulation (Karim *et al*., 2011). If efforts are made to determine the optimum inoculum concentration thresholds for the bio pathogens for formulation in the corn oil, there would be a likelihood of recommending a corn oil based mycoherbicide. These formulations may be used with an adjuvant (e.g. a phytotoxic compound or a registered chemical herbicide at low rates). The oil formulations have been reported to be effective as bio pesticide formulation materials (Boyette and Hoagland, 2013).

The germination ability of conidia mixed with eight vegetable oils was evaluated after 1, 2 and 3 week storage at 25℃ by spreading over SDA (Mola and Afkari, 2012). Results showed a significant difference between tolerances of conidia to different vegetable oils formulation with the highest germination proved at 25℃.Multiple applications of the formulated pathogens may be used, but the economics of the applications would have to be assessed (Dagno *et al*., 2012; Devika *et al.*, 2017)). A commonly used vegetable formulation material is corn oil(Rebolleda *et al*., 2012). This is a vegetable oil that is extracted from maize germ (Kadioglu *et al.*, 2011). It is harmless to the environment, highly biodegradable and used domestically in foods (Kaltragadda*et al*., 2010). A quality that qualifies corn oil as a formulation agent is its low viscosity(Bai *et al*., 2017). So far there has not been studies focusing on formulation of *C. piaropi* and *M. roridum* in corn oil for the control of water hyacinth. This has been in spite of the fact that maize, the farm

produce from which corn oil is extracted is a common crop grown by both the large scale and small scale farmer. Shawiza (2017) reported that maize is grown by 98 percent of Kenya's 3.5 million smallholder farmers. Small and medium scale farmers produce about 75 percent of the nation's' maize crop, while large-scale farmers produce the remaining 25 percent. In normal years, 25-35 percent of total marketed maize is sold directly to the National Cereals and Produce Board (NCPB) by medium and large producers. Smallholder producers sell 96 percent of their maize to private traders/brokers or consuming households. This high production of maize means that the raw material for the production of corn oil to be used in the formulation of the bio pathogens will be available.

In Mali, work to determine the effects of oil-water emulsion (prepared with *Carapa procera* (L) oil or refined palm oil amended with soybean lecithin and Tween 20 on the germination of *C malorum* (isolate Min715) and *A. jacinthicala* (strain MUCL53159) spores on the severity of the damage they cause to water hyacinth was done. The results suggested that the substrates were good for mass production of the mycoherbicides and demonstrated the ability of vegetable oil to improve the efficacy of the pathogen (Dagno *et al*., 2012). However, optimum concentration threshold for *C. procera* was not determined so that recommending the development of *C. procera*/palm oil as a mycoherbicide would be met with resistance.

Only two mycoherbicides have been developed to control water hyacinth with the intension of becoming commercialized (Dagno *et al*., 2012)). The first mycoherbicide was registered by the United States Environmental Protection Agency (US-EPA) under the patent US4097261nin the USA (Bolton *et al*., 2013). This product contains *Cercospora rodmanii,* which is a fungal pathogen specific for *Eichhornia crassipes.* Abbot Laboratories in the USA have developed an experimental formulation of *C.rodmanii*

named ABG-5003 for use against *E. crassipes*. The second mycoherbicide that has been developed is called Hyakill, which contains *Scleronitiasclerotiorum* (Charudattan, 2014). This mycoherbicide was submitted to the European Patent Office in 2003. *Sclerotinia* is not a pathogen specific to water hyacinth; it has been recognized as a plant pathogenic fungus on several crops such as beans sunflower and carrots as well as other dicotyledonous plant families. This disadvantage is the reason why Hyakill has not obtained commercial authorization use from the European Patent Office.

Boyette and Hoagland (2013) tested several surfactants, plant extracts and fatty acids for stimulation of conidial germination, appressorial formation and virulence of *Colletotrichum trumcatum*, a bio herbicide of the weed hemp sesbania (*Sesbania exaltata*). Conidia formulated in either water, a surfactant or, an emulsion of refined corn oil were ineffective on the plant in the absence of dew or when dew was delayed. However, formulations of conidia combined with a surfactant in emulsified corn oil did exhibit bioherbicidal activity when dew or free moisture was available. Overall, the results showed that formulations containing an emulsion of conidia had the potential for enhancement of the efficacy of *C. truncatum.*

2.7 Effect of fungal spores concentration on disease intensity

Chiang *et al*. (2017 described disease intensity as encompassing disease severity and disease incidence. Piyaboon *et al*. (2016) working on disease intensity of *M. roridum*spores at higher suspensions in 10% palm oil or 1% Tween 20 as bio agent for water hyacinth control reported that a higher level of disease severity was caused on water hyacinth plants compared to the spores applied in water alone. Bo *et al*. (2019) reported a similar scenario in studies with *Streptomyces* metabolites as bio herbicides. According to Mendgen and Hahn (2002) disease infection is attributable to the initial formation of

penetration and infection hyphae also known as appresoria that invade the plant with minimal damage to the host cells. The higher the concentration of inocula the more the appresorial structures that will be available to penetrate the host plant.

2.8Effect of leaf spot intensity on shoot length and biomass of water hyacinth

Fungal pathogens manipulate plant metabolism in their own favour therefore denying the plant the necessary resources for tissue growth with subsequent reduction on growth (Doehlemann *et al*., 2017). The bio pathogens are thus seen as important in lessening the detrimental effects of the normally luxuriant water hyacinth growth (Sharma *et al.*2016; Waithaka, 2013). Necrotrophic pathogens such as *C. piaropi*and *M. roridum*secrete toxins to kill water hyacinth plant tissues (To-Anun *et al*., 2011). These toxins are; cercosporin and roridin for *C. piaropi* and *M. roridum* respectively and are able to lower the growth rate of water hyacinth.

Joost van den Brink *et al.* (2013) and Moran (2005) in studies of plant biomass degradation by *Myceliophthora heterothallica* reported that fungal pathogens are able to degrade the water hyacinth plants as demonstrated in field plots with*C. piaropi*. The lessened biomass was able to curtail interference and put the biomass at manageable levels (Eid and Shaltout, 2017). Robles *et al*. (2015) also reported that biomass reduction in water hyacinth biomass is useful and effective as a control method.

2.9 Biomass estimation in water hyacinth control systems

The expansion of biomass of water hyacinth resultant from its fast growth is the major reason why water hyacinth infested waters are rendered unusable economically (Su *et al*., 2018). There has been lack of knowledge on how pathogen control measures impact the biomass of the plants in question. Typically, the biomass of water hyacinth is estimated

using quadrats with a specific unit area placed over the plant mat. The problem with this quantitative approach is that it is labour and time intensive (Robles *et al*., 2015). It is also a destructive method that removes plant material from the system which may be problematic for longer term studies of plant growth. As an alternative, Robles *et al.* (2015) reported that estimation of the biomass of emergent aquatic plants could be based on morphometric parameters such as plant height. These kind of nondestructive methods require less time and effort while maintaining adequate accuracy (Robert and James, 1991; Robles *et al.*, 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The inoculation study was carried out at the Kenya Agricultural and Livestock Research Organization (KALRO) center at Kibos in Kisumu city (Figure 3.1).

