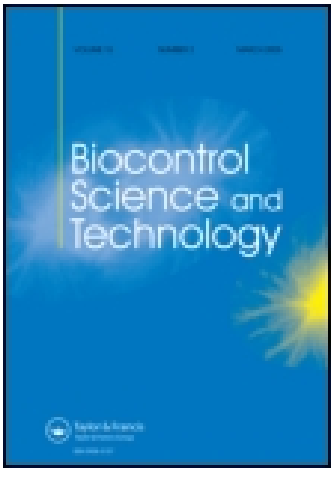

Biocontrol Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/cbst20

Occurrence and effectiveness of an indigenous strain of Myrothecium roridum Tode: Fries as a bioherbicide for water hyacinth (Eichhornia crassipes) in Nigeria

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Accepted author version posted online: 02 Sep 2013.Published online: 04 Oct 2013.

To cite this article: Wahab O. Okunowo, Akinniyi A. Osuntoki, Adedotun A. Adekunle & George O. Gbenle (2013) Occurrence and effectiveness of an indigenous strain of Myrothecium roridum Tode: Fries as a bioherbicide for water hyacinth (Eichhornia crassipes) in Nigeria, Biocontrol Science and Technology, 23:12, 1387-1401, DOI: 10.1080/09583157.2013.839981

To link to this article: http://dx.doi.org/10.1080/09583157.2013.839981

Biocontrol Science and Technology, 2013
Vol. 23, No. 12, 1387–1401, http://dx.doi.org/10.1080/09583157.2013.839981

RESEARCH ARTICLE

Occurrence and effectiveness of an indigenous strain of *Myrothecium roridum* Tode: Fries as a bioherbicide for water hyacinth (*Eichhornia crassipes*) in Nigeria

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(*Received 27 May 2013; returned 25 June 2013; accepted 27 August 2013*)

In a study to isolate fungal pathogens with potential for the biocontrol of water hyacinth (*Eichhornia crassipes*), some lakes in the Lagos State and its environs, Nigeria, were surveyed for diseased water hyacinth (*E. crassipes*). The fungi present in the diseased tissue were isolated and identified as: *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp., *Curvularia pallescens*, *Fusarium solani* and *Myrothecium roridum*. The pathogenicity of isolates of these fungi on fresh, non-diseased water hyacinth plants was investigated. *Myrothecium* was the only species capable of inducing disease symptoms. Necrosis was observed on water hyacinth leaves three days post inoculation (DPI) with *M. roridum* (1×10^6 spores/ml). The leaves and the petioles were withered at the end of day 24, and the disease incidence and disease severity were 100% and 8.67%, respectively. Molecular analysis of the internal transcribed spacer rDNA of the *M. roridum* isolate from water hyacinth showed >98% homology to authenticated sequences of *M. roridum*. The isolate, deposited at the International Mycological Institute, UK, as *M. roridum* Tode: Fries (IMI 394934), possesses the level of virulence needed in a potential mycoherbicide for use in the management of water hyacinth.

Keywords: biocontrol; mycoherbicide; fungi; pathogen

1. Introduction

Humans have facilitated the spread of water hyacinth, *Eichhornia crassipes* (Marts.) Solms-Laubach, from its native environment in South America to many regions throughout the world because of its attractive flowers. It made its entry into Nigerian waters via the Southwestern coastal border of Badagry around 1984 (Oso, 1988).

Water hyacinth forms dense impenetrable mats that impede the recreational use of water and economic activities such as agricultural irrigation, navigation, fishing and power generation (loss of electricity production) (Mailu, 2001). These mats competitively exclude native aquatic plants and create good conditions for breeding disease vectors, particularly mosquitoes (Harley, 1990; Center, Hill,

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Cordo, & Julien, 2002). The losses caused by the weed in the several key sectors of some African countries is estimated to be in the order of billions of dollars (Mailu, 2001). The negative socio-economic and environmental impacts of this weed in many areas of the world are well documented in the literature (Mailu, 2001; Schmitz et al., 1993; Center et al., 2002). The indirect costs are enormous.

Various control measures such as manual, mechanical, chemical and biological control are employed to check this water hyacinth. Some of these methods are, however, expensive and not environment-friendly. The use of conventional control measures such as mechanical removal, chemical herbicides and classical biological control using herbivorous insects are not entirely adequate and are probably expensive measures to apply on a large scale (Bateman, 2001).

Biocontrol involves the use of host-specific natural enemies to minimise the population of a target pest. Several fungi and insects have been reported as control agents for aquatic weeds such as water hyacinth (Bateman, 2001; Charudattan, 2001, 1997; Coetzee, Hill, Julien, Center, & Cordo, 2009; Venter, Hill, Hutchinson, & Ripley, 2013). All biological control agents are specific on the target weed, generally persist at the site of infestation and tend to be self-regulating. Therefore, biological control is considered to be environmentally safe.

