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Isolation and Identification *Rhizoctonia Solani* On The Water Hyacinth (*Eichhornia Crassipes* [Mart] Solms. Laubach) in the Winam Gulf (Lake Victoria, Kenya).

George T. Opande[1], David M. Musyimi[2], Hillary T Nyang,anga[3].
[1]*Department of Botany, School of physical and biological sciences, Maseno University, Kenya.*
[2]*Department of Botany, School of physical and biological sciences, Maseno University, Kenya.*
[3]*Department of Agri. Economics, Faculty of Agriculture, University of Nairobi, Nairobi, Kenya.*
Corresponding Author: George T. Opande

Abstract*: Rhizoctonia solani is one of the most widely distributed fungi throughout the world, it is a soil inhabiting fungus of which several strains are known to exist, it is a known phytopathogen of the water hyacinth (Eichhornia crassipes [Mart.] Solms Laubach), a floating aquatic weed invasive in the Winam gulf (L. Victoria) that has created serious weed problems. In the Winam gulf little information pertaining to R. solani occurrence and pathogenicity existed before this study, therefore this study was initiated to provide knowledge on this phytopathogen. Surveys were conducted along the shoreline of the Winam gulf along an area stretching from the Kisumu pier, Kusa and Kendu bay pier, where plants showing known symptoms of the Rhizoctonia disease were collected sorted and transported to the plant pathology laboratory at Maseno University for further observations. Each infected plant leaf showing the Rhizoctonia symptom was cut into 1mm^2 pieces, surface sterilized in 0.5% NaOCl for 2 minutes and thoroughly rinsed in sterile distilled water. These sterile portions were then transferred to sterile PDA (Potato dextrose agar) plates that were kept in the laboratory at room temperature (≅ 25° C) for any emergent fungal growth. Emergent fungal growth was transferred to new PDA media until pure cultures obtained. Pure cultures were reinoculated on to healthy plants to establish pathogenicity on conformity to Koch´s postulations and the relationship between the pathogen and its host. Identity of R. solani was confirmed both symptomatologicaly and morphologically using an identification key. This study was meant to provide scientific knowledge that would prove to be important in future studies on suitability of R. solani as a biological control agent of the water hyacinth in the Winam gulf (L. Victoria) and other water bodies in E, African water bodies where the water hyacinth is a problem*

Keywords; *mycelium, pathogenicity, water hyacinth, biological control, weed, isolation*

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I. Introduction

The Winam gulf is a large section of the Lake Victoria located geographically within Kenya. The L. Victoria is the second largest fresh water lake in the world covering three major East African countries of Kenya, Uganda and Tanzania. It is considered to be one of the major sources of the river Nile that stretches all the way to the Mediterranean Sea by traversing more than 5 countries from Uganda to Egypt.

Rhizoctonia solani is one of the most widely distributed fungi throughout the world; it is a soil inhabiting fungus of which several strains are known to exist [1]. It is among the few known phytopathogens of the water hyacinth that is able to cause a debilitating disease capable of developing into an epiphytotic if prevailing environmental factors are suitable for disease development [2]; it may also be pathogenic to different plants or may be parasitic on other fungi [2]. This fungus was classified in 1956 as a member of the sub division deuteromycotina, belonging to the imperfect order mycelia sterilla [1]. Its perfect stage is classified in the sub division basidiomycotina, a species known as *Thanatophorus cacumeris* [=*Aquathanatophorus pendulus*] [3]. The imperfect stage basically is made up of sterile mycelia that reproduce when the vegetative hyphae breakup. The imperfect mycelium is made of septate hyphae with sharp and almost right-angled bends in the hyphae, while the perfect stage is characterized by accumulations of spherical sexual spores at the tip of the fertile hyphae known as the sporangiophore [4].

One of the main hosts of this fungus is the water hyacinth (*Eichhornia crassipes* [Mart] Solms. Laubach) a free-floating aquatic weed of worldwide importance [5]. This weed is one of the most productive aquatic plants when the conditions are favorable for growth, it reproduces very quickly thus forming a large weed carpet and massive colonies which floats on the water surface within L. Victoria because of the human interference that have upset the natural balance [6]. Occasionally these carpets are translocated by wind

seasonally from one side of the lake to the other [6]. The amount of biomass determines the nuisance value and impact on the water quality in the lake, where they are able to grow under a wide range of nutrient levels and environmental conditions [6].

R. solani is a facultative parasite and occur on water hyacinth in several countries in different continents [7]. Its occurrence and distribution with the water hyacinth in different water bodies throughout the world has not been completely documented. In the Winam gulf studies have been conducted on other fungal pathogens of the water hyacinth e.g. *Myrothecium roridium* [8] and in other parts of the world fungi studied as partial biological control agents of the water hyacinth in different regions include: *Uredo eichhorniae; Uromyces ponteridae; Cercospora piaropii, Cercospora rodmani* and *Myrothecium roridium* among others [9]. In view of the very high reproduction rate, the water hyacinth is capable of out-growing the *Cercospora* disease or any other fungal disease for that matter [10] [11]. It was recognized from such studies that the pathogenicty of more fungal species should therefore be established to show their suitability for use as biocontrol agents of the water hyacinth with a lot of success in different water bodies.