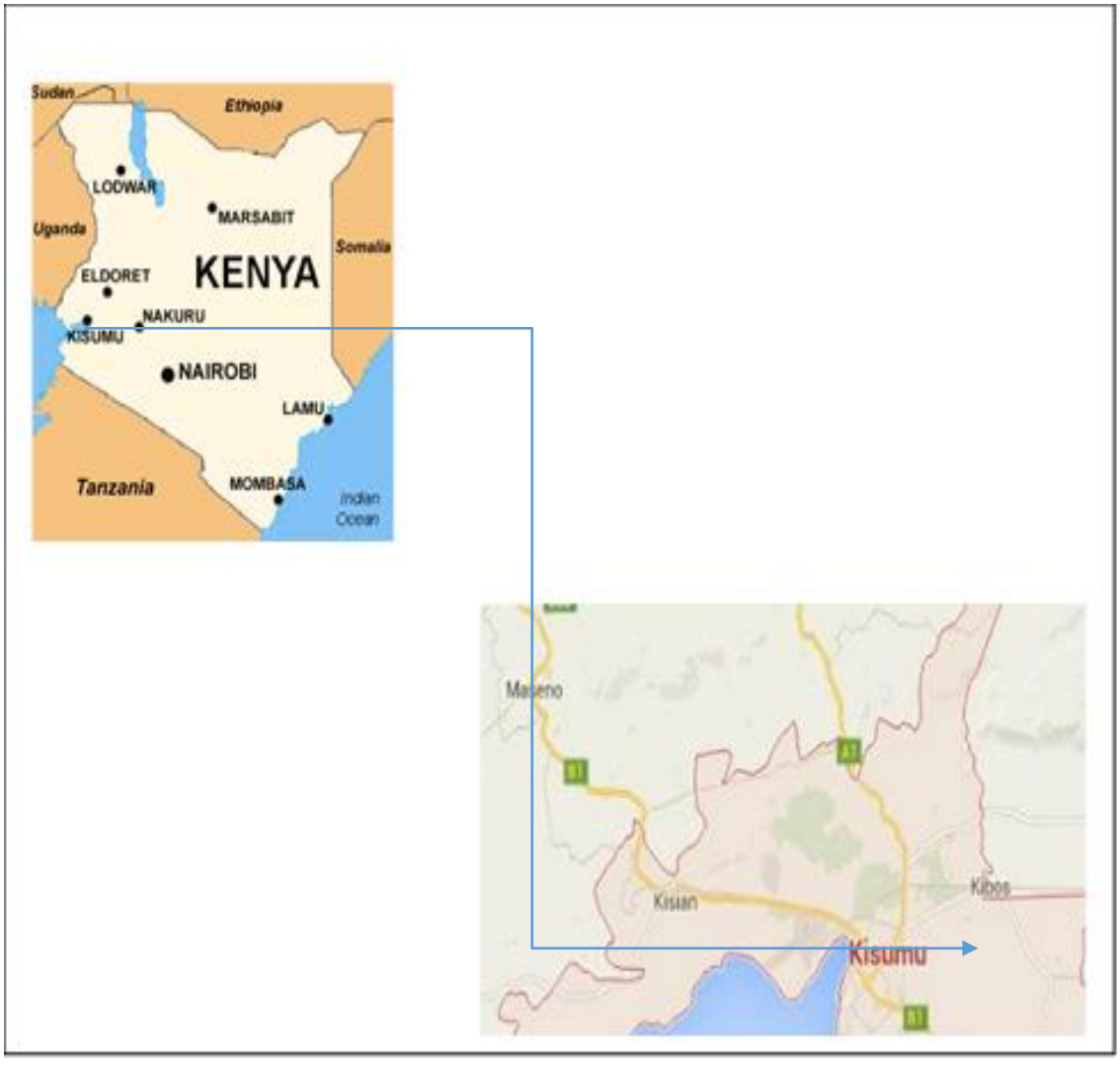

Figure 3.2: Study area (Source: Maplandia on google)

The center is situated at latitude $0^0$37'S and longitude $37^0$20'E. It is about 10km from Lake Victoria.

Both symptomatic and asymptomatic to *C. piaropi* and *M. roridum* water hyacinth plants for the study were collected from Dunga beach at the Kisumu city water front on the shores of Lake Victoria. The greenhouse conditions were not controlled. Temperature averages were 25 to 30^0C and 22 to 27^0C during the day and night respectively while the relative humidity averages varied from 60 to 69%.

3.2 Source of *C. piaropi* and *M. roridum* pathogens

Cercospora piaropi symptomatic plants with leaf spots characterized by pale centers with darker necrotic regions and/or brown and necrotic leaves as described by Groenewald *et al*. (2013) were sampled from naturally infested lake fronts at Dunga Beach. As for *Myrothecium roridum* symptomatic plants, the descriptions of Kwon *et al.* (2014) of tear drop leaf spots on the leaf margins were used to sample the plants from the same naturally infested lake fronts at Dunga Beach. The collected samples were put in a bucket containing water, covered and transported to the greenhouse.

3.3 Isolation of pathogens

3.3.1 Isolation of *C. piaropi* pathogen

Following the procedure used by Jimenez (2010), *C.piaropi* infected leaf was excised from the plant using a sharp sterile scalpel. Isolation was made with a little green area around the lesion left. In order to avoid contamination, the isolates were washed in running tap water for one minute to remove dust and other dirt particles. They were then rinsed with sterile distilled water in petri dishes and transferred to other petri dishes containing 10% sodium hypochlorite. The pieces were shaken for 1 minute and rinsed

twice in sterile distilled water. The washed leaf pieces were aseptically placed in petri dishes with filter papers soaked in sterile distilled water. The petri dishes were observed for red coloration indicating spore formation. The red coloration appeared within 8 to 12 days.

Upon spore formation, a flame sterilized platinum needle was used to transfer the spores to commercial quality sterilized Potato Dextrose Agar (PDA) medium under aseptic conditions provided by a running laminar flow. The cultures were incubated at room temperature on the laboratory benches for eight days and observed for red mycelia colonies. Sub cultures were made from the colonies by aseptically picking and transferring material to other petri dishes with fresh PDA using the needle, sealing the petri dishes with parafilm and incubating at room temperature on the laboratory benches until spore formation occurred. After fourteen days, multiplication and purification of the colonies was done by cutting about 1.2 x 1.2 cm blocks from the margins of sporulating colonies and inverting them over fresh PDA in other petri dishes. The cultures were incubated for fourteen days when colony growth spread and covered the entire media.

3.3.2 Isolation of *M. roridum* pathogen

In the laboratory, the leaves of *M. roridum* symptomatic were excised from the plants using a sharp sterile scalpel. The leaves showing leaf spots were washed in running tap water and rinsed in sterile water in order to remove dust and other dirt particles that could be a source of contamination. Excess water was shaken off and the leaves cut from the plants and placed on a sterile blotting paper. Following the procedure used by Piyaboon *et al.*(2016) small pieces of about 1 mm^2 were cut from the margins of the spots on the leaves. The pieces were disinfected in 0.5% sodium hypochlorite for 1 minute and in 70% ethanol for 30 seconds to kill any microorganisms growing on the surface. The sterilized

leaves were transferred to Potato Dextrose Agar (PDA) medium in plastic plates under aseptic conditions provided by a running lamina flow. The plated leaf pieces were sealed with parafilm membrane and incubated at room temperature on the laboratory benches. They were observed for *M. roridum* colony growth and sporulation evidenced by darkening of the colony at around twenty five days.

3.4 Harvesting of pathogen spores

Following the method of Tahlan (2014), 100mls of refined domestic grade corn oil obtained from a local shopping mall was measured and put into a sterilized cone flask and topped up to 1000 mls (1 liter) with sterilized distilled water. One milliliter of 1% polysorbate was added to the contents of the cone flask and the mixture thoroughly shaken to form a 10% corn oil emulsion. After the surface of *C. piaropi* turned red and *M. roridum* turned dark indicating sporulation for the two pathogens, the corn oil emulsion was repeatedly pipetted over the surface of each of the cultures emulsion in the pipette became cloudy. The contents of the pipettes were then separately plunged into sterilized beakers as *C. piaropi* and *M. roridum* stock solutions. The solutions were refrigerated at 5^0C awaiting usage.

