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Bio control of water hyacinth with *Cercospora piaropi* and *Myrothecium roridum* corn oil formulations in the greenhouse for enhanced water resources management and conservation

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Abstract

Water hyacinth hinders extraction and supply of clean water by clogging intake systems. Despite control efforts, it has remained resurgent and difficult to manage under current methods. Efficacious control for the weed is therefore necessary. This study's goal was determination of effect of corn oil spore formulations of *Cercospora piaropi* Tharp and *Myrothecium roridum* Tode Fries on; disease intensity, relative shoot length and relative biomass of water hyacinth. It was conducted in Maseno University and in a greenhouse at Kibos in Kisumu situated at latitude 0^0 37′ S and longitude 37^0 20′ E. The conditions were 25 to 30^0 C and 22 to 27^0 C temperature averages during the day and night respectively and 60 to 69% relative humidity. Isolations of *C. piaropi* and *M. roridium* were made, grown on PDA, harvested and formulated in corn oil at $1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$ and $1x10^9$ spores/ml. Healthy plants were misted with the corn oil formulations and placed in 90 cm diameter and 1.5 cm depth plastic basins filled with 20 liters of water that had been kept in a container for 24 hours for chlorine to be released. The experimental design was completely randomized design with three replications. Disease intensity, relative shoot length and relative biomass were determined biweekly for 6 weeks. Analysis of variance on the means was done using SAS Institute, Inc.1999 computer software and LSD ($p \leq 0.05$) used for mean separation. Disease intensity, AUDPS, relative shoot length and relative biomass scores for both pathogens showed a significant ($p \leq .05$) increase as the concentration of spores in the formulations increased. *Cercospora piaropi* and *M. roridum* in corn oil formulation at $1x10^8$ and $1x10^9$ spores/ml were found effective for lowering water hyacinth biomass and shoot growth respectively and with potential for use in open waters for water hyacinth control.

Keywords: *Biopathogen; disease intensity; fungal pathogen; kibos; mycoherbicides; petiole elongation; water resources*

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Introduction

Water hyacinth, known with scientific name *Eichhornia crassipes* (Martius) Solms Laubach has been rapidly spreading in the local water bodies. It is induced by the high soil erosion and nutrient runoff with the addition of urban pollution that comes from industries. Nasution *et al.* (2016)

reports that atmospheric deposition also adds to the pollution. The weed is a native of the Amazon Basin in South America. It has been reported to have become invasive all-over fresh water bodies within the tropical and subtropical regions (Dersseh and Dessalegn, 2019). Its negative impact is aided by the fact that it is highly adaptable to a wide variety of water bodies within the humid tropics and subtropics. The water bodies that are affected include; running stream water, drainage ditches, stagnant water ponds such as those used for rice cultivation and irrigation canals (Fawad and Jamal, 2019). The weed growth in terms of stem and petiole elongation and biomass accumulation makes it well adapted to the niches it invades. These compromises the economic use of the waterways as reported by Tobias *et al*. (2019). It therefore negatively impacts water quality, reduces the supply of clean potable water and hinders water extraction by clogging water intake systems. Local fresh water lakes particularly Lake Victoria and Lake Naivasha have continued to experience the water hyacinth menace due to eutrophication, one of the major ecological concerns over the world in recent times (Biswajit and Kamal, 2019). Reports by Tobias *et al*. (2019) have indicated that in the tidal water systems water quality directly outside the patch in all directions is impacted. Though the weed is a bio cleaning agent that would be necessary to have in such waters as reported by Ambastha *et al*. (2017), its growth and biomass accumulation have given it the ability of blocking the economic use of various water bodies. This calls for concerted efforts aimed at its control. In spite of the application of control methods, as reported by Ongore *et al*. (2018) and Segbefia *et al*. (2019), the fact is that water hyacinth has remained resurgent and difficult to manage. This has negatively affected a lot of livelihood activities. Tobias *et al.* (2019) have reported that the luxuriant petiole elongation and biomass accumulation of water hyacinth are among the properties that make the weed capable of blocking economic use of the waterways. The principal drawback with biological control particularly with unformulated bio-pathogens has been the period required to achieve control (Yigermal *et al*., 2020). Though formulation is recognized as a way of increasing both efficiency of application and efficacy as reported by Bo *et al*. (2019), there has been lack of empirical data on the effect of formulating *C. piaropi* and *M. roridum* and other fungal pathogens in different vegetable oils under different spore densities. This kind of data could be used in determining not only the optimal application rate in terms of inoculum density but also qualify local vegetable oils such as maize germ oil as formulation materials. It would also aid in determining the best mode of inoculation with the fungi in order to obtain their maximum efficacy.

