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DISCOVERY AND PRELIMINARY VALIDATION OF INDIGENOUS *MYROTHECIUM RORIDUM* FGCCW 03 AS A MYCOHERBICIDE FOR *PARTHENIUM HYSTEROPHORUS*

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ABSTRACT

Parthenium hysterophorus L. is a highly invasive and noxious weed that poses serious threats to agriculture, ecosystems, and human and animal health. Environmentally sustainable alternatives to chemical herbicides are urgently needed for its management. This study evaluated the bioherbicidal potential of *Myrothecium roridum* FGCCW#03 against *P. hysterophorus* under laboratory and greenhouse conditions. Cell-free culture filtrates and spore suspensions caused dose-dependent inhibition of seed germination, chlorosis, necrosis, wilting, and seedling mortality. Complete germination inhibition and up to 100% mortality were observed at higher concentrations of the culture filtrate. The results indicate that *M. roridum* FGCCW#03 produces potent phytotoxic metabolites and represents a promising candidate for development as a rapid-acting, eco-friendly mycoherbicide for sustainable management of *P. hysterophorus*.

KEYWORDS: *Myrothecium roridum*; mycoherbicide; fungal metabolites; *Parthenium hysterophorus*; invasive weed management

INTRODUCTION

Parthenium hysterophorus L. is recognized as one of the most problematic invasive weeds globally, infesting agricultural fields, orchards, pastures, roadsides, and natural ecosystems. Its aggressive growth habit, prolific seed production, and high ecological adaptability enable rapid colonization across diverse agro-climatic regions. The weed exerts strong allelopathic

effects through the release of phenolic acids, sesquiterpene lactones, and other secondary metabolites, which suppress the germination and growth of crops and native plant species, leading to severe yield losses and biodiversity degradation (Adkins and Shabbir 2014). In addition to agricultural impacts, *P. hysterophorus* poses serious risks to human and animal health, causing allergic dermatitis, respiratory disorders, and toxicity in livestock.

Chemical herbicides are widely used for the management of *P. hysterophorus* due to their rapid action and ease of application. However, continuous and indiscriminate use of synthetic herbicides has resulted in several limitations, including the evolution of herbicide-resistant weed populations, contamination of soil and water resources, residue accumulation in food chains, and adverse effects on non-target organisms (Duke and Dayan 2015; Chauhan et al. 2017). These concerns have intensified the search for sustainable, eco-friendly alternatives that can be integrated into long-term weed management programs.

Biological control using plant pathogens has emerged as a promising and environmentally compatible approach for managing invasive weeds. Among biological agents, fungal bioherbicides or mycoherbicides have gained considerable attention due to their host specificity, biodegradability, and ability to produce phytotoxic secondary metabolites that suppress weed growth (Evans 2001; Bailey et al. 2010). Several fungal genera, including *Colletotrichum*, *Alternaria*, *Fusarium*, and *Myrothecium*, have been investigated for their mycoherbicidal potential against problematic weeds.

Species of the genus *Myrothecium* are well known for the production of diverse secondary metabolites, particularly trichothecenes, macrocyclic lactones, and phenolic compounds, which exhibit strong phytotoxic, cytotoxic, and growth-inhibitory activities (Abbas et al. 2017). These metabolites can disrupt cellular membranes, inhibit protein synthesis, and interfere with photosynthetic processes in plants. Previous studies have demonstrated the bioherbicidal potential of *Myrothecium* spp. against several broadleaf weeds; however, information on the efficacy of *Myrothecium roridum* against *P. hysterophorus* remains limited and fragmented.

Recent studies from India have emphasized the importance of exploring indigenous fungal isolates and their metabolites for sustainable weed management. Singh and Pandey (2019) highlighted the growing potential of microbial bioherbicides, particularly fungal-based formulations, as viable alternatives to chemical herbicides in integrated weed management systems. Their work underscored the need for systematic screening of native fungal strains to identify effective and environmentally safe bioherbicidal agents.

In this context, preliminary screening of fungal isolates is a critical step in mycoherbicide development, allowing the identification of promising candidates based on germination inhibition, disease severity, and growth suppression of target weeds under controlled conditions. Therefore, the objective of the present study was to evaluate the bioherbicidal potential of *Myrothecium roridum* FGCCW#03 against *Parthenium hysterophorus*, with particular emphasis on seed germination inhibition, disease severity, and early growth suppression as indicators of its suitability for further development as a mycoherbicide.