Several plant pathogens have been tested and developed as biocontrol agents for large scale field application and over 15 have been used for biological control of weeds worldwide (Evans & Reeder, 2001). Several pathogens have also been tested for water hyacinth control though no commercial mycoherbicide was eventually developed (Dagno, Lahlali, Diourte, & Jijakli, 2011; El-Morsy, 2004; Praveena, Naseema, & George, 2007; Shabana & Mohamed, 2005; Tessmann, Charudattan, & Preston, 2008).

However, the success of a fungal pathogen used as a biocontrol agent is influenced by environmental factors (Kirkpatrick, Templeton, TeBeest, & Smith Jr, 1982; TeBeest & Templeton, 1985; Walker, 1981), and one of the goals of biocontrol strategies is that potential biocontrol agents of pests should be isolated and studied in the region of the origin where the target organisms were suppressed naturally (Hong, Ryu, Hyun, Uhm, & Kim, 2002). This work reported here was carried out to survey, isolate, evaluate and identify an indigenous fungal agent which is biologically active under Nigerian climatic conditions for use locally and regionally as a biocontrol agent for water hyacinth.

2. Materials and methods

2.1. Survey for pathogens

Field trips were undertaken to observe and examine waterways and lagoons of Badagry, Mile 2, Lagos and Ogun River (Isheri) (Figure 1) and to collect fungal pathogens from diseased water hyacinth. Sampling was done randomly at each sampling station: Lagos Lagoon, Mile 2, Ogun River and Badagry creeks using a motorised canoe at intervals of three months for a period of three years to collect fungal pathogens that attack the plant at various seasons of the year. The total area surveyed was 125 km^2, 44 km^2, 300 km^2 and 45 km^2 in Badagry, Mile 2, Lagos and Ogun River, respectively.

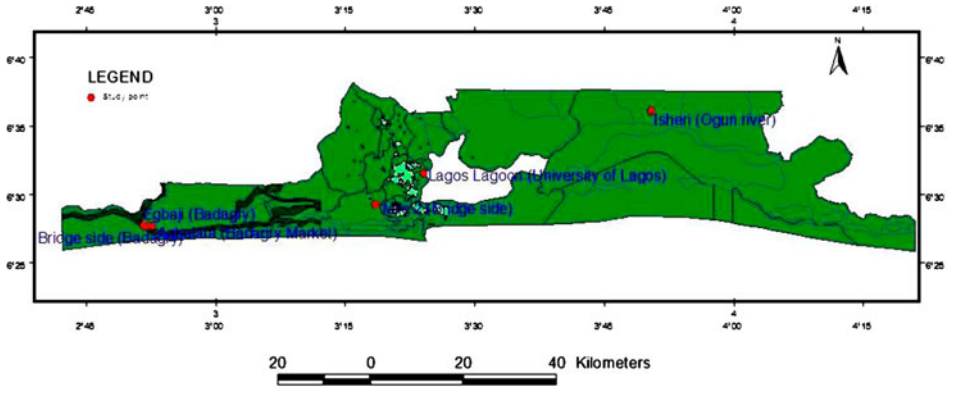

Figure 1. (Colour online) The lagoons and creeks of Lagos and its environs surveyed for diseased water hyacinth.

2.2. Fungal isolation from diseased water hyacinth leaves

Two square millimetre pieces were cut from the margins of necrotic lesions on the diseased leaf sample. These were surface sterilised in a solution of 0.26% sodium hypochlorite solution for 1 min and rinsed thrice in sterile water to remove traces of the disinfectant (Jimenez & Charudattan, 1998). Similarly, other pieces cut from typical lesions were sterilised in a solution of 1.4% sodium hypochlorite as described above. Five leaf pieces were placed on potato dextrose agar (PDA) and tap water agar (TWA), each containing an antibiotic (ampicillin; 500 mg/l), in petri plates and incubated at 25°C, with a 12 h dark/light regime to stimulate sporulation. All emerging fungi were isolated in pure cultures by the single hyphal-tip technique (Jimenez & Charudattan, 1998).

2.3. Morphological identification of fungal strains

The pure cultures obtained were subcultured on plates containing 2% Malt Agar, one plate of TWA containing a single piece of sterile wheat straw and one plate of PDA. Cultures in plates were grown for 14 days under black light (wavelength 300–380 nm; 12 h alternating cycles black light/darkness) at 22°C to induce sporulation. At the end of this period, squash mounts of sporulating material were stained with lactophenol stain and examined under a light microscope. The fungal pathogens were identified according to their morphological appearance on the plates and the characteristics of spores under the light microscope. The growth (average diameter) of some of the fungi was determined on six replicate plates of PDA and the results are presented as mean ± standard deviation (SD). All isolates were screened for their pathogenicity on fresh non-diseased water hyacinth plants.

