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# 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 $0^0$37'S and longitude $37^0$20'E with average temperature of 25 to $30^0$C and 22 to $27^0$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 $1x10^9$, $1x10^8$, $1x10^7$, $1x10^6$ and $1x10^5$spores/ml. The control plants were not inoculated. The experiment was set up in completely randomized 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 $1x10^9$ spores/ml while relative biomass was 73.53 for *C. piaropi* and 37.60 at $1x10^9$ spores/ml. Inoculation suppressed shoot elongation and biomass with $1x10^9$ 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 farm produce 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 **(Berestetskiy 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-Anun *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)**, a

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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 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 oil easy during spore harvesting and formulation **(Boyette and Hoagland, 2013)**.

The purpose of this study was to compare effect of C*ercospora piaropi* Tharp and *Myrothecium roridum* Tode Fries formulated as corn oil emulsion on water hyacinth shoot growth under greenhouse and make a choice of the pathogen between them that can be used as corn oil formulation for the control of water hyacinth.

## 2. Material and methods

The study was carried out in a greenhouse at Kibos in Kisumu situated at latitude $0^0$37'S and longitude $37^0$20'E. It is about 10 km from Lake Victoria. Temperature averages were 25 to $30^0$C and 22 to $27^0$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 **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 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 $5^0$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 $1x10^9$, $1x10^8$, $1x10^7$, $1x10^6$ and $1x10^5$spores/ml according to **Admas *et al*. (2017)**.

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 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; $1x10^9$, $1x10^8$, $1x10^7$, $1x10^6$ and $1x10^5$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 $45^0$ according to the method used by **Opande *et al*. (2013)**. 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 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:

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

**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.

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 $80^0$C ffor 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:

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

**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.

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 $1x10^9$ 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 $1x10^9$ 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

| | Spore conc. ($ml^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| **Pathogen** | **$1x10^5$** | **$1x10^6$** | **$1x10^7$** | **$1x10^8$** | **$1x10^9$** | **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** | **$1x10^5$** | **$1x10^6$** | **$1x10^7$** | **$1x10^8$** | **$1x10^9$** | **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 $1x10^9$ 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.

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## Declaration 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.