3.5 Source of healthy experimental plants

The sampling for the healthy plants was done in locations with healthy water hyacinth growth that did not display any disease symptoms. This was done to ensure that the plants collected did not carry any spores. Healthy water hyacinth plants with the broadest leaves having 50–100 cm^2 in size and of approximately the same age as determined by their architecture were collected from Kisumu City shoreline of Lake Victoria at Dunga beach. In order to estimate the leaf size, the method of Kuzmenko (2016) was adopted. In this

method water hyacinth stolons were gently lifted and daughter plants as described by Mujere (2015) plucked from the main plants. Daughter plant broadest leaves were held against a graph paper with two concentric squares, the smaller square with 7.1x7.1 cm (50.4cm^2) and the lager one at 10x10 cm (100 cm^2). The plants sampled were only those whose outline of the broadest leaves overshadowed the small squares but fell within the larger square.

Following the method of Daddy and Owotunse (2002), tap water was put in 40 liter plastic basins and aged for 3 days to allow the available chlorine to escape before use in the greenhouse. The sampled plants were put into the aged water to acclimatize for two days (Piyaboon *et al*., 2016) before being inoculated.

3.6 Setting up the experiments

The experiment was set up in the greenhouse at Kibos. The acclimatized healthy plants from the 20 liter plastic containers were surface-sterilized in 10% sodium hypochlorite followed with sterilized distilled water as a spray and transferred to smaller plastic basins with 3 foot in diameter and 1.5 foot depth filled with 20 liters of aged tap water according to the procedure followed by Daddy and Owotunse (2002). Three healthy plants with leaf size of 50 – 100 cm^{2}were placed in each of the basins including the basins for the control plants. There were thirty three basins in total; thirty to cater for *C. piaropi* and *M. roridum* to be inoculated with the five levels of spore concentrations and three for the control. The basins were topped up at the beginning of subsequent weeks to maintain the water level at 20 liters according to Daddy and Owotunse (2002).

3.6.1 Treatment formulation

A haemocytometer was used to determine the concentration of the spores in the suspension employing the method created by Caprette (2000).

A droplet of the corn oil/spore emulsion was mounted on the chamber of the haemocytometer and the cover slip affixed on top. The cells in the suspension were viewed under a microscope at 100x magnification. The microscope was focused two of the large grids with sixteen smaller squares. All the cells in the large grid were counted and the average taken. To arrive at a suspension with 10^8 spore/ml concentration:

Number of cells counted on large counting grid of haemocytometer = 109

Sample diluted 100 times

Concentration of cells in diluted stock = Average number of cells/volume of grid where

counting was done

= 109 cells/ ml $1x10^4$ml (or 109x10, 000)

= 1.09×10^6 cells/

But the original stock was diluted 100 times

Therefore: [Cell] x dilution factor = (1.09×10^6 cells/ml) x 100 = 1.09×10^8 cells/ml

The concentration of the stock solution was adjusted and by serial dilution to $1x10^9$, $1x10^8$, $1x10^7$, $1x10^6$ and $1x10^5$spores/ml according to Admas *et al.* (2017).

3.6.2. Inoculation of the experimental plants and experimental design

Healthy plants were placed in 20 liter basins at the rate of three plants per basin. Application of the six treatments or formulations of *C. piaropi* and *M. roridum* with; $1x10^9$, $1x10^8$, $1x10^7$, $1x10^6$ and $1x10^5$spores/ml of each of the pathogens was done with a single treatment being applied to a single basin. The application of the formulations involved uniformly applying 100mls of the formulation to the plants in the basins. The spray pump was held at 20 cm from the plant and inclined at 45^0 according to the method used by Opande *et al*. (2013). This was repeated until each formulation was applied to three separate basins. The formulation with the lowest concentration ($1x10^5$spores/ml) was sprayed first and subsequent concentrations sprayed in ascending order. The leaves of the plants were fully wetted by the spray. The control was sprayed with sterile distilled water. To ensure sufficient moisture for infection, a fine mist of sterile water was sprayed upon the leaves after the formulation spray droplets had evaporated according to procedure followed by Admas *et al*. (2017). The control plants were sprayed with 100ml of the corn oil emulsion without any of the two antagonists according to the methodology of Admas *et al*. (2017). The spraying of the control was done with a sprayer that had not been used to spray any of the formulations.

The five formulations and the non-treated control were replicated 3 times using the table of random numbers and randomly arranged on the greenhouse floor in a completely randomized design (CRD).

3.7 Determination of disease severity

Determination of disease severity was done at biweekly intervals up to the sixth week after inoculation by direct estimation of severity, by assigning a severity value to individual plant leaves based on closeness of perceived severity on each of the leaves. The

descriptive scale of 0 - 5 as used by Manandhar *et al.* (2016)was adopted. The rating scales were as follows:

0 = no symptoms (0%)

1 = 1 to 10%

2 = 11 to 25%;

3 = 26 to 50%

4 = 51 to 75%

5 = ≥75% area covered by leaf spot

This scale was adopted due to the fact that it is reproducible with severity scores matched to corresponding percentages that enhances interpretation. In addition the data obtained from the scale lends itself well to analysis of variance (ANOVA).

Based on the recorded mean disease severity scores, area under disease progress curves (*AUDPC*) was calculated at each scoring. The *AUDPC* values were then converted to area under disease progress stairs (AUDPS) in the following steps (Simko and Piepho, 2012):

Step 1: Calculation of *AUDPC*:

$$AUDPC = \frac{obs1 \mathrm{X} t1}{2} + obs2 \mathrm{X} t2 + \frac{obs3 \mathrm{X} t3}{2}$$

Where:

*obs*1, 2 and 3 are scores after time intervals 1, 2 and 3

*t*1, *t*2 and *t*3 are time intervals between scorings.

Step 2: Calculation of *AUDPS*:

$$AUDPS = AUDPC + \frac{y1 + yn}{2} \text{X} \frac{D}{n-1}$$

Where:

AUDPC = Area under disease progress curve for the respective biweekly DS score, *AUDPS* = Area under disease progress stairs,

*y*1 = DS score at first time

*y*n = Disease score at last time

D = *t*n-*t*1

n = number of observations

3.8 Determination of disease incidence

Determination of disease incidence was done at weeks 0, 2, 4, and 6.In doing this, all the leaves with observable leaf spot lesions were counted and recorded. The total number of leaves were also counted. Disease incidence percentage was then calculated following the formula used by Kone *et al*. (2017) as follows:

$$IC = \frac{n}{N} \text{X}100$$

Where: *IC* = Incidence

n = number of diseased leaves

N = number of leaves assessed

3.9 Determination of relative shoot length

At weeks 2, 4, and 6after inoculation, and following the method of Sharma *et al*. (2016), the lengths of the three plants in each basin were individually measured. This was done using a centimeter ruler and the average for each basin recorded. The average shoot length for the treated basins was compared with the average length of the control basins. Relative shoot length for each treatment was determined by adopting the formula of Robert and James (1991) as follows:

$$R = \frac{yp - yt}{yp} \text{X}100$$

Where:

R = relative shoot length in water hyacinth

yp = average shoot length from the control treatment

yt = average shoot length from the respective treatments.

The relative shoot length for each treatment was therefore the percentage by which the average length of the inoculated shoots varied from the average shoot length of the control plants.

3.10 Determination of relative biomass

Following the method of Daddy and Owotunse (2002), at the end of the sixth week the plants from each basin. For each of the basins, the plants were removed from the water, the roots disentangled gently. The stalks were removed from the roots by hand. They were blotted with a serviette to remove excess water and immediately weighed on an electronic scale. Harvested leaves, stalks and whole plants were taken to the laboratory and oven

dried at 80^0C to a constant weight for 24 hours. The dry matter was removed from the oven and weighed. The plants from the control basin were also removed and subjected to the excess water removal, weighing, oven drying and weighing again. The weights of each treatment were subjected to comparison to the weight of the control treatment by calculating the relative biomass using the formula developed by Robert and James (1991) as follows:

$$I = \frac{Ap - At}{Ap} X100$$ **Where:**

I = relative biomass

Ap = water hyacinth dry weight from control treatment

At = water hyacinth dry weight from the respective treatment

The relative biomass for each treatment was therefore the percentage by which the average biomass of the inoculated shoots varied from the average biomass of the control plants.

