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Evaluation of selected *Acremonium zonatum* Saw. Gams. mycoherbicide-oil mixtures for *Eichhornia crassipes* (Mart.) Solms. Laubach) biocontrol in Lake Victoria (Kenya).

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Abstract: *The use of microbial innoculum oil formulations has shown great promise for enhancing the efficacy of fungal agents that show potential as insecticides, fungicides and herbicides. When used in mycoherbicide formulation, oil encloses the moisture lost from the leaves, which provide the moist micro climate necessary for infection. The innundative ability of this phytopathogen using three oil types (i.e. petroleum oil (mineral in origin), corn oil (in vegetative tissue) and glycerol (occurring in animal tissue) having been confirmed, a new study was initiated at the Maseno University glasshouse to evaluate their specific suitability in enhancing the efficacy of Acremonium zonatum for the biocontrol of water hyacinth in Lake Victoria. A. zonatum was isolated aseptically following standard procedures. Pure cultures obtained were mass produced on PDA. Spores were then harvested after the surface of the cultures turned brown. A haemocytometer was used to prepare a stock solution with 1x10^{7}spores/ml. in sterile distilled water using Caprette's method. Healthy water hyacinth plants arranged in a RCBD within the glasshouse were inoculated with the mycoherbicide in the three selected oil formulations. A control experiment was mounted using sterile distilled water. Disease score recording was done three times at fourteen day intervals. Analysis of the results indicated that glycerol appeared to be the most suitable oil formulation of A. zonatum as a mycoherbicide followed by corn oil. Petroleum oil emulsion showed the least efficacy for the same purpose.*

Keywords*: Biocontrol, efficacy, mycoherbicide, oil formulations, zonate leaf spot, fungicide.*

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I. Introduction

The mycoherbicidal approach has demonstrated that an endemic (i.e. native) pathogen might be rendered completely destructive to its weed host by applying a massive dose of innoculum at a particular susceptible stage of weed growth [1] [2]. The application of an inundative dose of innoculum and its proper timing would shorten the lag period for innoculum build-up and pathogen distribution, essential for natural epiphytotics [2]. To be successful in this approach it must be possible to produce abundant and durable innoculum in artificial culture, the pathogen must be genetically stable and specific to the target weed, and it must be possible to infect and kill the weed in environments of reasonably wide latitude. The fungus would be applied annually shortly after the weeds emergence when conditions for disease were favorable [2] [1]. The spore innoculum for this purpose is raised in artificial media, harvested, prepared in a manner to withstand storage and handling such that it can be applied like a chemical herbicide. Thus, the in-vitro culturing of the pathogen to obtain large quantities of innoculum and the inundative application of the innoculum to achieve rapid epidemic buildup and a high level of disease are two distinctive aspects of the mycoherbicidal concept.

Before this study, little was known about the success rate of any mycoherbicide formulations used with or without oil in the Winam gulf (L. Victoria), so the use of an oil formulation to enhance the innundative biocontrol of the water hyacinth using *Acremonium Zonatum* [3] provided knowledge and created the study question; Which of the three selected mycoherbicide-oil formulation is most effective for use in water hyacinth control in L. Victoria?. The use of oil formulations has shown great promise for enhancing the efficacy of fungal agents that show potential as herbicides [3]. Womack *et al*. [4] while investigating the use of less viscous vegetable oil suspension emulsions in the formulation of biocontrol agents including fungal pathogens also realized the potentials of such mixtures. The oil encloses the moisture lost from the leaves and this becomes available to provide the moist micro climate that is necessary for infection to effectively take place. Fernandez et al. [5] found that temperature and humidity are the main constraints to the effectiveness of fungi as phytopathogens. Karim *et al*, [6] further proved that vegetable oil formulations generally improve the

efficacy of mycoherbicides. Several adjuvants improve the germination of fungal spores, as is the case of pure corn oil, which enhances the activity of fungal pathogens and reduces moisture requirements necessary for spore germination. [6]. The innundative capability of a mycoherbicede in oil formulation having been established [3]; this study was carried out to answer the study question and fill gaps of unknown in enhancement of the efficacy of *Acremonium zonatum* for use in biocontrol of water hyacinth in Lake Victoria.

II. Materials And Methods

Infected leaves showing the zonate leaf spot disease symptoms having been collected from the Winam gulf (L. Victoria) were processed [3][2][9], pathogen identified, pure cultures obtained grown in large scale on agar plates until sporulation occurred [3]. Such agar plates with *A. zonatum* colonies were flooded with 2 ml of sterile water[3], the spores were removed from the agar surface using a flame sterilized glass rod and the suspension produced filtered through a nylon mesh sieve to remove large mycelia particles as earlier described [3].

2.1 Mycoherbicide in oil preparations

A haemocytometer count chamber was used to prepare a suspension containing 10^7 spores/ml using sterile water [3] [8]. 10mls of such suspension was added to 30mls of each of the experimental oil types (i.e. commercial grade corn oil, mineral oil and glycerol) to make mycoherbicide in oil mixtures.

