



Bioherbicidal Potential of Metabolites from Endophytic *Alternaria* and *Gibberella* Species against *Parthenium hysterophorus*

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Abstract

Parthenium hysterophorus is an aggressive invasive weed that threatens agricultural productivity, biodiversity and ecological stability. This study explores the bioherbicidal potential of crude metabolites derived from endophytic *Alternaria* and *Gibberella* species as sustainable alternatives to synthetic herbicides. Fungal strains were isolated from the stems of *Sageretia thea*, identified based on morphological and microscopic characteristics, and cultured in a yeast extract-supplemented medium to enhance metabolite production. Crude extracts were obtained using ethyl acetate and evaluated for their weedcidal efficacy against *P. hysterophorus* under a Completely Randomized Design (CRD). The synthetic herbicide 2, 4-dichlorophenoxyacetic acid (2, 4-D) was used as a standard for comparison. The results revealed significant inhibitory effects of the fungal extracts on seed germination and seedling growth. The extract from *Alternaria* reduced germination by 60% and markedly suppressed shoot and root growth at concentrations ranging from 250 to 1000 µg/mL. The *Gibberella* extract demonstrated even greater potency, achieving complete inhibition of germination and growth at 1000 µg/mL. In contrast, 2, 4-D exhibited a gradual inhibitory effect but failed to completely suppress germination at the highest tested concentration. These findings highlight the promising potential of fungal metabolites as eco-friendly bioherbicides that can offer an effective and sustainable approach to managing *P. hysterophorus*.

Keywords: 2, 4-D, Bioherbicide, Fungal natural products, *Sageretia thea*, Slide culture technique, Weed management.

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1. INTRODUCTION

Invasive weed species are often termed "silent threats" that pose a profound challenge to agricultural systems globally, including Pakistan ^{1, 2}. These weeds significantly impair agricultural productivity by competing with crops for essential resources such as water, nutrients, soil space and sunlight, while releasing allelopathic substances that inhibit crop growth ³. In countries like Pakistan, where agricultural production is already strained due to salinity, climate change, and limited resources, the proliferation of invasive weeds has compounded the challenge ⁴. Weeds such as *Parthenium hysterophorus*, *Sonchus oleraceus*, *Silybum marianum* and *Avena fatua* are increasingly problematic, causing substantial economic losses and threatening food security ^{5, 6}. Among these, *P. hysterophorus* has emerged as the most invasive weed in Pakistan, as highlighted by the Ministry of Climate Change and the Food and Agriculture Organization of the United Nations ⁷. Native to the Americas, *P. hysterophorus* has spread rapidly across Asian countries, where it has become a highly destructive and pervasive species. This weed significantly reduces crop yields and disrupts agricultural ecosystems by displacing native vegetation, affecting biodiversity, clogging waterways, and harboring pests and pathogens that threaten both crops and livestock ⁸. Additionally, its impact extends to human health, causing severe allergic reactions and dermatitis. Farmers frequently cite inadequate weed control as a critical issue contributing to significant agricultural losses ⁹.

Conventional methods to manage *P. hysterophorus* include manual removal and the use of chemical herbicides such as 2, 4-D and glyphosate ¹⁰. However, these approaches have limitations. Chemical herbicides pose serious risks, including environmental pollution, harm to soil microbiota, negative effects on human health, and the development of herbicide-resistant weed species ¹¹. Globally, over 500 cases of resistance have been documented in 260 weed species, posing a growing challenge to sustainable agriculture ¹². In response, there is a need for innovative, eco-friendly alternatives such as bioherbicides. Bioherbicides, derived from natural sources, offer numerous advantages, including target specificity, environmental safety, and effectiveness at low concentrations without inducing resistance ¹³. Fungi, renowned for their capacity to produce bioactive secondary metabolites, have shown great promise in weed control research ¹⁴. Genera such as *Alternaria*, *Botrytis*, and *Colletotrichum* have demonstrated herbicidal potential, and fungal-derived metabolites have been successfully used in historical bioherbicide applications, such as the commercialization of *Alternaria* *cuscutacidae* spores in Russia during the 1950s to control dodder species ^{15, 16 17}.

In this study, two endophytic fungal strains were isolated from the young stem of *Sageretia thea* and identified morphologically and microscopically as *Alternaria* and *Gibberella* species. Crude ethyl acetate extracts of these fungi were tested on *P. hysterophorus* seeds using the Completely Randomized Design (CRD) method to evaluate their effects on seed germination. The results were

promising, demonstrating that these crude fungal metabolites have the potential to serve as effective bioherbicides, pending further purification and characterization of the active compounds. This study highlights the potential of fungal secondary metabolites as eco-friendly solutions for sustainable weed management.

2. MATERIALS AND METHODS

2.1 Processing of plant samples and isolation of endophytic fungi

The young stems of the collected plant (*S. thea*) were first thoroughly rinsed with tap water, followed by washing with Tween 80 to remove surface impurities. After air-drying, the plant material was sterilized using a 1% mercuric chloride solution. The sterilized stems were then cut into small segments approximately 2 cm in length using a sterile blade and carefully placed on Potato Dextrose Agar (PDA) plates supplemented with tetracycline (100 µg/mL) to suppress bacterial contamination. The plates were incubated at a temperature range of 18–28°C to encourage fungal growth, which was closely monitored throughout the incubation period ¹⁸.