3.11 Data Analysis

In order to assess the water hyacinth response after inoculation with the two pathogens over the experimental period, the data were subjected to Analysis of Variance (ANOVA) and least significant difference separately for each pathogen to compare their performances at different sampling dates. Combined analyses were done with spore formulation treatments and pathogen effects considered on all the data using PRO GLM in SAS (Institute, Inc.1999).

3.12 Disposal of Water Hyacinth Plants

At the end of the study period, water was drained from the basins. The water hyacinth plants were thoroughly sprayed with glyphosate (Roundup 5.4) at 300ml. per 20 liters of water when they started drying, they were buried at 3ft depth.

CHAPTER FOUR

RESULTS

4.1. Effect of corn oil formulations of *C. piaropi* and *M. roridum* on disease severity

It was observed that up till week 4, increasing spore concentrations of *C. piaropi* and *M. roridum* incorn oilsignificantly ($p \leq 0.05$)increased leaf spot disease severity on water hyacinth plants. These significant differences were established from the control except for *M. roridum* at week 2 and *C. piaropi* at week 4 where the $1x10^5$ spores/ml treatment were not significantly different from the control (Table 4.1.1). The highest disease severity scores were 5.00 and 4.67 for *C. piaropi* and *M. roridum* at $1x10^9$ spores/ml respectively, both being registered at week 6. The two aforementioned scores suggest that the leaf spot severity on the leaves was 51 to 75% on the Manandhar disease severity scale. Further there was increase in disease severity for both pathogens at subsequent biweekly intervals. The means for disease severity for the two pathogens were not significantly different with 2.76 and 2.55 for *C. piaropi* and *M. roridum* respectively (Appendix 1c).

Table 4.1.1: Effect of corn oil formulations of *C. piaropi* and *M. roridum*on disease severity of water hyacinth plants during the study period

	DISEASE SEVERITY					
	Week 2		**Week 4**		**Week 6**	
Form.	***C. piaropi***	***M. roridum***	***C. piaropi***	***M. roridum***	***C. piaropi***	***M. roridum***
$1x10^5$	0.67a	0.33a	1.67a	1.33a	3.33b	3.00a
$1x10^6$	1.00a	0.67a	2.33b	1.67a	4.33c	3.33a
$1x10^7$	1.00a	1.33b	3.00c	2.33b	5.00d	4.33c
$1x10^8$	1.67b	2.00c	3.67d	3.33c	5.00d	4.67c
$1x10^9$	2.67c	2.33c	4.33e	4.00d	2.33a	3.67 b
CV (%)	**22.23**					
LSD	**0.36**					

Numbers followed by the same letters down the column are not significantly different at p≤0.05

With regards to the progress of the disease as observed from area under disease progress stair (AUDPS) values, it was observed that increasing spore concentrations of *C. piaropi* and *M. roridum* incorn oilsignificantly (p≤0.05)increased disease progress on water hyacinth plants (Table 4.1.2). For both *C. piaropi* and *M.roridum,* as time at biweekly intervals increased, there was significant (p≤0.05) increase in AUDPS with increasing spore concentration within the formulations. The highest AUDPS value was 20.67 for *C. piaropi* formulated at $1x10^9$ spores/ml while that for *M.roridum* was 18.50.

Comparative effect of the pathogens on AUDPS showed that the AUDPS means for the two pathogens were significantly different (Appendix 1f). *Cercospora piaropi* performed better with respect to AUDPS eliciting a value of 7.17 as compared to *M. roridum* with

6.56. However at $1x10^9$ spores /ml the two pathogens were not significantly different in terms of AUDPS with both registering the same value of 10.11.

Table 4.1.2: Effect of corn oil formulations of *C. piaropi* and *M. roridum*on AUDPS during the study period

	AUDPS					
	Week 2		Week 4		Week 6	
Form.	*C. piaropi*	*M. roridum*	*C. piaropi*	*M. roridum*	*C. piaropi*	*M. roridum*
$1x10^5$	0.67a	0.00a	3.50a	2.50a	7.83b	6.67a
$1x10^6$	1.00b	0.67b	5.00b	3.50b	11.17c	10.00b
$1x10^7$	1.00b	1.33c	6.00c	5.50c	14.00d	11.67c
$1x10^8$	1.67c	2.00d	8.00d	8.00d	17.33e	16.17d
$1x10^9$	2.67d	2.33e	7,00e	9.50e	20.67f	18.50e
%CV	13.70					
LSD	0.33					

Numbers followed by the same letters down the column are not significantly different at $p \leq 0.05$

4.2: Effect of corn oil formulations of *C. piaropi* and *M. roridum* on disease incidence

Increasing spore concentrations of *C. piaropi* and *M. roridum* incorn oilsignificantly ($p \leq 0.05$)increased disease incidence in the water hyacinth plants at all the biweekly intervals except for *M. roridum* at week 6 where the no significant differences were noticed. The highest disease incidence percentages were 82.23 and 88.90% for *C. piaropi* and *M. roridum* respectively at $1x10^9$ spores/ml for week six (Table 4.2). At all the spore concentrations the *C. piaropi* had significantly lower disease incidence percentages than *M. roridum*.

The interaction between formulation and weeks was significant while that between the pathogens and weeks was not significant (Appendix 2b).

Comparative effect of the pathogens on disease incidence showed that the mean disease incidence for *C. piaropi* was significantly lower at 57.98 as compared to 86.22 for *M. roridum* (Appendix 2c).

Table 4.2: Effect of corn oil formulations of *C. piaropi* and *M. roridum*on disease incidence during the study period

	DISEASE INCIDENCE					
	Week 2		**Week 4**		**Week 6**	
	Disease incidence (%)					
Form.	***C. piaropi***	***M. roridum***	***C. piaropi***	*M. roridum*	***C. piaropi***	***M. roridum***
1X10^5	15.27a	42.5a	48.13a	59.37a	67.23a	83.33a
1X10^6	46.30a	44.43b	53.33a	61.30a	71.67a	83.33a
1X10^7	48.13c	48.13ab	53.33a	71.33ab	73.90a	87.77a
1X10^8	51.87d	51.47b	55.20ab	75.80b	76.67bc	87.77a
1X10^9	54.23e	66.40c	55.93 b	78.70bc	82.23c	88.90a
%CV	16.90					
LSD	7.42					

Numbers followed by the same lettersdown the column are not significantly different at p≤0.05

4.3:Effect of corn oil formulations of *C. piaropi* and *M. roridum* on relative shoot length

For both pathogens, as the corn oil emulsion formulation increased in amount of spores, there was a significant increase in relative shoot length. The highest relative shoot length

were 55.07 and 51.93 for *C. piaropi* and *M. roridum* respectively at $1x10^9$ spores/ml (Table 4.3).At each of the biweekly intervals, the relative shoot length significantly increased starting from week 2 up to week 6.

The results for comparative effect of pathogens on relative shoot length (Appendix 3c) indicated that the mean relative shoot length for *C. piaropi* was significantly higher than that of *M. roridum* at 41.31 and 38.51 respectively. This scenario was repeated at all spore concentration levels.