2.4. Pathogenicity test

Healthy water hyacinth plants were collected, washed with 0.26% sodium hypochlorite solution and rinsed three times to eliminate insect infestation. Three plants (each average height 60–70 cm) were maintained in 20 l pot (30 cm diameter by 30 cm depth) containing 8 l of 50% Hoaglands solution (Jimenez & Charudattan, 1998) and allowed to equilibrate in the solution for one week prior to inoculation

with the pathogen. Inoculum was formulated by harvesting fungal spores from PDA culture plates in sterile distilled water containing 0.1% v/v Tween 80 solution. Leaves and petioles of experimental plants were inoculated with 200 ml of 1×10^6 spores/ml spore suspension containing 0.1% v/v Tween 80 using a hand-held low-pressure atomiser at a distance of 20 cm from the plant. The fungus was sprayed until run off on the leaves and stem of the plants. This experiment was conducted in six replicate pots and in three different experiments A, B and C in the University of Lagos. Control plants were also set up in the three different experiments by spraying leaves and petioles with sterile distilled water containing Tween 80 (0.1% v/v). Inoculated and control plants were immediately covered with sterile polythene bags for 48 h to maintain high relative humidity. The plants were then left in the open experimental field under the conditions of average temperature ranging between 24°C and 31°C, relative humidity between 68% in the night and 86% in the day and at an average rainfall of 25 mm for the month. The average sunlight/intensity was 7 h per day. Plants were monitored at three-day intervals for symptoms development. The isolates were ranked on the basis of the severity of the disease inflicted. Disease severity was assessed according to Freeman and Charudattan (1984). Finally, the pathogens were reisolated and identified from the inoculated and dead plants as well as from the control plants to fulfil Koch's postulates.

2.5. Host range examination

Host range of the most pathogenic isolate from the pathogenicity trial was tested on several local and economically important agricultural crops under field conditions (described above). The plants were sprayed with 200 ml fungal suspension (1×10^6 spores/ml in 0.1% v/v Tween 80 solution) and monitored for about three weeks for disease development and host plant reactions. Three individual plants in each pot were examined in triplicate experimental pots. Disease symptom rating was assessed by visual examination as: − = not susceptible (leaves healthy, no disease symptom observed), + = slightly susceptible (scanty leaf spotting or slight chlorosis no necrosis), ++ = susceptible (leaf spots/leaf necrosis at 30–50% leaf is dead) and +++ = highly susceptible (severe leaf spotting/necrosis at >50% leaf is dead).

2.6. Molecular characterisation of the pathogenic isolate

The pathogenic isolate which was tentatively identified as *Myrothecium* sp. was further characterised by the Centre for Agriculture and Bioscience International (CABI), Egham, Surrey, UK using standard molecular identification technique to analyse the ITS1 rDNA sequence. The sequence and the isolate were deposited, respectively, in the GenBank (accession no. GQ853401) and the CABI microbial collection (Deposit no. IMI 394934).

2.7. Blast and phylogenetic analysis of the isolate

The ITS1 rDNA sequence of our isolate obtained from CABI was subjected to homology analysis against the holdings of the GenBank using the software BLASTN 2.2.28+ (Zhang, Schwartz, Wagner, & Miller, 2000) and the phylogenetic relationship among taxa (for >97% homology) were determined using the neighbour-joining method (Saitou & Nei, 1987).

2.8. Data analysis

The data presented in this study are the results obtained from six replicate determinations and are expressed as mean ± SD. To determine the reproducibility of the experimental results, disease progression between the different experiments were compared using the general linear model (GLM) regression analysis with days post inoculation (DPI) set as continuous predictor. The analyses were done at critical *P* value of 0.05 using the GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, California USA).

3. Results

3.1. Survey for pathogens

The continuous survey of the water bodies over the period of investigation showed that water hyacinth was prevalent at different periods in the year, particularly in the rainy season between late May and late September and absent in the dry season. Initial cursory observations in the field revealed that there was little evidence of occurrence of fungal pathogens on water hyacinth except during September to November when plants with unique pattern of infection were found in two of the four sampling stations: Badagry Creeks (N6.41950° E2.86019°, N6.42066° E2.86630° and N6.41748° E2.87552°) and Ogun River (N6.64091° E3.8406°) (Figure 2A). The disease appeared as a leaf spot, with concentric rings rounded on the side facing the petiole and narrowing towards the laminar tip. Older leaf spots turned necrotic with dark brown margins, with the centre of the spot containing white and black fungal spores. The diameter of each spot appeared to be proportional to the age of the spot. The disease was easy to identify as brownish necrotic leaf blight, forming massive brownish patches on water hyacinth leaves in the field. The total area infected was 0.0396 km^2 in Badagry and 0.0074 km^2 in Ogun River. The fungal prevalence or number of infection was 3.17×10^{-2}% of the total number of plants in Badagry Creeks and 1.64×10^{-2}% of the total number of plants surveyed in Ogun River. There were no infection in Mile 2 and Lagos Lagoon.

3.2. Fungal isolation from diseased water hyacinth leaves

Five different fungi (*Fusarium* sp., *Aspergillus niger, Aspergillus flavus, Curvularia* sp., *Penicillium* sp.) were isolated from water hyacinth leaf pieces sterilised with 0.26% sodium hypochlorite and plated on PDA. These fungi grew out within 24 h while a *Myrothecium* sp. appeared within 36 h. From leaf pieces sterilised with 1.4% sodium hypochlorite solution, *Myrothecium* sp. appeared conspicuously on day 3, while the other fungi appeared between days 5 and 6. Of these organisms, *Myrothecium* sp. occurred most frequently (45%) of total isolation (350) followed by *Fusarium* sp. (30%), *Curvularia* sp. (14.7%), *Penicillium* sp. (5%), *A. niger* (3.5%) and *A. flavus* (1.8%). The use of TWA medium yielded *Myrothecium* sp. after 24 h, although this appeared as transparent hyphae on the medium as compared to the fluffy, whitish, conspicuous appearance on PDA. The other fungi such as *Fusarium* sp. and *Curvularia* sp. on TWA were not noticeable until the fourth or fifth day. The growth of the other organisms appeared not to be well supported by TWA.

