Effect of *Cercospora piaropi* Tharp and *Myrothecium roridum* Tode Fries Formulated as Corn Oil Emulsion on Water Hyacinth Shoot Growth under Greenhouse Conditions

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**Abstract**

A study was done to find out the comparative effect of *Cercospora piaropi* Tharp and *Myrothecium roridum* Tode Fries formulated as corn oil emulsion on water hyacinth shoot growth and biomass under greenhouse conditions. The study site was located in Kibos at latitude 0°37’S and longitude 37°20’E with average temperature of 25 to 30°C and 22 to 27°C during the day and night respectively, and 60 to 69% relative humidity. Healthy water hyacinth plants were inoculated with the pathogens formulated in corn oil at 1x10⁶, 1x10⁷, 1x10⁸ and 1x10⁹ spores/ml. The control plants were not inoculated. The experiment was set up in completely randomised design (CRD) with each treatment replicated three times. At weeks 2, 4, and 6 after inoculation, the average shoot length and biomass for the treated basins were separately compared to the average shoot length and biomass of the control plants. Increase in spore density for both pathogens significantly increased relative shoot length and relative biomass. Relative shoot length was 55.07 and 51.93 for *C. piaropi* and *M. roridum* respectively at 1x10⁶ spores/ml while relative biomass was 73.53 for *C. piaropi* and 37.60 at 1x10⁹ spores/ml. Inoculation suppressed shoot elongation and biomass with 1x10⁸ spores/ml being most effective. *Cercospora piaropi* formulated in corn oil lowered shoot length and biomass of water hyacinth more than *M. roridum* did.

1. Introduction

Water hyacinth invasion and its associated effects to riparian communities poses challenges to activities like fishing and farming along invaded water bodies. Destruction of farmland by flooding due to blocked drainage channels, increasing travel time used to access farms and consequent reduction in farmers income are some of the adverse effects of water hyacinth invasion (*Honla et al., 2018*). Though physical, chemical and biological control methods have been tried out, *VonBlank et al. (2018)* has stated that reoccurrence of the weed relies on biomass reintroduction by humans. The weed has therefore remained resurgent and difficult to manage (*Ongore et al., 2018; Segbefia et al., 2019*) courtesy of its 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 (*Bereestetskly and Sokornova, 2018*). These formulations have not been effective due to reasons related to rapid water hyacinth luxuriant growth in terms of shoot growth and biomass accumulation with the added advantage of ecological adaptability (*Worku and Sahile, 2018*). *Tobias et al. (2019)* reported that the weed growth in terms of stem elongation and biomass accumulation makes it have a propensity for compromising the economic use of the waterways. Management of shoot growth and biomass would open up the water for economic use (*Eid and Shaltout, 2017*).

Studies have been carried out on using various vegetable oils from plants as formulation material for pathogens for water hyacinth control (*Boyette and Hoagland, 2013*). 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 bio control. Cercosporin produced by *Cercospora* is able to lower the growth rate of water hyacinth (*To-Anunu et al., 2011*) while phytotoxins roridin A and roridin E produced by *Myrothecium* have been reported to be similar to paraquat and can be used for water hyacinth control (*Okunowo et al., 2019*). Generally, foliar pathogens working under natural disease pressure 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 (*Charudattan, 2014; Mutebi et al., 2013*).
formulation being the form of a specific product that is used to control a pest (Libs and Salim, 2017). Recent trends in the implementation of bio herbicide use in the control of water hyacinth have depended primarily on several strategies (Okunowo et al., 2019). The use of bio formulations 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 mycopathogens as they reduce or eliminate the dew requirement for fungal colonization (Berestetskaya 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 (Kal tragadda et al., 2010). A quality that qualifies corn oil as a formulation agent is its low viscosity that makes dispersal of spores within the oil easy during spore harvesting and formulation (Boyette and Hoagland, 2013).

2. Material and methods

The study was carried out in a greenhouse at Kibos in Kisumu situated at latitude 0°37’S and longitude 37°20’E. It is about 10 km from Lake Victoria. Temperature averages were 25 to 30°C and 22 to 27°C during the day and night respectively while the relative humidity averages varied from 60 to 69%. Cercospora piaropi and Myrothecium roridum were isolated from infected plants and aseptically cultured, sub cultured and spores harvested following procedure by Groenewald et al. (2013) and of Kwon et al. (2014) for the two pathogens respectively. Following the method of Tahan (2014), 100 ml 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 ml (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 until the emulsion in the pipettes 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°C awaiting usage. A haemocytometer was used to determine the concentration of the spores in the suspension employing the method created by Caprette (2000). The concentration of the stock solution was adjusted and by serial dilution to 1x10^5, 1x10^6, 1x10^7 and 1x10^8 spores/ml according to Admas et al. (2017).

Okunowo et al. (2019) prepared the 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 according to the method of Kuzmenko (2016) and Mujere (2015). The sampled plants were put into the aged water to acclimatize for 2 days (Piyaboon et al., 2016) before being inoculated. The healthy plants were placed in 20 liter basins at the rate of 3 plants per basin. The plants were applied with the 6 treatments or formulations of C. piaropi and M. roridum with; 1x10^5, 1x10^6, 1x10^7, 1x10^8 and 1x10^9 spores/ml of each of the pathogens using 100mls of the formulation on the plants with a spray pump held at 20 cm from the plant and inclined at 45° according to the method used by Oparde et al. (2013). The formulation with the lowest concentration (1x10^9 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 plants were 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 Admas et al. (2017). The experimental setup was completely randomized design (CRD).