Table 4.3Effect of corn oil formulations on relative shoot length of water hyacinth plants during the study period

	RELATIVE SHOOT LENGTH					
	Week 2		**Week 4**		**Week 6**	
Form.	***C. piaropi***	***M. roridum***	***C. piaropi***	***M. roridum***	***C. piaropi***	***M. roridum***
$1X10^5$	27.33b	26.1a	37.83a	31.4a	50.00a	49.57a
$1X10^6$	25.67a	26.73a	40.27b	34.7b	49.53a	49.87a
$1X10^7$	29.23cd	28.80c	42.90c	36.70c	50.73a	50.60a
$1X10^8$	30.47de	27.33b	44.33d	40.17d	52.40b	50.30a
$1X10^9$	31.53e	30.90d	52.43e	42.57e	55.07c	51.93b
CV (%)	16.9					
LSD	0.78					

Numbers followed by the same letters down the column are not significantly different at $p \leq 0.05$

4.4Effect of corn oil formulations of *C. piaropi* and *M. roridum* on relative biomass

It was observed that as the concentration level of the spores for both *C. piaropi* and *M. roridum* increased, there was a significant increase in relative biomass. The highest

relative biomass was 73.53 for *C. piaropi* at $1x10^9$ spores/ml (Table 4.4) .Comparison of the two pathogens with regards to relative biomass showed that *C. piaropi* had a significantly higher mean relative biomass at 64.81 as compared to 32.34 of *M. roridum.*

Table 4.4: Comparative effect of the pathogens on relative biomass

	RELATIVE BIOMASS					
Pathogen	**$1x10^5$**	**$1x10^6$**	**$1x10^7$**	**$1x10^8$**	**$1x10^9$**	**Mean**
C. piaropi	57.40	60.77	63.83	68.53	73.53	**64.81a**
M. roridum	39.53	24.73	26.73	33.13	37.60	**32.34b**
LSD						3.40
%CV						11.10

Means followed by different letters down the column are significantly different at p≤0.05

CHAPTER FIVE

DISCUSSIONS

5.1. Effect of corn oil formulations of *C. piaropi* and *M. roridum* on disease severity

The results of disease severity agreed with those reported by Piyaboon *et al*. (2016) that fungal colonization on the host plants takes time. This, according to Mendgen and Hahn (2002) is attributable to the initial formation of penetration and infection hyphae also known as appresoria that invade the plant with minimal damage to the host cells. This was the possible reason for the low level disease severity scores at week 2. The results were also in conformity with the findings of Piyaboon *et al.* (2016) who reported that *M. roridum* spores at higher suspensions in 10% palm oil or 1% Tween 20 caused a higher level of disease severity on water hyacinth plants compared to the spores applied in water alone. Fungal bioactivity in the study for both *C. piaropi* and *M. roridum* increased at higher inoculum concentration in agreement with the findings of Bo *et al*. (2019).The significant interaction between the pathogens and treatments as opposed to the non-significant one between the pathogens and weeks suggests that the pathogens were more reactive on the host with increasing spore concentration and that spore concentration was the more important factor as compared to time interval after inoculation. The fact that the mean disease scores for the pathogens at week 6 were not significant suggested that the pathogens had equal pathogenicity on water hyacinth plants at the same concentrations.

Increase in AUDPS with time following inoculation suggested that the volume of disease on the water hyacinth plants increased with increasing time. These results of progressive increase in infection also suggested that the pathogens were sustainable. This was in agreement with the findings of Tegene *et al.* (2014) on the sustainability of fungal

pathogens. Further, the increase in area under disease progress stairs (AUDPS) with time was in agreement with the findings of Sharma *et al.* (2016) that these fungi have a potential of being self-sustaining.

The results reported indicated that the two fungi; *C.piaropi* and *M. roridum* isolated from water hyacinth plants and formulated at $1x10^9$spores/ml were able to infect healthy water hyacinth plants and cause debilitating leaf spot symptoms that rapidly spread across the leaves of inoculated plants.

5.2: Effect of corn oil formulations of *C. piaropi* and *M. roridum* on disease incidence

The results for effect of formulations on disease incidence were suggestive of the fact that the disease spread as the pathogens were able to establish on the plants. Higher spread in higher spore concentration within the formulations was in conformity with the findings of Mendgen and Hahn (2002) who reported that increasing inoculum concentration has the potential of making the bio pathogen in question more efficacious. These results suggested that at $1x10^8$ and $1x10^9$spores/ml for both *C. piaropi* and *M. roridum* disease spread was highest than at lower spore concentration levels healthy water hyacinth plants and caused debilitating leaf spot symptoms that rapidly spread across the leaves of inoculated plants.

The significant interaction between formulation and weeks and the non-significant interaction between the pathogens and weeks was a compelling reason for the suggestion that for the formulations to elicit observable disease incidence, time was essential and that the pathogens did not change their mode of action with changing time. Disease incidence could therefore be said to be a time dependent activity and that spread of inocula within the water hyacinth mats required time. These results conform to the findings of Bo *et al.* (2019) that epidemiology of leaf spot disease in the water hyacinth host is time dependent.

5.3:Effect of corn oil formulations of *C. piaropi* and *M. roridum* on relative shoot length

This results of effect of corn oil formulations on relative shoot length suggested that the inoculated plants had suppressed shoot elongation as compared to the control plants on which no antagonist had been applied. The significant interactions between the pathogens and formulation and formulations and weeks was indicative that relative shoot length was strongly dependent on the pathogen, formulation and time period. The importance of this result was that both reduced growth and resurgence of the weed disallowed the weed to build huge populations that form dense mats on water surfaces. This finding agree with Asmare (2017) and Worku and Sahile (2018) who reported similar results in Lake Tana. Fungal pathogens manipulate plant metabolism in their own favour therefore denying the plant the necessary resources for tissue growth with subsequent reduction on growth (Doehlemann *et al*., 2017). The bio pathogens were thus seen as important in lessening the detrimental effects of the normally luxuriant water hyacinth growth (Sharma *et al.*2016; Waithaka, 2013). The reduction in shoot length was attributed to the severe stress caused by the pathogens, which affected the ability of the mature plants to produce strong fresh leaves and daughter plants. Necrotrophic pathogens such as *C. piaropi*and *M. roridum* secrete toxins to kill water hyacinth plant tissues. This is agreed with the findings of To-Anun *et al*.(2011) that the pathogens produce toxins; cercosporin and roridin for *C. piaropi* and *M. roridum* respectively that are able to lower the growth rate of water hyacinth.

5.4:Effect of corn oil formulations of *C. piaropi* and *M. roridum* on relative biomass

The results are in agreement with the findings of Admas *et al*. (2017) who reported that fungal pathogens cause diseases upon water plants that reduces their biomass. For all the

spore concentration levels, *C. piaropi* had significantly higher relative biomass reduction as compared to *M.roridum*. These results also conformed to the findings of Joost van den Brink *et al.* (2013) who in a study of plant biomass degradation by *Myceliophthora heterothallica* reported that fungal pathogens are able to degrade the biomass of plants. Moran (2005) who demonstrated similar results in field plots with *C. piaropi*. This lessened biomass will curtail interference and put it at manageable levels (Eid and Shaltout, 2017).This agreed with the findings of Robles *et al*. (2015) that biomass reduction is useful and effective as a control method for water hyacinth.

CHAPTER SIX

CONCLUSION, RECOMMENDATIONS ANS SUGGESTIONS FOR FURTHER RESEARCH

6.1 CONCLUSION

Cercospora piaropi and *M.roridum* leaf spot disease severity in water hyacinth increased with increasing spore concentration in the corn oil. The inherent disease volume as evidenced from AUDPS values also increased as the spore concentration increased. These increase in disease infection parameters compromised the water hyacinth growth. Corn oil formulation with spore concentrations of $1x10^8$ and$1x10^9$ spores/ml of *C. piaropi* and *M.roridum* were the most effective.

Increasing spore concentration within the corn oil formulations of *C. piaropi* and *M. roridum* in corn oil increased the leaf spot disease incidence in water hyacinth. This increase was time dependent and the incidence got higher with increasing time up to six weeks.

Both *C. piaropi* and *M. roridum* lowered shoot length of water hyacinth plants. The most effective concentration for the corn oil formulation were$1x10^8$ and $1x10^9$ spores/ml. The strategy of implementing biological control of water hyacinth by *C. Piaropi* and *M.roridum*in inundative/augmentative bioherbicide approach that involves the use of corn oil as carrier material was deemed feasible and with the potential of addressing the water hyacinth menace in water bodies such as Lake Victoria.