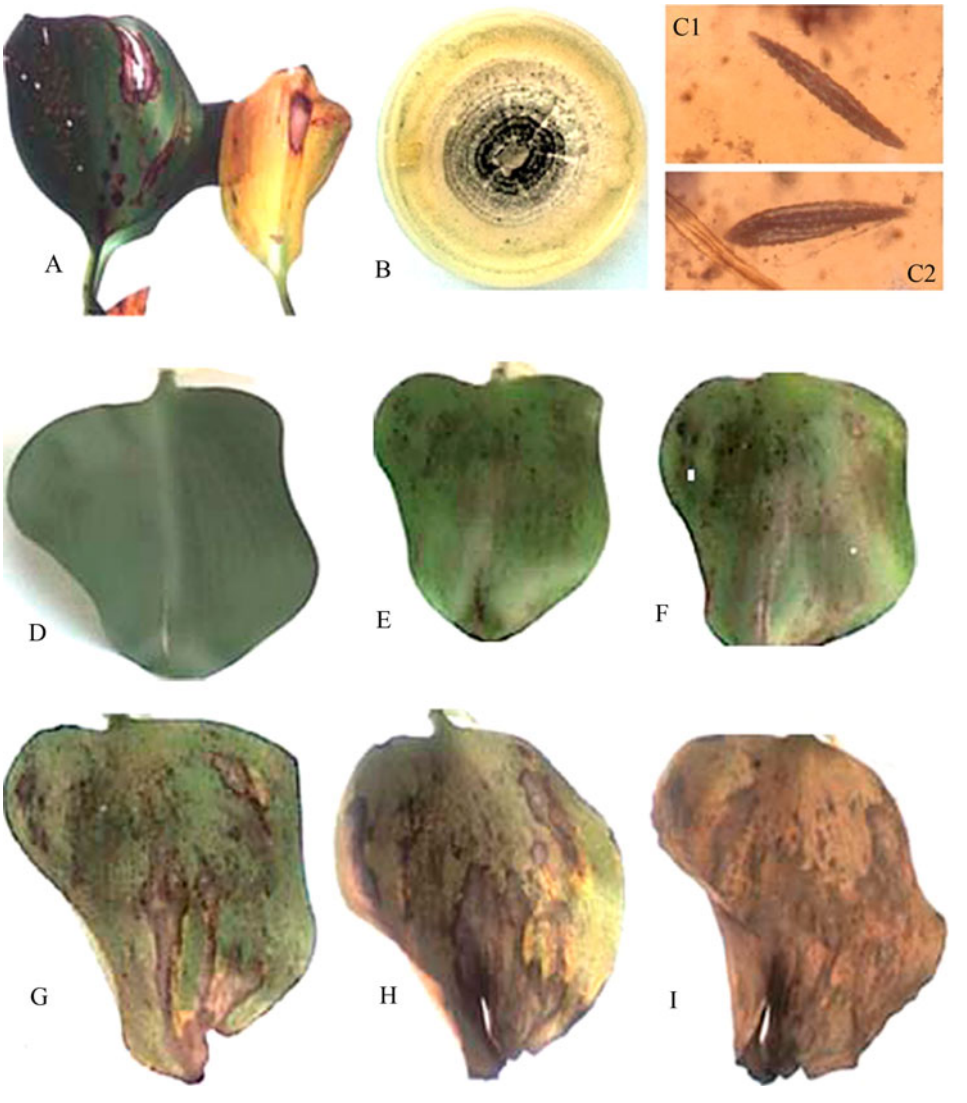

Figure 2. (Colour online) (A) Pattern of natural infection of water hyacinth by *Myrothecium* species at the survey site. (B) Morphological appearance of *Myrothecium roridum* (IMI 394934) on PDA plate. (C1 & C2) Photomicrograph of *Myrothecium roridum* (IMI 394934) conidia × 1000. (D–I) Disease progression in water hyacinth leaf post inoculation with *Myrothecium roridum* (IMI 394934) (1×10^6 spores/ml): (D) Day 0; (E) Day 3 (F) Day 6; (G) Day 9; (H) Day 12; (I) Day 21.

3.3. Fungal identification

Cultures of one of the isolates on PDA plates (using an 8 mm diameter cork borer) reached 56 ± 3 mm diameter in six days at 25°C and appeared brownish-black with irregular border and concentric zones. Conidia were slightly curved, septate and the central cells were broader than the end cells. The isolate was identified and authenticated as *Curvularia pallescens* Boedijn by Dr Markus N. Thormann

(Northern Forestry Centre, Natural Resources Canada, 5320-12251, Edmonton, AB T6H 3S5, Canada).