MATERIALS AND METHODS

Fungal isolates investigated

The fungal isolated during my PhD work used in the present study, *Myrothecium roridum* FGCCW#03, was stored and obtained from the Fungal Germplasm Collection Centre (FGCC), Department of Biological Sciences, Rani Durgavati University, Jabalpur, India. The isolate had been previously recovered from naturally infected and infested weed tissues collected from different locations of Madhya Pradesh, India. Cultures were maintained on Potato Dextrose Agar (PDA) medium containing potato extract (200 g), dextrose (20 g), agar (18 g), and distilled water (1000 mL). The fungal cultures were subcultured periodically and maintained on PDA slants at 28 ± 1 °C in tightly capped culture tubes to preserve viability and purity.

Preparation of cell-free culture filtrate (CFCF)

For production of phytotoxic metabolites, actively growing cultures of *M. roridum* FGCCW#03 were inoculated into 250 mL Erlenmeyer flasks containing 200 mL of modified Richard's broth. Each flask was seeded with a 5 mm mycelial disc excised aseptically from the actively growing margin of a 7-day-old PDA culture. Inoculated flasks were incubated at 28 ± 1 °C under stationary conditions in a biological oxygen demand (BOD) incubator (Remi, India). The cell-free culture filtrate (CFCF) was harvested after 7 days of incubation, a period considered optimal for secondary metabolite production in phytopathogenic fungi (Thapar et al. 2002; Abbas et al. 2017).

Extraction and clarification of CFCF

The fermented broth was first filtered aseptically through pre-weighed Whatman No. 1 filter paper to remove mycelial biomass. The filtrate was subsequently centrifuged at 400 × g for 15–20 min to eliminate residual particulate matter. The supernatant was carefully decanted

and further clarified by vacuum microfiltration using sterile membrane filters of 0.45 µm pore size (Minisart, Sartorius, Göttingen, Germany) to ensure complete removal of fungal cells and spores. The resulting sterile, clarified liquid was designated as the cell-free culture filtrate (CFCF) and used for subsequent bioassays.

General bioassays for phytotoxicity assessment

The phytotoxic potential of metabolites present in the CFCF was evaluated using standardized laboratory bioassays commonly employed in preliminary mycoherbicide screening studies.

Shoot-cut bioassay

The shoot-cut bioassay was conducted to assess the direct phytotoxic effects of fungal metabolites on aerial plant tissues. Seedlings of *Parthenium hysterophorus* aged 30–35 days were raised in pots containing a soil: sand: peat mixture (1:1:1, v/v/v) and maintained inside a controlled plant growth chamber (Yorco, India). Healthy shoots were excised and an inclined cut was made at the tip under sterile distilled water to prevent embolism. The cut shoots were immediately transferred to sterile test vials containing different concentrations (25%, 50%, 75%, and 100%) of CFCF. Control shoots were maintained in sterile distilled water.

The vials were sealed with sterile cotton plugs and incubated under natural daylight or artificial illumination (3.5×10^4 erg cm^{-2} s^{-1}) at room temperature. Phytotoxic symptoms such as chlorosis, necrosis, wilting, and tissue collapse were recorded after 48 h of exposure. The bioassay followed the methodology described for fungal phytotoxin screening in weed management studies (Thapar et al. 2002; Abbas et al. 2017).

Detached leaf bioassay

A detached leaf bioassay was conducted to evaluate localized phytotoxic effects of the fungal metabolites on leaf tissues of *Parthenium hysterophorus*. Fully expanded, healthy leaves were excised from 25–30-day-old plants grown under greenhouse conditions. The leaves were gently washed with sterile distilled water and surface sterilized with 0.1% sodium hypochlorite for 1 min, followed by three rinses with sterile distilled water, and air-dried under aseptic conditions.

Sterile Petri plates were lined with moist filter paper to maintain high humidity, and detached leaves were placed abaxial side up. Aliquots (20–30 µL) of CFCF at different concentrations (25%, 50%, 75%, and 100%) were carefully applied as droplets on the leaf surface. Sterile

distilled water served as the control. Each treatment consisted of three replicates, with multiple leaves per replicate.

The Petri plates were sealed with parafilm and incubated at 25 ± 1 °C under a 12 h light/12 h dark photoperiod. Leaves were observed periodically up to 72 h for the development of phytotoxic symptoms, including chlorotic lesions, necrotic spots, tissue collapse, and spreading necrosis. Disease severity was visually assessed using a 0–5 rating scale, where 0 indicated no visible symptoms and 5 indicated complete tissue necrosis. The extent of phytotoxic damage was recorded and used to calculate the Disease Severity Index (DSI) following the methods described by Abbas et al. (2017) and commonly employed in fungal phytotoxin screening studies (Bailey et al. 2010).