2.2 Innoculation with mycoherbicide-oil mixture.

Young plants with relatively small leaves and short petioles and of approximately the same age with 6-10 leaves per plant were selected. Individual plants were placed into 3 ft plastic basins containing pond water and arranged in a randomized complete block design (RCBD). The young fully expanded leaves were injured by being slightly pricked 25 times using a sterile needle. Hand held sprayers positioned at 45^0 and 20cm from the plants were used to apply the mycoherbicide on the healthy plants. Spraying was done until all the leaf surfaces were covered. A fine mist of sterile water was sprayed upon the leaves after the spray droplets had evaporated. The inoculated plants were not covered with polythene. A control experiment was mounted using sterile distilled water. .After 42 days, water hyacinth plants were observed for zonate leaf spot symptoms and disease scoring conducted three times at fourteen day intervals using a scale [9].

III. Results And Discussion

Following inoculation by the different mycoherbicides, infection was observed as shown in "Table 1". There was no infection in the control experiment. All the mycoherbicides elicited rising disease score at each of the three subsequent DAI (Days after inoculation) during the study period. The corn oil and glycerol mycoherbicide caused higher disease score than mineral oil. At day 14, there was significant difference ($p>0.05$) in disease score in the three mycoherbicides formulations. At the 28^{th} and 42^{nd} days, mineral oil disease score was significantly different ($p>0.05$) from glycerol and corn oil. Overall, the mean disease score for corn oil was highest (51.7) followed by that of glycerol (49.9). Mineral oil had the least disease score (32.5).

Table 1: Disease score different concentrations of *A. zonatum* in different oil emulsions.

Treatment	Days after inoculation (DAI)			
	14	28	42	Mean
Corn oil	33.1^a	47.1^b	74.8^b	51.7^b
Glycerol	28.9^b	44.5^b	76.4^b	49.9^b
Mineral oil	18.0^c	35.8^d	43.8^d	32.5^d
Control	0.0	0.0	0.0	0.0

For each scoring date and, means bearing the same superscript are not significantly different (P=0.05)

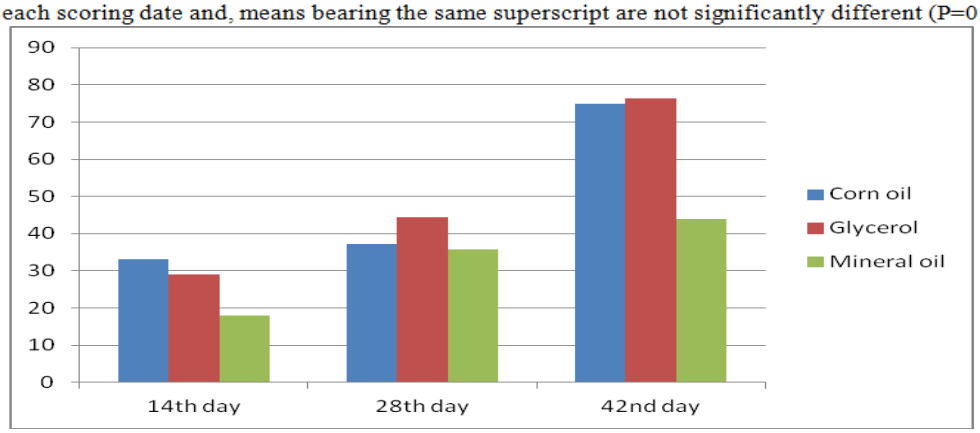

Figure 1: Disease score for the different mycoherbicides during study period

The findings of this study indicate that vegetable oil (corn oil) is better than oil deriveved from mineral or and/or animal sources in formulating *A. zonatum* as a mycoherbicide. This agrees with the finding of Whomack *et al*. [4] that shows that vegetable oil is advantageous in as far as overcoming the need to physically cover the infected leaves with polythene material to increase moisture for enhanced infection after inoculation.

Petroleum oils or mineral oil on the other hand has mostly been used in plant disease control with better results than oil derived from plants. This fact has been demonstrated by the dismal performance of petroleum oil as a formulation for mycoherbicide in this study. Indeed, even though petroleum oil has formerly been proved to have antifungal activity apart from being pesticidal, emulsified petroleum oils could be used in control of foliar fungal pathogens by suffocation or deprivation of oxygen from fungi and reduction of attachment of fungal spores to plant surfaces [7]. Glycerol has been proved to be a preservative that has little chemical effect on fungal spores. It immobilizes the spores making infectivity go down. Its more important role is therefore in preserving fungal spores rather than being used in mycoherbicide formulation. However, when the spore inoculums are increased to a suitable level as was done in this study at 10^7spores/ml., infectivity is observed.

IV. Conclusion

It is now clear form the results obtained in this research that plant based oils are more suitable to use in mycoherbicide formulation in L. Victoria for the biocontrol of water hyacinth, in preference to mineral oils that seems to be least suited for this purpose. Glycerol can be used in formulation of mycoherbicides if the spore or conidia concentration is increased to a suitable level way above the inoculum potential of the other two formulations. In a comparative study [10], $1x10^6$ spores/ml was the threshold inoculum concentration for the common fungal pathogens used to infect water hyacinth. Therefore for glycerol to act as a suitable oil formulation for *A. zonatum* mycoherbicide the spore or conidia concentration must be maintained above the 10^6 spore/ml level.

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