Cultures of a second isolate on PDA reached 77.5 ± 4.2 mm diameter in six days at 25°C, slightly whitish at first and later turning pinkish in colour. Conidia were sickle shaped and septate. The isolate was authenticated as *Fusarium solani* by Prof. A.A. Adekunle (Botany and Microbiology Department, University of Lagos).

The third isolate on PDA reached 77.13 ± 1.6 mm diameter in 14 days at 25°C. The isolate produced white, floccose colonies with sporodochia in dark green-to-black concentric rings (Figure 2B). Conidia were sub-hyaline and cylindrical with rounded ends (Figure 2C1 & C2). All characteristics were consistent with the description of *Myrothecium roridum* Tode ex Fr. (Ellis, 1971; Fitton & Holliday, 1970). This was authenticated as *M. roridum* and was given the accession number (IMI 394934) at the Centre for Agriculture and Bioscience International (CABI), Egham Surrey, UK.

3.4. Pathogenicity screening

No disease symptoms were observed on water hyacinth plant infected with *C. pallescens* and *F. solani* 24 DPI. Of the six different fungal species tested for their ability to infect healthy water hyacinth plants *in vitro*, the result showed that *M. roridum* was the only candidate which infected and produced disease symptoms on water hyacinth leaves. The disease started as scanty patches which developed into pale-to-dark brown heavy necrotic spots on the leaves. The necrotic spots expanded in diameter between 5 and 10 mm. With disease progression, the necrotic spots coalesced and the necrotic area increased. The resultant effect was a decrease in the green leaf area and leaf death (Figure 2D–I). The symptom produced in the pathogenicity test was similar to that seen in the field (Figure 2A).

The *M. roridum* isolate was ranked on the basis of the severity of the damage it caused (Table 1). The disease progression was monitored over time in terms of disease severity and disease incidence (Table 1). The disease incidence on day 4 was greater than 60% in experiments A–C, respectively, and 100% in these experiments on day 7. Similarly, the disease severity became prominent on day 4 in all experiments and the mean values were greater or equal to 2.60. The average disease severity on day 24 was maximum in experiment A and least in experiment C. However, regression analysis indicates that there was no significant difference in the rate of disease progression in all experiments ($F_{2,27} = 0.95$, $P = 0.4$). This is an indication that the result is reproducible. Based on the result obtained, the isolate of *M. roridum* (IMI 394934) was chosen for further study.

3.5. Host specificity test.

The host range plant response to *M. roridum* showed that 74.19% of the test plants were not susceptible (Table 2). Slightly susceptible plants account for 16.13% and plants health status were not compromised. Duckweed was susceptible to the fungus resulting in necrosis and death of the plants. Water lettuce was highly susceptible. Water hyacinth was highly susceptible showing heavy leaf spotting and necrosis with more than 50% of leaf area coalescing with a resultant death of the plant in less than 21 days.

Table 1. Pathogenicity profile of *Myrothecium roridum* (IMI 394934) on water hyacinth.

DPI	Disease incidence (Exp. A)	Mean disease severity (Exp. A)	Disease incidence (Exp. B)	Mean disease severity (Exp. B)	Disease incidence (Exp. C)	Mean disease severity (Exp. C)
0	0.00	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00	0.00 ± 0.00
3	0.00	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00	0.00 ± 0.00
4	83.33	2.60 ± 0.62	66.67	2.75 ± 0.45	66.67	2.75 ± 0.47
5	100	3.17 ± 0.72	83.33	3.00 ± 0.52	83.33	3.11 ± 0.62
7	100	4.17 ± 0.66	100	4.00 ± 0.57	100	3.94 ± 0.50
10	100	5.00 ± 0.57	100	4.67 ± 0.51	100	4.72 ± 0.50
11	100	5.17 ± 0.54	100	4.67 ± 0.51	100	4.78 ± 0.60
14	100	5.83 ± 0.62	100	5.53 ± 0.47	100	5.33 ± 0.83
17	100	6.83 ± 0.62	100	5.94 ± 0.54	100	5.83 ± 0.93
20	100	7.83 ± 0.77	100	6.67 ± 0.52	100	6.50 ± 0.87
24	100	8.67 ± 0.62	100	7.67 ± 0.62	100	7.28 ± 0.75

Data represent mean ± SD of six replicate determinations. *$P < 0.05$ = Significant difference in data exist between different experiments (Exp. A–C) when subjected to GLM regression analysis with DPI set as continuous predictor. Data for control plants were excluded as disease symptoms were absent. DPI, days post inoculation; GLM, general linear model.

Disease incidence = (number of leaves with disease symptoms/total number of leaves present on plants in six replicate pots) × 100.