In the Winam gulf before this study, little information existed pertaining to *R. solani* distribution, pathogenicity and occurrence, therefore this research was initiated to provide this scientific knowledge that would prove important to future studies on the suitability of *R. solani* as a suitable biological control agent of the water hyacinth not only in the Winam gulf but other water bodies throughout E. Africa where the water hyacinth is a problem.

II. Materials and Methods

2.1 Study area

This was done at the Winam gulf of L. Victoria within that area stretching between Kisumu pier and Kendu bay pier, in field sites with severe water hyacinth infestations that were selected for this purpose because the probability of finding infected plants and the frequency of disease incidences could be better studied in such heavily infested sites than in locations where the infestation were sparse. Plants that showed disease symptoms were picked from shoreline along the fish-landing site of Kisumu pier, Kusa and Kendu bay after which they were sorted according to the symptoms that they exhibited.

Source: Wesonga C. (2016)

2.2 Isolation of the *R. solani*

Plant parts that had visible signs on their leaves of the *Rhizictonia* disease were collected from the Winam gulf. After the collection process was completed, each infected plant part was cut into $1mm^2$ pieces, surface sterilized in 0.5% NaOCl for 2 minutes and thoroughly rinsed in sterile distilled water as earlier described [12]. Sterile portions were then transferred to PDA (Potato dextrose agar) plates that were kept in the laboratory at room temperature (≅ 25° C) for any fungal growth present. Any emergent fungal growth was transferred to new PDA media [12], [13]

2.3 Pure cultures of *R. solani.*

Pure cultures were obtained from these blooming mycelial growths by transferring emergent mycelia to fresh PDA plates. Pure cultures obtained were subjected to microscopic examinations to determine the characteristics of these blooming mycelia such as type of hyphae and vegetative plant body and all these PDA isolates stored under strict sterile conditions for future use while confirmative and identification placement into the correct systematic position for the PDA isolate was done using an identification key [14].

2.4 Pathogenicity trials.

Pathogenicity trials were conducted at the botany laboratory (Maseno University) in conformity to Koch's postulations. Plants meant for this exercise were kept in sterile plastic buckets of 30-cm diameter, filled with water. Pure inoculum was transferred onto healthy leaves areas that appeared clean and free of any visible disease symptoms. The inoculum used for this purpose consisted of a drop of dense suspension made up of fungal spores and mycelium. Four leaves were inoculated using these inoculums' made from *R. solani.* Each leaf was inoculated at two spots, one on each surface [2]. Control plants were treated in a similar manner except that they were inoculated with a drop of sterile distilled water. After the inoculation process was complete, each plant and its bucket were covered with a plastic bag in order to maintain the high level of humidity that is a requirement for the disease establishment

III. Results

Water hyacinth plants were found floating as mats, attached to vegetation on lakeshores, or as islands of plants floating freely on the lake waters. Such observations have been reported by [4]. Plants were found at times above the water level on damp lakeshores. Both healthy and infected plants were spotted at the lakeshores.

The *Rhizoctonia* disease symptoms were spotted at various points of the lakeshores within the area of study. Several plant leaves were seen having prevalent symptoms of the *Rhizoctonia* disease as earlier described by other workers [2], [12]. Feeding damage and symptoms possibly attributed to other microorganisms and *Neochetina* weevils associated with the water hyacinth were seen on the many water hyacinth plants. Suspected water hyacinth diseases symptoms exhibited by several plants that that were unconfirmed included symptoms of *Cercospora sp., Acremonium zonatum Alternaria sp., Myrothecium roridium, Helminthosporium* and *Uromyces ponteridae* diseases. Damage to weed due to feeding effects of the water hyacinth weevils, i.e. *Neochetina eichhorniae* and *Neochetina bruchii* introduced into the lake by KARI (Kenya Agricultural Research Institute) now KALRO (Kenya Agricultural and Livestock Research Organization) were visibly seen in many locations within the study area particularly at the Kisumu pier, Kusa and Kendu bay. Clear signs of bacteria initiated chlorotic halo that have been reported to be associated to the *Neochetina* sp. feeding signs were widespread.

3.1 Identification of the *Rhizoctonia* **disease**

Identification of the disease was done by spot-checks which were done along the shoreline within the study area to distinguish the disease from other diseases of the weed. The symptoms of the fungi may have confused with damage due to desiccant type of chemical herbicides. They damage was characterized by irregular, necrotic spots and broad lesions. However, unlike chemical damage, the brown necrotic areas were more or less surrounded by noticeable, thin water-soaked margins of darker brown color than the rest of the necrosis. Plants showing symptoms of the *Rhizoctonia* disease were found at all the collection spots at the study area i.e. starting Kisumu, Kusa and Kendu bay along the Winam gulf (L. Victoria).

3.2 Mycelial characters of the *R. solani* **isolate**

This was observed in cultures and in the host and the following important features were noted: -

3.2.1 In the host leaf.

A transverse section (TS) of the infected water hyacinth leaf showed short lateral infection hyphae traversing the intercellular spaces of the leaf. The hyphae penetrate the host epidermis and then grow within the host. In the host tissue the hyphae branches followed various directions between the cells. Oil-like globules

appeared to extend into the host's cell cytoplasm, which later replaces the whole cytoplasm if the disease is allowed to progress.