High proliferation coupled with high seed production rate, ability for both sexual and asexual reproduction, high expenses have been reported to make physical control and herbicide application to be non-sustainable (Worku and Sahile, 2018). This has made researchers to devote a lot of effort in developing control measures. Much of the research on bio control with pathogens 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 (Asmare, 2017). The bio control efforts have however hit a snag due to among other reasons; poor efficiency of application of the mycoherbicides, 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. In the aforementioned research efforts, *M. roridum* has been identified as an effective fungal agent against water hyacinth. In pathogenicity tests involving disease severity scoring and the extent of disease incidence, Piyaboon *et al*. (2016) qualified the bioagent as one of the best in the control of water hyacinth. However, the shortcoming in the study that qualified the fungus, only the density of the spore suspension at $1x10^9$ spores /ml was used. From the results of the study, it could not be ascertained if this level of spore density was the optimum level for maximum disease severity, disease incidence, and suppressed biomass and shoot elongation. The same scenario has been noticed in *C. piaropi* inoculation studies where variation of inoculum

concentration was never carried out. There is a need therefore to obtain formulations of known inoculum concentration for the control of water hyacinth.

As it has been reported, 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 and other waters (Worku and Sahile, 2018). Putting a check on the biomass and petiole growth would go a long way in opening up the affected waters for economic use as reported by Eid and Shaltout (2017). A summary of the major constraints in biological control with fungal pathogens such as *C. piaropi* and *M. roridum* has been that there has been no comparative data so far on leaf spot disease severity and incidence for different vegetable oils that could be used to qualify the vegetable oil formulations in question as being efficacious substrates for mass production as mycoherbicides. In addition, as far as slowing down water hyacinth spread to uninfected areas is concerned, there is a lack of information about how different oil formulations of *C. piaropi* and *M. roridum* impact on leaf spot disease incidence within the water hyacinth marts. Whereas bio pathogens have been reported to be important in lessening the detrimental effects of the normally luxuriant shoot growth of water hyacinth (Sharma *et al*., 2016), there is lack of data based on comparative studies that can be used to come up with suitable vegetable oil(s) having potential as formulation material for *C. piaropi* and *M. roridum* biocontrol agents. 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. Robles *et al*. (2015) reported that biomass estimation is useful in determining the effectiveness of water hyacinth control measures. However, there is currently lack of comparative data on biomass estimation for different vegetable oils upon which recommendation of the most effective vegetable oil for mycoherbicide development can be based. Biological herbicides such as vegetable oil fungal formulations are more effective when incorporated into weed management programs particularly in the mechanical management (Berestetskiy and Sokornova, 2018). Knowledge of appropriate inoculum density will be crucial in understanding and standardizing the design of appropriate prevention and control policies. Water hyacinth being a fast-growing plant can easily use escape mechanism to evade disease caused by pathogens with low incubation period. This coupled with information generated on leaf spot disease intensity as it impacts petiole growth and biomass would be used as an appropriate tool by the stakeholders for the management of this obnoxious weed. Epidemiologists in water hyacinth management would not be misled about the viability of initiating fungal biocontrol strategies. In line with the foregoing exposition of the knowledge gaps in the management of water hyacinth via fungal pathogens, the goal of this study therefore was to determine the effect of *C. piaropi* and *M. roridum* corn oil formulations in the greenhouse on water hyacinth for enhanced water resources management and conservation. The study was set out with the specific objective of determining 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 inoculated with corn oil formulations of the two bio pathogens. The hypotheses to be tested were that increase in corn oil spore concentrations of *C. piaropi* and *M. roridum* do not significantly reduce leaf spot disease severity, disease incidence, relative shoot length and relative biomass of water hyacinth.

Materials and methods

Study location

Sampling of plants to be used in the study was done at the Lake Victoria water fronts at Kisat river entry point into the lake, Lwang'ni beach and at the Dunga fish landing site. Laboratory based activities involving isolation, culturing and formulation of the pathogens were conducted in Maseno University Botanic Garden and laboratory. The geographical co-ordinates in degrees minutes seconds are latitude -1^{0}0′ 00′′ S and longitude 34^0 36′ 00′′E. The climate is tropical with an average temperature of 26.6^0 C. The temperatures in February average 21.4^0 C while July has the lowest average of 19.6^0 C. Generally, the variation in temperatures throughout the year is 1.8^{0}C. Greenhouse studies were carried out in Kibos located at latitude 00 38′S and longitude 38′21′E. The greenhouse conditions were 25 to 30^0

C and 22 to 27 ^{0}C temperature averages during the day and night respectively and 60 to 69 % relative humidity.

Isolation of C. piaropi

Following the procedure used by Jimenez (2010), *C. piaropi* infected leaves were 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 in 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.

Isolation of M. roridum

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.