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:

\[ R = \frac{y_p - y_t}{y_p} \times 100 \]

Where:

- \( R \) = relative shoot length in water hyacinth
- \( y_p \) = average shoot length from the control treatment
- \( y_t \) = 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.

Following the method of Daddy and Owotunse (2002), at the end of the sixth week the plants from each basin were removed from the water and the roots disentangled gently. The stalks were removed from the roots by hand and 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°C for 24 hours to a constant weight. 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:

\[ I = \frac{A_p - A_t}{A_p} \times 100 \]

Where:

- \( I \) = relative biomass
- \( A_p \) = water hyacinth dry weight from control treatment
- \( A_t \) = 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. 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. Results and discussion

For both pathogens, as the concentration of spores increased, there was a corresponding significant (p≤0.05) increase in relative shoot length (Table 1). Cercospora piaropi recorded significantly higher relative shoot length at all the spore concentrations. The highest relative shoot length for C. piaropi was 46.34 while for M. roridum was 41.80, both being recorded for 1x10⁹ spores/ml. In addition, the mean relative shoot length for C. piaropi was significantly higher at 41.31 as compared to that of M. roridum which was 38.51. The increasing relative shoot length with increasing spore concentration for both pathogens suggested that the inoculated plants had suppressed shoot elongation as compared to the control plants. The significantly higher relative shoot length for C. piaropi as compared to M. roridum was compelling evidence to suggest that C. piaropi elicited a higher suppression of shoot length on water hyacinth than M. roridum did. The importance of these results was that both pathogens reduced growth and resurgence of the weed disallowing the potential of the weed to build huge populations that form dense mats on water surfaces. This was in conformity with the findings of Asmare (2017) and Work and Ashlie (2018) who reported similar results in Lake Tana. The results also agreed with the findings of Doehlemann et al. (2017) that fungal pathogens manipulate plant metabolism in their own favour therefore denying the plant the necessary resources for tissue growth with subsequent reduction on growth. The bio pathogens were thus seen as important in lessening the detrimental effects of the normally luxuriant water hyacinth growth in agreement with similar results with Sharma et al. (2016) and Waithaka (2013) who reported that reduction in shoot length is attributable to the severe stress caused by the pathogens, which affect the ability of the mature plants to produce strong fresh leaves and daughter plants. It was observed that as the concentration level of the spores for both C. piaropi and M. roridum increased, there was a significant (p≤0.05) increase in relative biomass. The highest relative biomass for the two pathogens were 73.53 for C. piaropi and 37.60 for M. roridum at 1x10⁹ spores/ml (Table 2). 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. The increased relative biomass with increased spore concentration for both pathogens suggested that the inoculated plants had suppressed biomass accumulation as compared to the control plants. The significantly higher relative biomass for C. piaropi as compared to M. roridum was compelling evidence to suggest that C. piaropi elicited a higher suppression of biomass in water hyacinth than M. roridum did. The results were in agreement with the findings of Admas et al. (2017) who reported that fungal pathogens cause diseases upon water plants that reduce their biomass. 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. The results further agreed with the findings of Moran (2005) who demonstrated similar results in field plots with C. piaropi. This lessened biomass curtailed interference of the weed and put it at manageable levels in accordance with Eid and Shaltout (2017). In addition, the results agreed with the findings of Robles et al. (2015) that biomass reduction is useful and effective as a control method for water hyacinth.

Table 1. Effect of corn oil formulations on relative shoot length of water hyacinth plants during the study period

| Pathogen  | Spore conc. (ml⁻¹) | 1x10⁵ | 1x10⁶ | 1x10⁷ | 1x10⁸ | 1x10⁹ | Mean |
|-----------|--------------------|-------|-------|-------|-------|-------|------|
| C. piaropi |                   | 38.89f| 38.49c| 40.95h| 42.40k| 46.34m| 41.31i|
| M. roridum |                   | 35.69a| 37.10b| 38.70e| 39.27g| 41.80j| 38.51d|

%CV 16.9
LSD 0.78

Numbers followed by different letters are significantly different at p≤0.05

Table 2. Comparative effect of the pathogens on relative biomass

| Spore conc. (ml⁻¹) | Pathogen  | 1x10⁵ | 1x10⁶ | 1x10⁷ | 1x10⁸ | 1x10⁹ | Mean |
|--------------------|-----------|-------|-------|-------|-------|-------|------|
| C. piaropi         |           | 57.40d| 60.77e| 63.83f| 68.53g| 73.53h| 64.81f|
| M. roridum         |           | 39.53c| 24.73a| 26.73a| 33.13b| 37.60c| 32.34b|

LSD 3.40
%CV 11.10

Numbers followed by different letters are significantly different at p≤0.05

5. Conclusion

Of the two fungal pathogens C. piaropi and M. roridum, the former is the better bio control option. Its application at rates of 1x10⁹ spores/ml has the potential to lower water hyacinth shoot growth and biomass accumulation and can therefore be recommended to be used in water hyacinth management efforts.

Acknowledgements

We the authors acknowledge Maseno University Botany department for providing me with the necessary technical back up that made authorship of this paper possible. We also acknowledge the invaluable facilitation from Kibos KALRO center.
Declarations of interest

We, the authors report no conflict of interest and they are the only ones responsible for the content and writing of the paper.

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