Water hyacinth biomass was lowered with high concentration of up to $1x10^9$ spore/ml. In order to weaken the water hyacinth vegetative structure for easier management,

application augmentative use of *C, piaropi* and *M. roridum* was considered a viable option in water hyacinth management.

6.2 RECOMMENDATION

Based on increased leaf spot severity, incidence and physical stress upon the water hyacinth plants, this study recommends that any of the two bio pathogens, *C. piaropi* and *M.roridum* can be formulated in corn oil at spore concentrations of $1x10^8$and $1x10^9$spores/ml for adoption in the control of water hyacinth in Lake Victoria.

6.3 SUGGESTIONS FOR FURTHER STUDIES

The following studies are suggested:

1. More studies should be done in the greenhouse using $1x10^8$and $1x10^9$spores/ml formulated in other oils before field trials in Lake Victoria.

2. Other vegetable oils such as palm, sesame and sunflower oil could be used for the formulation of the pathogen and the disease intensity manifested compared across oils.

3. The pathogens could be tried out in an integrated approach with *Neochetina* arthropod pests to determine the effect on water hyacinth shoot length and biomass.

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APPENDICES

Appendix 1a: Biweekly disease severity scores

Pathogen	Week 2			Week 4			Week 6		
	Form	**Rep**	**DS score**	**Form**	**Rep**	**DS score**	**Form**	**Rep**	**DS score**
Untreated	Contr.	1	0	Contr.	1	0	Contr.	1	2
C. piaropi	$1x10^5$	1	0	$1x10^5$	1	1	$1x10^5$	1	3
C. piaropi	$1x10^6$	1	1	$1x10^5$	1	2	$1x10^5$	1	4
C. piaropi	$1x10^7$	1	1	$1x10^5$	1	3	$1x10^5$	1	5
C. piaropi	$1x10^8$	1	1	$1x10^5$	1	4	$1x10^5$	1	5
C. piaropi	$1x10^9$	1	2	$1x10^5$	1	5	$1x10^5$	1	2
M. roridum	$1x10^5$	1	0	$1x10^5$	1	1	$1x10^5$	1	3
M. roridum	$1x10^6$	1	0	$1x10^5$	1	1	$1x10^5$	1	4
M. roridum	$1x10^7$	1	1	$1x10^5$	1	2	$1x10^5$	1	4
M. roridum	$1x10^8$	1	2	$1x10^5$	1	3	$1x10^5$	1	4
M. roridum	$1x10^9$	1	3	$1x10^5$	1	4	$1x10^5$	1	0
untreated	Contr	2	0	Contr	2	0	Contr	2	2
C. piaropi	$1x10^5$	2	1	$1x10^5$	2	2	$1x10^5$	2	4
C. piaropi	$1x10^6$	2	1	$1x10^5$	2	3	$1x10^5$	2	5
C. piaropi	$1x10^7$	2	1	$1x10^5$	2	3	$1x10^5$	2	5
C. piaropi	$1x10^8$	2	2	$1x10^5$	2	3	$1x10^5$	2	5
C. piaropi	$1x10^9$	2	3	$1x10^5$	2	4	$1x10^5$	2	3
M. roridum	$1x10^5$	2	0	$1x10^5$	2	2	$1x10^5$	2	3
M. roridum	$1x10^6$	2	1	$1x10^5$	2	2	$1x10^5$	2	3
M. roridum	$1x10^7$	2	1	$1x10^5$	2	3	$1x10^5$	2	4
M. roridum	$1x10^8$	2	2	$1x10^5$	2	4	$1x10^5$	2	5
M. roridum	$1x10^9$	2	2	$1x10^5$	2	4	$1x10^5$	2	0
untreated	Contr	3	0	Contr.	3	0	Contr.	3	3
C. piaropi	$1x10^5$	3	1	$1x10^5$	3	2	$1x10^5$	3	3
C. piaropi	$1x10^6$	3	1	$1x10^5$	3	2	$1x10^5$	3	4
C. piaropi	$1x10^7$	3	1	$1x10^5$	3	3	$1x10^5$	3	5
C. piaropi	$1x10^8$	3	2	$1x10^5$	3	4	$1x10^5$	3	6
C. piaropi	$1x10^9$	3	3	$1x10^5$	3	4	$1x10^5$	3	2
M. roridum	$1x10^5$	3	1	$1x10^5$	3	1	$1x10^5$	3	3
M. roridum	$1x10^6$	3	1	$1x10^5$	3	2	$1x10^5$	3	3
M. roridum	$1x10^7$	3	2	$1x10^5$	3	2	$1x10^5$	3	5
M. roridum	$1x10^8$	3	2	$1x10^5$	3	3	$1x10^5$	3	5
M. roridum	$1x10^9$	3	2	$1x10^5$	3	4	$1x10^5$	3	0

Appendix 1b: ANOVA for disease severityscores

Source	*df*	*ss*	*ms*	*F value*	*Pr>f*
Form.	5	117.741	23.548	88.92	<.0001
Path.	1	3.703	3.703	13.99	0.0013
Path. xFormulation	5	2.296	0.459	1.73	0.1728
Week	2	100.352	50.176	189.48	<.0001
Form. x week	10	29.870	2.987	11.28	<.0001
Path. x Week	2	1.685	0.843	3.18	0.0631
Path. x Form. x Week	10	7.648	0.765	2.89	0.0209

t Tests (LSD) for disease severity scores

Alpha	**0.05**
Error Degrees of Freedom	20
Error Mean Square	0.264815
Critical Value of t	2.08596
Least Significant Difference (LSD)	0.3578

t Grouping	**Mean**	**N**	**Trt**
A	3.4444	18	$1x10^{8}$
A	3.2778	18	$1x10^{9}$
B	2.8333	18	$1x10^{7}$
C	2.222	18	$1x10^{6}$
D	1.7222	18	$1x10^{5}$
E	0.000	18	Control

Means with the same letter are not significantly different

Appendix 1c: Comparative effect of the pathogens on disease severity

Pathogen	$1x10^5$	$1x10^6$	$1x10^7$	$1x10^8$	$1x10^9$	Mean
C. piaropi	1.67a	2.55a	3.00a	3.45a	3.11a	**2.76a**
M. roridum	1.55a	1.89a	2.66a	3.33a	3.33a	**2.55a**
LSD						3.40
%CV						11.10

Numbers followed by the same letter down the column are not significantly different at p≤0.05