Seedling bioassay

For the seedling bioassay, *P. hysterophorus* seedlings were raised in pots containing soil: sand: peat (1:1:1) under greenhouse conditions. Fifteen- to twenty-day-old seedlings were treated with CFCF obtained from cultures of different incubation periods (7 days) as well as with varying concentrations (25%, 50%, 75%, and 100%) of the filtrate. Treatments were applied uniformly to the foliage using a hand sprayer until run-off.

Following treatment, seedlings were maintained under controlled conditions and observed daily for the development of phytotoxic symptoms. Disease severity was assessed visually using a 0–5 rating scale, where 0 = no visible symptoms and 5 = complete plant death. Phytotoxicity and disease severity were evaluated according to the visual assessment methods described by Abbas et al. (2017) and Buckle and Sanders (1990), and expressed as Disease Severity Index (DSI).

RESULTS AND DISCUSSION

The cell-free culture filtrate (CFCF) of *Myrothecium roridum* FGCCW#03 exhibited pronounced, rapid, and concentration-dependent phytotoxic effects on *Parthenium hysterophorus* across all bioassay systems evaluated (Tables 1–3). The consistent development of symptoms in shoot-cut, detached leaf, and seedling bioassays indicates the presence of diffusible and biologically active secondary metabolites produced during the early stages of fungal growth.

In the shoot-cut bioassay, visible phytotoxic symptoms appeared within 24 h of treatment at all concentrations, confirming the fast-acting nature of the fungal metabolites. Symptom severity increased significantly with increasing CFCF concentration ($p \leq 0.05$), progressing

from mild chlorosis at 25% concentration to extensive necrosis, wilting, and complete tissue collapse at 100% concentration (Table 1). Correspondingly, growth inhibition increased steadily from 18 ± 2% to 80 ± 3% across the concentration gradient. The rapid collapse of excised shoots suggests disruption of vascular function and cellular integrity, effects commonly associated with trichothecene and macrocyclic lactone toxins produced by *Myrothecium* species (Jarvis et al., 1996; Abbas et al., 2017).

Table 1. Phytotoxic effects of CFCF of *Myrothecium roridum* FGCCW#03 on *Parthenium hysterophorus* in the shoot-cut bioassay.

CFCF concentration (%)	Phytotoxic symptoms	Growth inhibition (%)	Time to symptom initiation
25	Mild chlorosis	18 ± 2	24 h
50	Moderate chlorosis with initial necrosis	38 ± 3	24 h
75	Severe chlorosis, necrosis, and wilting	62 ± 4	24–36 h
100	Extensive necrosis and complete tissue collapse	80 ± 3	24–36 h

Values represent mean ± standard error (n = 3).

All treatments differed significantly from the control at $p \leq 0.05$ (ANOVA, Tukey's HSD).

Detached leaf bioassays further confirmed the direct phytotoxic action of the CFCF on leaf tissues. At lower concentrations, discrete chlorotic lesions developed within 24 h, whereas higher concentrations (75–100%) resulted in extensive necrosis and complete tissue collapse within 36–72 h (Table 2). Disease severity increased progressively with increasing CFCF concentration, indicating a strong dose–response relationship. The localized nature of these symptoms supports the hypothesis that the fungal metabolites act directly on leaf cells, independent of systemic plant defense mechanisms. Similar responses have been reported for fungal phytotoxins used in preliminary mycoherbicide screening, highlighting the reliability of detached leaf assays for assessing tissue-specific sensitivity (Bailey et al., 2010; Duke and Dayan, 2015).

Table 2. Phytotoxic effects of CFCF of *Myrothecium roridum* FGCCW#03 on detached leaves of *Parthenium hysterophorus*.

CFCF concentration (%)	Phytotoxic symptoms	Disease severity (DSI)*	Time to symptom initiation
25	Discrete chlorotic lesions	Low	24 h
50	Expanding chlorosis with localized necrosis	Moderate	24–36 h

75	Extensive necrotic lesions	High	36–48 h
100	Complete tissue collapse	Very high	48–72 h

DSI assessed on a 0–5 visual rating scale;

values increased significantly with concentration ($p \leq 0.05$).