Disease severity keys:

0 = no spots on lamina or petiole.
1 = 1–4 spots on lamina, no petiolar spotting.
2 = Less than 25% of lamina surface with spots, no coalescence or petiolar spotting.
3 = Less than 50% of laminar surface with spots, some coalescence, no petiolar spotting.
4 = Less than 50% of leaf surface with spots, coalescence, some tip dieback, and petiolar spots.
5 = Less than 50% of leaf surface with spots, coalescence, 10% tip dieback, and petiolar spotting.
6 = Less than 75% spots, coalescence, 30% tip dieback, and petiolar spotting.
7 = Greater than 75% spots, coalescence, 60% tip dieback, coaleascing spots on petiole.
8 = Dead lamina, petiole green, but heavily spotted.
9 = Dead lamina and petiole (submerged).

3.6. Molecular identification of the pathogenic isolate

Results from CABI microbial identification service indicated that the morphology of the strain conforms in all respects to standard descriptions of *Myrothecium* species and the ITS1 rDNA sequence data of 533 base pairs revealed that it is a new strain of *M. roridum* (GenBank accession no. GQ853401).

3.7. Blast and phylogenetic analysis

Sequence alignment of the ITS rDNA of the isolate IMI394934 did not produce any species with 100% homology (Table 3). The closest species which were from marine sources and agricultural crops were 99% homologous and differed by four base pairs. However, we could not find any sequence data for previously reported isolates of water hyacinth in the GenBank/alignment search.

The strain is clustered with a number of other, previously described, *M. roridum* isolates (Figure 3). Moreso, this isolate was most related to a strain identified as

Table 2. Plant response to *Myrothecium roridum* (IMI 394934) 21 days post inoculation.

Plant family	Botanical name	Common name	Host response/disease rating
Amaranthaceae	*Amaranthus viridis*	Green amaranth	–
Amaranthaceae	*Celosia argentea*	Plumed celosia	–
Anacardiaceae	*Mangifera indica*	Mango	–
Apiaceae	*Daucus carota*	Carrot	–
Araceae	*Colocasia esculenta*	Cocoyam	–
Araceae	*Lemna minor*	Duckweed	++
Araceae	*Pistia stratiotes*	Water lettuce	+++
Arecaceae	*Phoenix dactylifera*	Date palm	–
Asteraceae	*Lactuca taraxacifolia*	Lettuce	–
Asteraceae	*Vernonia amygdalina*	Bitter leaf	+
Brassicaceae	*Brassica oleracea*	Cabbage	–
Bromeliaceae	*Ananas comosus*	Pineapple	–
Caricaceae	*Carica papaya*	Pawpaw	–
Cucurbitaceae	*Citrullus lanatus*	Water melon	–
Dioscoreaceae	*Dioscorea alata*	Yam	–
Euphorbiaceae	*Acalypha cordifolia*	Acalypha	–
Euphorbiaceae	*Manihot esculenta*	Cassava	–
Euphorbiaceae	*Euphorbia milii*	Crown of thorn	–
Fabaceae	*Vigna unguiculata*	Beans	+
Fabaceae	*Arachis hypogaea*	Groundnut	+
Hydrocharitaceae	*Hydrilla verticilata*	Hydrilla	–
Musaceae	*Musa paradisiacal*	Banana	–
Nymphaeaceae	*Nymphaea caerulea*	Water lilly	–
Poaceae	*Zea mays*	Corn	–
Poaceae	*Cymbopogun citrates*	Lemon grass	+
Pontederiaceae	*Eichhornia crassipes*	Water hyacinth	+++
Solanaceae	*Capsicum chinense*	Red savina	–
Solanaceae	*Capsicum annuum*	Chili pepper	–
Solanaceae	*Nicotiana tabacum*	Tobacco	–
Sparrmanniaceae	*Corchorus olitorius*	Jute	+
Xanthorrhoeaceae	*Aloe vera*	Aloe	–

Disease rating scale: – = not susceptible (leaves healthy, no disease symptom observed), + = slightly susceptible (scanty leaf spotting or slight chlorosis or no necrosis), ++ = susceptible (leaf spots/leaf necrosis at 30–50% leaf is dead), and +++ = highly susceptible (severe leaf spotting/necrosis at >50% leaf is dead). The fungal suspension was applied at 1×10^{-6} spore/ml in 0.1% v/v Tween 80 solution.

M. carmichaelii isolate IMI 199044 (GenBank accession no. AY254150). Our isolate has been registered in the International Mycological Institute (IMI) Culture Collection Center (*M. roridum* IMI 394934). The molecular sequence data of the internal transcribed spacer regions ITS1, ITS2 and the 5.8s rRNA genomic region of the isolate has also been deposited in the GenBank (accession no. GQ853401).

4. Discussion

The symptoms or diseased water hyacinth plants were peculiar to two of the sampled locations; Badagry Creeks (N6.41950° E2.86019°, N6.42066° E2.86630° and N6.41748° E2.87552°) and Ogun River (Isheri: N6.64091° E3.8406°). Previous explorative studies in Nigeria have shown the presence of some fungal isolates such

Table 3. Alignment analysis of ITS rDNA sequence of *Myrothecium roridum* (IMI 394934) with the closest (≥97% homology) fungi in the holdings of GenBank.