Appendix 1d: Disease severity progression by AUDPS

	Week 2			Week 4			Week 6		
Pathogen	**Form**	**Rep**	**AUDPS**	**Form**	**Rep**	**AUDPS**	**Form**	**Rep**	**AUDPS**
untreated	Contr.	1	0	Contr.	1	0	Contr.	1	0
C. piaropi	$1x10^5$	1	0	$1x10^5$	1	1.5	$1x10^5$	1	5
C. piaropi	$1x10^6$	1	1	$1x10^5$	1	4.5	$1x10^5$	1	10
C. piaropi	$1x10^7$	1	1	$1x10^5$	1	6	$1x10^5$	1	13.5
C. piaropi	$1x10^8$	1	1	$1x10^5$	1	7.5	$1x10^5$	1	17
C. piaropi	$1x10^9$	1	2	$1x10^5$	1	7	$1x10^5$	1	20.5
M. roridum	$1x10^5$	1	0	$1x10^5$	1	1.5	$1x10^5$	1	5
M. roridum	$1x10^6$	1	0	$1x10^5$	1	1.5	$1x10^5$	1	10
M. roridum	$1x10^7$	1	1	$1x10^5$	1	4.5	$1x10^5$	1	11.5
M. roridum	$1x10^8$	1	2	$1x10^5$	1	7.5	$1x10^5$	1	15
M. roridum	$1x10^9$	1	3	$1x10^5$	1	10.5	$1x10^5$	1	18.5
untreated	Contr.	2	0	Contr	2	0	Contr	2	0
C. piaropi	$1x10^5$	2	1	$1x10^5$	2	4.5	$1x10^5$	2	8.5
C. piaropi	$1x10^6$	2	1	$1x10^5$	2	6	$1x10^5$	2	13.5
C. piaropi	$1x10^7$	2	1	$1x10^5$	2	6	$1x10^5$	2	15
C. piaropi	$1x10^8$	2	2	$1x10^5$	2	7.5	$1x10^5$	2	16.5
C. piaropi	$1x10^9$	2	3	$1x10^5$	2	7	$1x10^5$	2	20
M. roridum	$1x10^5$	2	0	$1x10^5$	2	3	$1x10^5$	2	8.5
M. roridum	$1x10^6$	2	1	$1x10^5$	2	4.5	$1x10^5$	2	10
M. roridum	$1x10^7$	2	1	$1x10^5$	2	6	$1x10^5$	2	12
M. roridum	$1x10^8$	2	2	$1x10^5$	2	9	$1x10^5$	2	17
M. roridum	$1x10^9$	2	2	$1x10^5$	2	9	$1x10^5$	2	18.5
untreated	Contr.	3	0	Contr.	3	0	Contr.	3	0
C. piaropi	$1x10^5$	3	1	$1x10^5$	3	4.5	$1x10^5$	3	10
C. piaropi	$1x10^6$	3	1	$1x10^5$	3	4.5	$1x10^5$	3	10
C. piaropi	$1x10^7$	3	1	$1x10^5$	3	6	$1x10^5$	3	13.5
C. piaropi	$1x10^8$	3	2	$1x10^5$	3	9	$1x10^5$	3	18.5
C. piaropi	$1x10^9$	3	3	$1x10^5$	3	7	$1x10^5$	3	21.5
M. roridum	$1x10^5$	3	0	$1x10^5$	3	3	$1x10^5$	3	6.5
M. roridum	$1x10^6$	3	1	$1x10^5$	3	4.5	$1x10^5$	3	10
M. roridum	$1x10^7$	3	2	$1x10^5$	3	6	$1x10^5$	3	11.5
M. roridum	$1x10^8$	3	2	$1x10^5$	3	7.5	$1x10^5$	3	16.5
M. roridum	$1x10^9$	3	2	$1x10^5$	3	9	$1x10^5$	3	18.5

Appendix 1e: ANOVA for AUDPS

Source	*df*	*ss*	*ms*	*F value*	*Pr>f*
Form.	5	897.069	179.414	305.39	<.0001
Pathogens	1	8.403	8.403	14.30	0.0016
Week	2	2027.338	1013.669	1725.39	<.0001
Path. x Form.	4	3.583	0.896	1.52	0.2422
Form. x Week	10	415.939	41.594	70.80	<.0001
Path. x Week	2	11.006	5.503	9.37	0.0020
Path. x Form. x Week	8	14.300	1.788	3.04	0.0277

t Tests (LSD) for AUDPS

Alpha	0.05
Error Degrees of Freedom	16
Error Mean Square	0.5875
Critical Value of t	2.11991
Least Significant Difference (LSD)	0.328
Harmonic Mean of Cell Sizes	49.09091

Cell sizes are not equal

t Grouping	Mean	N	Pathogen
A	6.5556	45	*M. roridum*
B	5.0722	54	*C. piaropi*

Appendix1f: Comparative effect of the pathogens on AUDPS

Pathogen	$1x10^5$	$1x10^6$	$1x10^7$	$1x10^8$	$1x10^9$	Mean
C. piaropi	4.00b	5.72b	7.00b	9.00b	10.11a	**7.17b**
M. roridum	3.06a	4.72a	6.17a	8.72a	10.11a	**6.56a**
LSD						0.33
%CV						13.70

Means followed by different letters down the column are significantly different at p≤0.05

Appendix 2a: Biweekly disease incidence

WEEK	PATHOGEN	Form	REP 1	REP 2	REP 3	Mean DI
2	*C. piaropi*	$1x10^5$	22.2	12.5	11.1	**15.27**
2	*C. piaropi*	$1x10^6$	33.3	50	55.6	**46.3**
2	*C. piaropi*	$1x10^7$	50	44.4	50	**48.13**
2	*C. piaropi*	$1x10^8$	50	55.6	50	**51.87**
2	*C. piaropi*	$1x10^9$	57.1	50	55.6	**54.25**
2	*M. roridum*	$1x10^5$	33.3	50	44.4	**42.57**
2	*M. roridum*	$1x10^6$	50	33.3	50	**44.43**
2	*M. roridum*	$1x10^7$	50	44.4	50	**48.13**
2	*M. roridum*	$1x10^8$	44.4	50	60	**51.47**
2	*M. roridum*	$1x10^9$	62.5	66.7	70	**66.4**
4	*C. piaropi*	$1x10^5$	44.4	50	50	**48.13**
4	*C. piaropi*	$1x10^6$	50	60	50	**53.33**
4	*C. piaropi*	$1x10^7$	60	50	50	**53.33**
4	*C. piaropi*	$1x10^8$	50	55.6	60	**55.2**
4	*C. piaropi*	$1x10^9$	51.1	50	66.7	**55.93**
4	*M. roridum*	$1x10^5$	62.5	55.6	60	**59.37**
4	*M. roridum*	$1x10^6$	50	71.4	62.5	**61.3**
4	*M. roridum*	$1x10^7$	75	77.8	61.2	**71.33**
4	*M. roridum*	$1x10^8$	85.7	75	66.7	**75.8**
4	*M. roridum*	$1x10^9$	83.3	75	77.8	**78.7**
6	*C. piaropi*	$1x10^5$	75	60	66.7	**67.23**
6	*C. piaropi*	$1x10^6$	80	75	60	**71.67**
6	*C. piaropi*	$1x10^7$	66.7	75	80	**73.9**
6	*C. piaropi*	$1x10^8$	80	66.7	83.3	**76.67**
6	*C. piaropi*	$1x10^9$	100	80	66.7	**82.23**
6	*M. roridum*	$1x10^5$	100	75	75	**83.33**
6	*M. roridum*	$1x10^6$	100	50	100	**83.33**
6	*M. roridum*	$1x10^7$	83.3	100	80	**87.77**
6	*M. roridum*	$1x10^8$	80	100	83.3	**87.77**
6	*M. roridum*	$1x10^9$	100	66.7	100	**88.9**

Appendix 2b: ANOVA for disease incidence

Source	*df*	*ss*	*ms*	*F value*	*Pr>f*
Form.	5	62637.293	12527.459	109.91	<.0001
Path.	1	2615.669	2615.669	22.95	<.0001
Week	2	14035.500	7017.750	61.57	<.0001
Path. x Form.	5	897.426	179.485	3.21	0.2126
Form. x Week	10	3664.265	366.427	3.21	0.0126
Path. x Week	2	2235128	117.564	1.03	0.3747
Path. x Form. x Week	10	914615	91.462	0.80	0.6287

t Tests (LSD) for disease incidence

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	113.976
Critical Value of t	2.08596
Least Significant Difference (LSD)	7.4232

t Grouping	Mean	N	Trt
A	71.067	18	$1x10^9$
A	66.461	18	$1x10^8$
AB	63.767	18	$1x10^7$
BC	60.061	18	$1x10^6$
C	52.650	18	$1x10^5$
D	0.000	18	Control

Means with the same letter are not significantly different

Appendix 2c: Comparative effect of the pathogens on disease incidence

Pathogen	$1x10^5$	$1x10^6$	$1x10^7$	$1x10^8$	$1x10^9$	Mean
C. piaropi	43.58a	62.50a	58.45a	61.25a	64.14a	**57.98a**
M. roridum	83.33b	83.33b	87.77b	87.77b	88.90b	**86.22b**
%CV						16.90
LSD						7.42