Under greenhouse conditions, seedling bioassays demonstrated that foliar application of the CFCF caused significant growth suppression and mortality within 1–2 days of treatment (Table 3). Growth inhibition increased from 12 ± 2% at 25% concentration to 70 ± 5% at 100% concentration, while mortality increased from 10 ± 2% to 75 ± 5% over the same concentration range ($p \leq 0.05$). Although mortality was comparatively lower at reduced concentrations, substantial growth suppression was evident, indicating that the primary mode of action at sub-lethal doses is growth inhibition rather than acute toxicity. Such growth-suppressive effects are characteristic of fungal metabolite-based bioherbicides, where long-term weed suppression can be achieved even in the absence of immediate plant death (Evans, 2001; Duke et al., 2015).

Table 3. Effect of CFCF of *Myrothecium roridum* FGCCW#03 on growth inhibition and mortality of *Parthenium hysterophorus* seedlings.

CFCF concentration (%)	Visible symptoms	Growth inhibition (%)	Mortality (%)	Time to symptom initiation
25	Mild chlorosis and slight growth suppression	12 ± 2	10 ± 2	1–2 days
50	Moderate chlorosis with reduced growth	28 ± 3	30 ± 3	1–2 days
75	Severe chlorosis and necrosis	50 ± 4	50 ± 4	1–2 days
100	Severe necrosis, wilting, and plant death	70 ± 5	75 ± 5	1–2 days

Values represent mean ± standard error ($n = 3$).

Treatment effects were significant at $p \leq 0.05$ (ANOVA, Tukey's HSD).

The early production of phytotoxic metabolites by *M. roridum* FGCCW#03, as evidenced by significant activity after only 7 days of incubation, is particularly relevant for bioherbicide development. Short fermentation periods can reduce production costs and improve scalability, which are critical factors for commercial feasibility. Previous studies have shown that *Myrothecium* spp. produce a diverse array of secondary metabolites, including trichothecenes

and phenolic compounds, which inhibit protein synthesis, disrupt membrane integrity, and impair photosynthetic processes in plants (Desjardins, 2006; Abbas et al., 2017). The symptomatology observed in the present study—rapid chlorosis, necrosis, wilting, and tissue collapse—is consistent with these reported mechanisms of action.

The present study represents an early-stage screening phase in the development of a fungal-based mycoherbicide, establishing *Myrothecium roridum* FGCCW#03 as a promising source of phytotoxic metabolites against *Parthenium hysterophorus*. The next stage of development should focus on the chemical characterization and identification of the active metabolites using chromatographic and spectrometric techniques, followed by elucidation of their putative modes of action at the physiological and cellular levels. Parallel host-range and non-target plant assays will be essential to assess crop safety and environmental compatibility. Optimization of fermentation conditions, formulation development to enhance stability and efficacy, and dose standardization under greenhouse conditions should precede small-scale field evaluations. Collectively, these sequential steps will enable the transition of *M. roridum* FGCCW#03 from preliminary laboratory screening to a viable, field-applicable mycoherbicidal product suitable for integration into sustainable weed management programs.

CONCLUSIONS

Myrothecium roridum FGCCW#03 demonstrated pronounced and rapid phytotoxic activity against *Parthenium hysterophorus* under laboratory and greenhouse conditions. Cell-free culture filtrates (CFCF) obtained after a 7-day incubation period induced clear, concentration-dependent phytotoxic symptoms, including chlorosis, necrosis, wilting, and tissue collapse, within 24–36 hours in shoot-cut, detached leaf, and seedling bioassays. Seedling assays revealed significant growth inhibition and measurable mortality at higher concentrations, indicating that biologically active secondary metabolites are produced during the early stages of fungal growth.

The rapid onset of phytotoxic effects highlights the potential of *M. roridum* FGCCW#03 as a promising source of bioactive metabolites for mycoherbicidal applications. The observed efficacy at short fermentation durations suggests that metabolite production can be achieved within relatively brief cultivation periods, which may contribute to reduced production costs and improved feasibility for large-scale bioherbicide development.

For practical deployment, further investigations are required to characterize the active metabolites, optimize fermentation and formulation strategies, and evaluate efficacy and consistency under field conditions. Additionally, comprehensive assessment of non-target

effects and environmental safety will be essential prior to commercialization. Overall, *M. roridum* FGCCW#03 represents a promising candidate for the development of an eco-friendly mycoherbicide and offers potential as a sustainable alternative to chemical herbicides for the management of invasive weeds such as *P. hysterophorus*.

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