3.2.2 In culture.

After four days of culturing, fungal hyphae had grown and covered the entire surface of the petri dishes. The fungal colonies included mycelia, which had a raised elevation only being limited by the lid of the petri-dishes. The mycelia were white to brownish in color. The texture of the colonies was rough made of cylindrical strands of branched hyphae. The hyphae were septate (i.e. having cytoplasmic interruptions by walls which were regular throughout the length of the hyphae) the mycelia appeared as aggregates of 'threads' having right angled turns and they are branched extensively. Only mycelia were observed, no spores could be attained within cultures.

3.2.3 Pathogenicity test

By the fourth day after inoculation the disease had started developing in the areas where inoculation was done. Photographs were taken on the seventh day after inoculation and the disease symptoms were similar to those in the field. In the laboratory conditions, this disease was seen to spread very rapidly and entire leaves were consumed in less than two weeks time of observation. Re-isolated fungi were observed and found to have same mycelial characters as those observed in previous cultures.

After re-inoculation the same results were seen (i.e. the disease spread rapidly on the leaves as previously observed). The original *Rhizoctonia* disease was produced on the leaves of the water hyacinth plants.

VI. Discussion

The existence of mats of water hyacinth over the water surface within the Winam gulf (L. Victoria) calls for urgent control action. The massive infestation of the weed in the lake is an indication that the habitat is favorable for reproduction and proliferation of the weed as observed earlier [6]. Water hyacinth is one of the new species in the lake that has had severe impacts on the lake's indigenous species. Outside the water hyacinths natural habitat of Amazonian Brazil, this weed is freed from the controlling influence of their natural enemies and their unchecked growth results in thick mats of vegetation with harmful effects on the water quality, fish populations, aquatic plants and loss of biological diversity in the infested aquatic ecosystems [6].

The confirmed occurrence the *Rhizoctonia* disease in various locations within the Winam gulf is a clear indication that the weed has co-migrated with this fungal pathogen form its native range to its current adventive range of Winam gulf (L. Victoria). In every point where the weed was found there were plants that were showing symptoms of the *Rhizoctonia* disease. Presently, two weevils, *Neochetina eichhorniae* and *N. bruchii* were introduced in Kenya and Uganda where they were multiplied and released within the lake. However, considering the present magnitude and distribution of the weed, the effectiveness of the few weevils is highly limited. To be effective, billions of agents must be produced to match the rate of increase in population of the weed, which is highly expensive.

Due to their slow multiplication rate and action, the weevils can be considered as a major component of the long-term measures of maintenance in the control of the weed under an integrated plan. This will ensure that when the emergency removal is undertaken, the weevils will be available for deployment to prevent weed expansion [15]. Isolation of *R. solani* from its host is easily achievable in any simple laboratory set-up and once isolated it is easy to re-culture to obtain the organisms in mass to explore the possibility of exploring the use of this phytopathogen as a mycoherbicide candidate agent. It is important at this point to note that fruiting of *R. solani* could not be achieved in the laboratory as it was outside the scope of this particular work, though in order to fruit this fungus, re-culturing needed to be done in different culture medium containing potato dextrose marmite agar (PDMA). On attaining the perfect stage, the fungus is then not considered a member of the division Deuteromycotina but a member of Basidiomycotina known as *Thanetophorus cacumeris* [*Aquathanatophorus pendulus*] [3]. Experiments need to be conducted on the pathogenicity of this perfect stage on the water hyacinth to determine it efficacy and pathogenicity on the water hyacinth.

Since the pathogenicity test conducted during this study were done strictly following Koch's postulations to avoid the assumption that, since *R. solani* is associated with the leaf blights then it is necessarily the organism that caused this *Rhizoctonia* disease. This would have ignored the possibility of the organism being a secondary invader of an essentially saprophytic nature, which comes after the blights have been formed. The conditions laid down by Koch were fulfilled, as seen in the results, thus confirming *R solani* as the causative agent of this leaf blight on the water hyacinth.

IV. Conclusion and Recommendations.

The pathogenicity of this *R. solani* isolate on the water hyacinth was established to in this study, though it is important to continue with further studies on better ways to improve water hyacinth control using this organism either by a classical or a mycoherbicidal tactic. There is also a need to design control strategies in

the Winam gulf (L. Victoria) based on the level of water hyacinth infestation and national socio-economic constraints, such methods should be cheap long term self sustaining.

We wish to recommendation for the following three main activities to be undertaken urgently in the Winam gulf:

(1) To get rid of the massive biomass accumulation of the water hyacinth in the lake as soon as possible.
(2) Intensification and the multiplication of biological control agents to be used as a major component of the integrated long-term control of this weed in the Winam gulf (L. Victoria) is necessary.
(3) Urgent research on water hyacinth suitable biocontrol agents to control its growth, propagation rates and dispersal dynamics is required; We highly recommend *R. solani* to be considered as one of the biological control agents.

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