SN	Sources	Species	GenBank accession numbers	Homology percentage
1	Water hyacinth (Nigeria)	*M. roridum* (isolate IMI 394934)	GQ853401	100
2	Melon (Brazil)	*M. roridum* (strain 784)	JF724157	99
3	Marine (Spain)	*M. carmichaelii* (strain IMI 199044)	AY254150	99
4	Soybean leaf (Brazil)	*M. roridum* (strain 794)	JF724158	99
5	Soybean leaf (Brazil)	*M. roridum* (strain MA-73)	JF724153	99
6	Soybean root (Brazil)	*M. roridum* (strain 801)	JF724151	99
7	Soybean root (Brazil)	*M. roridum* (strain 802)	JF724150	99
8	Salvia sp. (USA)	*M. roridum*	EF151002	99
9	Melon root (Brazil)	*M. roridum* (strain 782)	JF724156	99
10	Cotton leaf (India)	*M. roridum* (strain CICR)	EU927366	99
11	Bean (China)	*M. roridum* (strain CD08072303)	GQ381291	99
12	Young fronds (India)	*Pteris ensiformis*	AM920397	99
13	Marine sponge (USA)	*Myrothecium* sp. (isolate HKB 34)	EF029818	99
14	*Hemionitis arifolia* leaf (China)	*M. roridum* (strain DGM01)	JF343832	98
15	(Germany)	*M. roridum* (strain BBA 71015 {CBS 212.92})	AJ302001	98
16	(Germany)	*M. roridum* (strain BBA 67679)	AJ301995	98
17	Soybean leaf (Brazil)	*M. roridum* (strain 781)	JF724155	98
18	Tomato leaf (China)	*M. roridum* (strain FQ07090401)	GQ162434	98
19	Surface dust (USA)	Uncultured fungus (clone f4HSc41)	GU722059	97
20	Soybean leaf (Brazil)	*M. roridum* (strain MA-20)	JF724152	97
21	Marine (Spain)	*M. lachastrae* (strain IMI 273160)	AY254159	97
22	Soil (Brazil)	Uncultured fungus (clone ASSA173)	JQ081552	97
23	Soil (China)	*Myrothecium* sp. (strain JZ-45)	HQ637275	97
24	(Germany)	*M. leucotrichum* (strain BBA 71014 {CBS 131.64})	AJ302000	97
25	Soil (Australia)	Uncultured fungus (clone RFLP type 6)	GQ921722	97

E value = 0, for all sequences in the table.

as *Cercospora piaropi* Tharp, *Cladosporium oxysporum* Berk. & Curt and *Phyllosticta* sp. on water hyacinth (Barreto & Evans, 1996). In this study, a different fungal species identified as *M. roridum* (IMI 394934) was obtained. Reports indicate that strains of this fungus have been isolated in India, Mexico, Philippines Thailand/Burma (IMI 79771) and Malaysia (IMI 277583) (Charudattan, 2001; Evans & Reeder, 2001). However, to the best of our knowledge this is the first documented report of *M. roridum* isolation from water hyacinth in Nigeria.

As a result of pathogenicity testing and on the basis of disease severity, *M. roridum* (IMI 394934) was found to be highly destructive on water hyacinth.

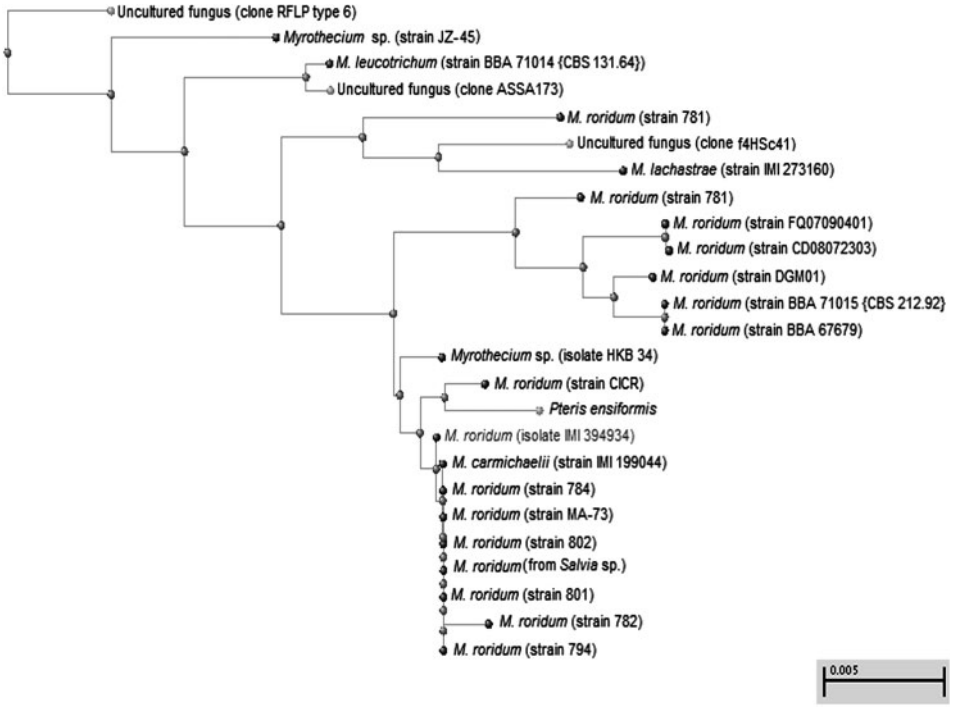

Figure 3. Phylogenetic analysis of ITS rDNA sequence data of *Myrothecium roridum* IMI 394934 (gb/GQ853401) with 24 most homologous sequences available in the GenBank.