Harvesting of pathogen spores

Following the method of Tahlan (2014), 100 mls 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^{0}C awaiting usage.

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 (50.4 cm^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.

Setting up the experiments

The experiment was set up in the greenhouse in 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).

Formulation of C. piaropi and M. roridum

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 on two of the large grids with sixteen smaller squares. All the cells in the large grid were counted and the average taken. The estimation of the cells in the droplet was arrived at and used to estimate the cells in 1 ml of suspension. Using serial dilution, the number of cells in the suspension was adjusted to $1x10^9$, $1x10^8$, $1x10^7$, $1x10^6$ and $1x10^5$ spores/ml.

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 Mutebi *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).

Collection of disease severity data

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 % of leaves show symptoms)

1 = 1 to 10 % leaves show symptoms

2 = 11 to 25 % leaves show symptoms

3 = 26 to 50 % leaves show symptoms

4 = 51 to 75 % leaves show symptoms

5 = ≥75 % area covered by leaf spot, leaf senescing

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:

$$AUDPC = \frac{obs1Xt1}{2} + obs2Xt2 + \frac{obs3Xt3}{2}$$

Where:

Obs 1 = observation of disease severity at time interval 1

Obs 2 = observation of disease severity at time interval 2

Obs 3 = observation of disease severity at time interval 3 and

t 1, t 2 and t 3 are time intervals between observation of disease severity.

Step 2: Calculation of AUDPS:

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

Where:

AUDPC = Area under disease progress curve for the respective biweekly disease severity score

AUDPS = Area under disease progress stairs,

y1 = Disease severity score at first time

yn = Disease severity score at last time

D = tn-t1

n = number of observations

Collection of disease incidence data

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

Collection of relative shoot length data

At weeks 2, 4, and 6 after 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.

Collection of relative biomass data

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 80^0 C 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, and 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:

$$I = \frac{Ap - At}{Ap} \text{X} 100$$

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.

Data Analysis

In order to assess the water hyacinth response after inoculation with the two pathogens over the experimental period, the data on disease severity, AUDPS, disease incidence, relative shoot length and relative biomass 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).

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* in corn oil significantly ($p \leq .05$) 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 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 *et al*. (2016) disease severity scale. Further there was increase in disease severity for both pathogens at subsequent biweekly intervals.

Results

Table 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**	**22.23**	**22.23**	**22.23**	**22.23**	**22.23**
LSD	**0.36**	**0.36**	**0.36**	**0.36**	**0.36**	**0.36**

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

Effect of corn oil formulations of C. piaropi and M. roridum on AUDPS

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* in corn oil significantly ($p \leq .05$) increased disease progress on water hyacinth plants (Table 2). For both *C. piaropi* and *M. roridum*, as time at biweekly intervals increased, there was significant ($p \leq .05$) 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. *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 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**	**13.70**	**13.70**	**13.70**	**13.70**	**13.70**
LSD	**0.33**	**0.33**	**0.33**	**0.33**	**0.33**	**0.33**

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

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

Increasing spore concentrations of *C. piaropi* and *M. roridum* in corn oil significantly ($p \leq .05$) 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 3). At all the spore concentrations the *C. piaropi* had significantly lower disease incidence percentages than *M. roridum*.

Effect 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 5). 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 3: 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**

Form.	Disease incidence (%)					
	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**	**16.90**	**16.90**	**16.90**	**16.90**	**16.90**
LSD	**7.42**	**7.42**	**7.42**	**7.42**	**7.42**	**7.42**

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

Table 4: Effects of A. zonatum formulations (treatments) on the disease incidence by mean value Area under Disease Progress Stairs (AUDPS) of zonate leaf spot on water hyacinth

	SPORE CONCENTRATION			
FORMULATION	**10^8**	**10^7**	**10^6**	**10^5**
Corn oil	1271.0a	1237.0a	961.8a	436.0ab
Glycerol	1083.6ab	932.4a	614.6a	705.0a
Mineral oil	704.8bc	306.4b	326.2b	159.6b
Tween 80	590.1c	450.1b	404.6b	268.8b
Control	0d	0c	0c	0c
CV (%)	**39.6**	**39.6**	**39.6**	**39.6**
LSD	**396.2**	**396.2**	**396.2**	**396.2**

In a column, means followed by the same letters are not significantly different (p=0.05)

Table 5: Effect 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	16.9	16.9	16.9	16.9	16.9
LSD	0.78	0.78	0.78	0.78	0.78	0.78

Numbers followed by the same letters down the column are not significantly different at p≤0.05 shoot length increased with increasing concentration

Discussion

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.

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.

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 agrees 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.

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.

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 bio herbicide 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.

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 fresh water bodies particularly in Lake Victoria.

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