Numbers followed by different letters down the column are significantly different at p≤0.05

Appendix 3a: biweekly relative shoot length

Week	Pathogen	Form	Rep 1		Rep 2		Rep 3		Total	MEAN *R*
			Length	*R*	Length	*R*	Length.	*R*		
2	Untreated	Contr.	157	0	160	0	162	0	**0**	**0.0**
2	*C. piaropi*	$1x10^5$	118	24.8	114	28.8	116	28.4	**82**	**27.3**
2	*C. piaropi*	$1x10^6$	120	23.6	119	25.6	117	27.8	**77**	**25.7**
2	*C. piaropi*	$1x10^7$	115	26.8	113	29.4	111	31.5	**87.7**	**29.2**
2	*C. piaropi*	$1x10^8$	110	29.9	111	30.6	112	30.9	**91.4**	**30.5**
2	*C. piaropi*	$1x10^9$	109	30.6	110	31.3	109	32.7	**94.6**	**31.5**
2	*M. roridum*	$1x10^5$	120	23.6	117	26.9	117	27.8	**78.3**	**26.1**
2	*M. roridum*	$1x10^6$	116	26.1	117	26.9	118	27.2	**80.2**	**26.7**
2	*M. roridum*	$1x10^7$	115	26.8	111	30.6	115	29	**86.4**	**28.8**
2	*M. roridum*	$1x10^8$	116	26.1	115	28.1	117	27.8	**82**	**27.3**
2	*M. roridum*	$1x10^9$	114	28.7	112	30	107	34	**92.7**	**30.9**
4	Untreated	Contr	206	0	210	0	211	0	**0**	**0.0**
4	*C. piaropi*	$1x10^5$	130	37	131	37.6	129	38.9	**113.5**	**37.8**
4	*C. piaropi*	$1x10^6$	128	37.7	126	40	120	43.1	**120.8**	**40.3**
4	*C. piaropi*	$1x10^7$	119	42.2	120	42.9	119	43.6	**128.7**	**42.9**
4	*C. piaropi*	$1x10^8$	116	43.7	119	43.3	114	46	**133**	**44.3**
4	*C. piaropi.*	$1x10^9$	109	47.1	90	57.1	99	53.1	**157.3**	**52.4**
4	*M. roridum*	$1x10^5$	143	30.6	147	30	140	33.6	**94.2**	**31.4**
4	*M. roridum*	$1x10^6$	139	32.5	136	35.2	135	36	**103.7**	**34.6**
4	*M. roridum*	$1x10^7$	130	36.9	133	36.7	134	36.5	**110.1**	**36.7**
4	*M. roridum*	$1x10^8$	127	38.3	126	40	122	42.2	**120.5**	**40.2**
4	*M. roridum*	$1x10^9$	120	41.7	119	43.3	121	42.7	**127.7**	**42.6**
6	Untreated	Contr.	220	0	223	0	225	0	**0**	**0.0**
6	*C. piaropi*	$1x10^5$	113	48.6	110	50.7	111	50.7	**150**	**50.0**
6	*C. piaropi*	$1x10^6$	115	47.7	112	49.8	110	51.1	**148.6**	**49.5**
6	*C. piaropi*	$1x10^7$	110	50	111	50.2	108	52	**152.2**	**50.7**
6	*C. piaropi*	$1x10^8$	107	51.3	100	55.2	111	50.7	**157.2**	**52.4**
6	*C. piaropi.*	$1x10^9$	100	54.5	101	54.7	99	56	**165.2**	**55.1**
6	*M. roridum*	$1x10^5$	116	47.3	110	50.7	111	50.7	**148.7**	**49.6**
6	*M. roridum*	$1x10^5$	110	50	112	49.8	113	49.8	**149.6**	**49.9**
6	*M. roridum*	$1x10^5$	110	50	106	52.5	109	49.3	**151.8**	**50.6**
6	*M. roridum*	$1x10^5$	110	50	110	50.7	112	50.2	**150.9**	**50.3**
6	*M. roridum*	$1x10^5$	110	50	107	52	104	53.8	**155.8**	**51.9**

Appendix 3b: ANOVA for relative shoot length

Source	*df*	*ss*	*ms*	*F value*	*Pr>f*
Form.	5	24448.733	4889.747	3861.43	<.0001
Path.	1	148.403	148.403	117.19	<.0001
Week	2	6385.087	3192.543	2521.15	<.0001
Path. x Form.	5	53.654	10.730	8.47	0.0002
Form. x Week	10	1453.451	145.345	114.78	<.0001
Pathogens x Week	2	125.735	62.868	49.65	<.0001
Path. x Form. x Week	10	54.167	5.417	4.28	0.0028

t Tests (LSD) for reduction in shoot length

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	1.266306
Critical Value of t	2.08596
Least Significant Difference (LSD)	0.7824

t Grouping	Mean	N	Trt
A	44.0722	18	$1x10^9$
B	40.8333	18	$1x10^8$
C	39.8278	18	$1x10^7$
D	37.7722	18	$1x10^6$
D	37.0389	18	$1x10^5$
E	0.000	18	Control

Means with the same letter are not significantly different

Appendix 3c: Comparative effect of the pathogens on relative shoot length

Pathogen	$1x10^5$	$1x10^6$	$1x10^7$	$1x10^8$	$1x10^9$	Mean
C. piaropi	38.89b	38.49b	40.95b	42.40b	46.34b	**41.31b**
M. roridum	35.69a	37.10a	38.70a	39.27a	41.80a	**38.51a**
%CV						16.9
LSD						0.78

Numbers followed by different letters down the column are significantly different at p≤0.05

Appendix 4a: Relative biomass

Form	Pathogen	Rep 1		Rep 2		Rep 3		Total I	*Mean (I)*
		Wt.	***I***	**Wt.**	***I***	**Wt.**	***I***		
Contr.	Untreated	205	0	301	0	240	0	**0**	**0.0**
$1x10^5$	*C. piaropi*	100	51.2	110	63.5	102	57.5	**172.2**	**57.4**
$1x10^6$	*C. piaropi*	95	53.7	97	67.8	94	60.8	**182.3**	**60.8**
$1x10^7$	*C. piaropi*	90	56.1	89	70.4	84	65	**191.5**	**63.8**
$1x10^8$	*C. piaropi*	70	65.9	75	75.1	85	64.6	**205.6**	**68.5**
$1x10^9$	*C. piaropi*	60	70.7	63	79.1	70	70.8	**220.6**	**73.5**
$1x10^5$	*M. roridum*	149	27.3	139	53.8	150	37.5	**118.6**	**39.5**
$1x10^6$	*M. roridum*	180	12.2	186	38.2	183	23.8	**74.2**	**24.7**
$1x10^7$	*M. roridum*	180	12.2	179	40.5	174	27.5	**80.2**	**26.7**
$1x10^8$	*M. roridum*	163	20.5	160	46.8	163	32.1	**99.4**	**33.1**
$1x10^9$	*M. roridum*	152	25.9	151	49.8	151	37.1	**112.8**	**37.6**

Appendix 4b: ANOVA for relative biomass

Source	***df***	***ss***	***ms***	***F value***	***Pr>f***
Form.	5	12392.647	2478.529	401.67	<.0001
Pathogens	1	6588.028	6588.028	1067.65	<.0001
Pathogens x Form.	5	1719.562	343.912		

t Tests (LSD) for relative biomass

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	6.170611
Critical Value of t	2.22814
Least Significant Difference (LSD)	3.1956

t Grouping	**Mean**	**N**	**Trt**
A	55.567	6	$1x10^9$
B	50.833	6	$1x10^8$
BC	48.467	6	$1x10^7$
CD	45.283	6	$1x10^6$
D	42.750	6	$1x10^5$
E	0.000	6	Control

Means with the same letter are not significantly different