Barreto and Evans (1996) and Charudattan (2001) reported that only a few fungi recorded on water hyacinth have been thoroughly tested and confirmed to be highly virulent pathogens. Of these fungi, *Acremonium zonatum, Alternaria eichhornia* and *C. piaropi* (= *C. rodmanii*), under experimental conditions have been shown as biocontrol agents effective against in water hyacinth (Charudattan, 2001). Also, Bateman (2001) reported some promising fungi as potential mycoherbicides for water hyacinth control in Africa. In order of potential utility based on the virulence, they include *A. eichhorniae*, *A. zonatum*, *C. piaropi, Rhizoctonia solani*, *Alternaria alternata* and *M. roridum*. However, the strain of *M. roridum* (IMI 394934) reported in this study appeared to show a greater disease incidence and disease severity than that reported for *A. alternata* (El-Morsy, Dohlob, & Hyde, 2006), since healthy water hyacinth used in this study died four weeks post inoculation with *M. roridum* (IMI 394934), taking into consideration that this study was carried out under different climatic and environmental conditions. *C. pallescens* and *F. solani* isolated in this study were not virulent and not considered potential candidates for water hyacinth control. This is in agreement with the previous reports from some other countries which also found these two organisms to be non-virulent fungi associated with water hyacinth (Barreto & Evans, 1996; El-Morsy et al., 2006).

The basic sequence alignment analysis of the internal transcribed spacer of the isolate IMI 394934 (GenBank accession no. GQ853401) showed no strains with 100% homology; an indication that it is a new strain of *M. roridum*. However, it was

99% homologous to a strain identified as the closely related species *M. carmichaelii* isolate IMI 199044 (GenBank accession no. AY254150). This suggests that the difference in homology is not sufficient to establish an unequivocal identification.

The paucity in the sequence data of the previous isolates of water hyacinth in Sri Lanka; IMI 261802 (Hettiarachchi, Gunasekera, & Balasooriya, 1983), India (Ponnappa, 1970), Mexico/India/Philippines Thailand/Burma (IMI 79771) and Malaysia (IMI 277583) (Barreto & Evans, 1996; Evans & Reeder, 2001) made it impossible to compare our isolate or perform phylogenetic relationship studies among isolates of *M. roridum* pathogenic to water hyacinth. Phylogenetic studies have been done on *Cercospora* species pathogenic to water hyacinth (Tessmann, Charudattan, Kistler, & Rosskopf, 2001), such studies could offer some insights into biogeographic hypothesis of *Myrothecium* on water hyacinth. Our isolate was able to weakly infect bean and groundnut; however, it is not clear if the isolates from bean, soybean and other agricultural crops can infect or be pathogenic to water hyacinth.

M. roridum has been previously reported as a pathogen of water hyacinth and some other host plants including some economically important crops (Fish, Bruton, & Popham, 2012; Gaikwad, 1988; Hettiarachchi et al., 1983; Ponnappa, 1970). The non-host nonspecificity was confirmed in this study by the ability to cause slight disease on bitter leaf, bean, groundnut, lemon grass and jute plants. The isolate studied caused no disease symptoms in corn unlike the report of Gaikwad (1988), this may be due to the differences in the source and origin of the isolates. Several studies indicate that the difference in the source or origin of microorganisms affects their performance (Anuna & Akpapunam, 1995; Anuna, Sokari, & Akpapunam, 1990; Okunowo & Osuntoki, 2007). We have previously reported its virulence on water lettuce (Okunowo, Osuntoki, & Adekunle, 2011). The efficacy of the fungus in the integrated management of water hyacinth is known to be enhanced by 2,4 D (Liyanage & Gunasekera, 1989). Hoagland, Weaver, and Boyette (2007) elucidated some possible strategies to reduce the non-target risk of a promising mycoherbicidal agent *Myrothecium verrucaria* which can be adapted to reduce the non-host specificity of *M. roridum*.

Conclusively, this study has isolated and identified a Nigerian indigenous strain of *M. roridum*, which is highly virulent to water hyacinth. This *M. roridum* isolate has potential for application in the biocontrol of water hyacinth. However, since it is not host specific, future studies should include extensive host range tests and strategies to reduce its non-target risk.

Acknowledgements

The authors are grateful to Raghavan Charudattan (Emeritus Professor, Plant Pathology Department, University of Florida, Gainsville, Florida, USA) and Hamed K. Abbas (Research Plant Pathologist/Lead Scientist, Biocontrol of Pest Research Unit, USDA-ARS, NBCL, Stoneville, MS, USA) for their helpful discussions and review of the draft version of the manuscript.

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