## References


1. Admas, A., Sahle, S., Belete, E., Agidie, A. & Alebachew, M. 2017. Controlling water hyacinth in Lake Tana using biological method at greenhouse and pond level. *European Journal of Experimental Biology*, 7, 5-29.
2. Asmare, E. (2017). Current trend of water hyacinth expansion and its consequence on the fisheries around North Eastern part of Lake Tana, Ethiopia. *Journal of Biodiversity and Endangered Species*, 5: 189-197.
3. Berestetskiy, A. & Sokornova, S. 2018. Production and stabilization of mycoherbicides. Biological Approach for controlling weeds. *Intec open publishers*, 1, 63-88.
4. Boyette, C. D. & Hoagland, R. E. 2013. Adjuvant and refined corn oil formulation effects on conidial germination, appressorial formation and virulence of the bioherbicide, *Colletotrichum truncatum*, *Plant Pathology Journal*, 12, 50-60. https://agris.fao.org
5. Caprette, R. D. 2000. Using a count chamber. Experimental biosciences. *Introductory laboratory*, 211, 1-3. https://www.ruf.rice.edu
6. Charudattan, R. 2014. Biological control of water hyacinth by using pathogens: opportunities, challenges, and recent developments. Center for aquatic and invasive plants, plant pathology department, University of Florida, Gainesville, FL 32611-0680, USA.
7. Daddy, F. & Owotunse, S. 2002. Effect of different sources of water on water hyacinth growth perfoer5t5r44rmance. A publication of the national institute for freshwater fisheries research, New Bussa, Nigeria. Department of fisheries, 1, 130-132.
8. Dagno, K., Lahlali, R., Diourte, M. & Haissam, J. 2012. Fungi occurring on water hyacinth (*Eichhornia crassipes* [Martius] Solms-Laubach) in Niger River in Mali and their evaluation as Mycoherbicides. *Journal of Aquatic Plant Management*, 50, 25-32.
9. Doehlemann, G., Okmen, B., Zhu, W. & Sharon, A. 2017. Plant pathogenic fungi. The Fungal Kingdom. ASM Press. Washington, DC, 1, 703-726.
10. Eid, E. M. & Shaltout, K. H. 2017. Growth dynamics of water hyacinth (*Eichhornia crassipes*): a modeling approach. *Rendiconti lincei*, 28, 169-181. Doi: 1007/s12210-016-0589-4
11. Groenewald, J. Z,, Nakashima., Nishikawa, J., Shim, H. D., Park, A. N., Jama, M. & Crous, P. W. 2013. Species concepts in *Cercospora*: Spotting the weeds among the roses. *Studies in Mycology*, 75, 115-170. www.nvbi.nih.gov
12. Honla, E., Zegbefia, A. Y., Odame, D. A. & Mensa, M. 2019. The effects of water hyacinth invasion on smallholder farming along River Tano and Tano lagoon, Ghana. *Cogent food and Agriculture journal*. 5, 1. https://doi.org/10.1080/23311932.2019.1567042
13. Joost van den Brink, Gonny, C. J., van Muiswinkel, Bart, T., Sandra, W. A., & Ronald, P. V. 2013. Efficient plant biomass degradation by thermophilic fungus *Myceliophthora heterothallica*. *Applied and Environmental Microbiology*, 1, 1316-1324. http://dx.doi.org/10.1128/AEM.02865-12
14. Kaltragadda, H. R., Fuliara, A. S., Sidhu, S. & Carbonell-Barachina, A. H. 2010. Emissions of volatile aldehydes from cooking oils. *Food Chemistry Journal*, 3, 120:59.
15. Kuzmenko, M. 2016. Four proven methods on how to measure leaf area. Plants under a microscope from the folks behind petiole. Dark green singularity, 64, 1.
16. Kwon, H. W., Kim, J. Y., Choi, M. A, Son, S. Y. & Kim, S. H. 2014. Characterization of *Myrothecium roridum* isolated from imported Anthurium plant culture medium. *Mycobiology*, 4, 82-85. https://doi.org/10.5941/MYCO.2014.42.1.82
17. Libs, S. E. R., & Salim, A. E. R. 2017. Formulation of essential oil pesticides technology and their application. *Agricultural Research and Technology Open Access Journal*, 2, 5-6.
18. Moran, P. 2005. Leaf scarring by the weevils *Neochetina eichhorniae* and *N. bruchi* enhances infection by the fungus *Cercospora piaropi* on water hyacinth, *Eichhornia crassipes*. *Biocontrol Journal*, 50, 3.
19. Mujere, N. 2015. Water hyacinth: Characteristics, problems, control and beneficial uses. *Journal of impact of water pollution on human health and environmental sustainability*, 12, 511.
20. Mutebi, C. M., Arama, P., Opande, G., Were, J. & Buyela, D. 2013. Innandative bio-control of water hyacinth using zonate leaf spot fungal agent. 11th African *Crop Science Society Conference*. 13th to 17th October, 2013, Entebbe, Uganda.
21. Okunowo, W., Adekunle, A. A., Akinniyi, O. & Gbenie, G. O. 2019. Optimization of *Myrothecium roridum* Tode: Fries phytotoxin production and bioactivity on water hyacinth (*Eichhornia crassipes*). *Toxin Reviews*, 1, 1-2. https://doi.org/10.1080/15569543.2018.1564772
22. Ongore, C. O., Aura, C. M., Ogari, Z., Njiru, J. M. & Nyamweya, C. S. 2018. Spatial-temporal dynamics of water hyacinth macrophytes and their impact on fisheries in Lake Victoria, Kenya. *Journal of the Great Lakes*, 8, 4. https://doi.org/10.1016/j.jglr.2018.10.001
23. Opande, G. O, Mutebi, C. M. & Arama, P. F. 2013. Innundative bio control of water hyacinth (*Eichhornia crassipes* (Mart.) Solms. Laubach) using zonate leaf spot (*Acremonium zonatum* Sawada Gams) fungal agent. IOSR *Journal of Agriculture and Veterinary Science*, 69, 2319-2372.
24. Piyaboon, O., Pawongrant, R., Unartngam, J. & Unartngam, A. 2016. Pathogenicity, host range and activities of a secondary metabolite and enzyme from *Myrothecium roridum* on water hyacinth from Thailand. Wiley Online Library, 16, 1-3. https://doi.org/10.1111/wbm.12104
25. Robert, G. D. & James, H. T. 1991. *A Biometrical Approach: Principles and Procedures of Statistics.* 2nd Ed. Elsevier, New York, 1, 527-529.
26. Robles, W., Madsen, J. D. and Wersal, R. M. 2015. Estimating the biomass of water hyacinth [*E. crassipess* (Mart.) Solms] using the normalized difference vegetation index derived from simulated Landsat 5TM. *Journal of Invasive Plant Science and Management,* 8, 203-211. https://doi.org/10.1614/IPSM-D-14-00033.1
27. Segbefia, A. Y., Honiah, E. & Appiah, D. O. 2019. Effects of water hyacinth invasion on sustainability of fishing livelihoods along the River Tano and Abby-Tano lagoon, Ghana. *Congent Food and Agriculture*, 5, 1.
28. Sharma, A., Neeraj, K., Aggarwal, N. K. Saini, A. & Yadav, A. 2016. Beyond Bio control: water Hyacinth-Opportunities and Challenges. *Journal of Environmental Science and Technology*, 9:26-48.
29. Shende, D. & Sidhu, G. K. 2014. Methods used for extraction of maize (*Zea mays*, L) germ oil-A review. *Indian Journal of Scientific Research and Technology*, 4, 48-54.
30. Tahlan, V. 2014. "Antimicrobial activity of essential oil emulsions and possible synergistic effect on food borne pathogens". Wayne State University Theses, 316, 1-100.
31. To-Anun, C., Hidayat I. & Meeboon, J. 2011. Genus *Cercospora* in Thailand: Taxonomy and phylogeny (with a dichotomous key to species). *Journal of plant pathology and quarantine*, 1, 11-87.
32. Tobias, V. D., Conrad, J. L., Mahardja, B. & Khanna, S. 2019. Impacts of water hyacinth treatment on water quality in a tidal estuarine environment. *Journal of Biological invasions*, 21:3479-3490. https://doi.org/10.1007/s10530-019-02061-2
33. VonBlank, J. A., Casper, A F., Pendleton E. J. & Hagy, H. M. 2018. Water hyacinth (*Eichhornia crasipess*) invasion and establishment in a temperate river systems. *Journal of River research and Applications*. 34, 10. https://doi.org/10.1002/rra.3362
34. Waithaka, E. 2013. Impacts of Water Hyacinth (*Eichhornia crassipes*) on the Fishing Communities of Lake Naivasha, Kenya. *Journal of Biodiversity and Endangered Species*, 4, 9-11.
35. Worku, M. & Sahile, S. 2018. Impact of Water Hyacinth, *Eichhornia crassipes* (Martius) (Pontederiaceae) in Lake Tana Ethiopia: A Review. *Journal of Aquatic Research Development*, 9, 520.