2.2 Morphological and microscopic characterization of the fungal isolates

Among the isolated endophytic fungi, only those fungal strains that exhibited bioactivity during the preliminary antibacterial and antifungal screening were selected for further identification. The identification process involved both morphological and microscopic analyses. Morphological characterization included observing colony texture, growth patterns and hyphal pigmentation on culture media. For microscopic identification, the slide culture technique was employed, allowing detailed examination of fungal structures. This included observing fruiting bodies, microconidia, conidiophores and the structure and arrangement of spores under a compound microscope at magnifications ranging from 40× to 1000×. To improve the visibility of fungal features, slides were stained with lactophenol cotton blue before analysis ^{19, 20}.

2.3 Fungal cultivation for secondary metabolite production in Yeast Extract Supplemented (YES) medium

To produce crude metabolites, fungal cultures were grown in a modified Yeast Extract Supplemented (YES) medium. This medium was prepared by dissolving yeast extract, peptone and glucose at concentrations of 2% (w/v) each in distilled water. To provide an alternative carbon source, 2% (w/v) sucrose was included, while 0.5% (w/v) ammonium sulfate was added to enhance nitrogen availability. To support fungal growth and metabolic activity, trace elements such as 0.01% (w/v) iron sulfate, 0.001% (w/v) zinc sulfate, 0.0001% (w/v) copper sulfate, and 0.0001% (w/v) manganese sulfate were incorporated into the formulation. The medium's pH was adjusted to 5.8 to ensure optimal conditions and was sterilized by autoclaving at 121°C. To verify sterility, the prepared medium was incubated overnight at 28°C before inoculation. Once sterility was confirmed, the

medium was inoculated with the selected fungal strain and placed in a shaking incubator at 28°C with constant agitation at 150 rpm. Cultivation was carried out for 14 days, providing the necessary conditions for fungal growth and metabolite production ²¹.

2.4 Separation and extraction of fungal secondary metabolites from the broth medium

To facilitate the separation and sedimentation of media components, approximately 300 µL of 35% hydrochloric acid (HCl) was added to each culture flask. This step ensured proper acidification, which aids in the precipitation of components and other undesirable materials. The fungal mycelia were then homogenized thoroughly using an electric blender to break down the cellular structure and release intracellular metabolites. Following homogenization, an equal volume of ethyl acetate was added to each flask as a solvent to extract the secondary metabolites. The mixture was intermittently stirred and gently shaken for 30 minutes to maximize the extraction of bioactive compounds from the fungal biomass.

The homogenized mixture was filtered through cheesecloth to separate the mycelial debris from the liquid extract. The filtrate, containing both the aqueous and organic layers, was transferred to a separating funnel. The organic layer, containing the ethyl acetate phase with dissolved metabolites, was carefully separated from the aqueous phase. To purify the organic extract, it was washed with a 2M brine solution, effectively removing water-soluble impurities. To ensure the removal of any residual moisture, the organic phase was dried over anhydrous sodium sulfate (Na₂SO₄). The dried organic layer was then filtered using filter paper to eliminate any solid residues. The purified organic extract was concentrated using a rotary evaporator set to 45°C, which evaporated the ethyl acetate under reduced pressure, leaving behind the crude metabolites. Finally, the dried crude extract was collected from the rotary evaporator flask by rinsing with methanol to ensure complete recovery of the metabolites. This crude extract was measured and stored in a suitable container for further analysis and herbicidal activity ²².

2.5 Herbicidal activity of the fungal crude metabolites against *Parthenium hysterophorus*

The herbicidal activity of crude metabolites produced by endophytic *Alternaria* and *Gibberella* species was evaluated against *Parthenium hysterophorus*, a highly invasive weed responsible for substantial agricultural damage in Pakistan. The experiment was designed using a Completely Randomized Design (CRD) and conducted in sterile, autoclaved Petri plates lined with two layers of sterile tissue paper. Surface sterilization of *P. hysterophorus* seeds was carried out with a 0.1% mercuric chloride solution to eliminate microbial contaminants. The sterilized seeds were then evenly spaced in the Petri plates, with 10 seeds per plate. Different concentrations of the fungal crude metabolites (10, 100, 250, 500, and 1000 µg/mL) were prepared and uniformly applied to the seeds using a sterile spray bottle. The plates were watered daily with sterile water as required,

ensuring consistent moisture levels. Each concentration was tested in triplicate, along with a control group treated with distilled water. To provide a benchmark for comparison, a parallel experiment was conducted under identical conditions using 2, 4-Dichlorophenoxyacetic acid (2, 4-D), a widely used selective herbicide. Seed germination was observed daily over a 27 day period, and key parameters such as germination percentage, shoot and root length (cm), and shoot and root weight (g) were recorded. Data analysis was performed using SPSS software, and significant differences between treatments were evaluated using the Least Significant Difference (LSD) test. Results were expressed as mean $\pm$ standard deviation to provide a clear comparison of the herbicidal efficacy of fungal crude metabolites versus the synthetic herbicide ²³.

3. RESULTS

3.1 Description of morphological and microscopic features of the identified fungal strains

3.1.1. Description of fungal strain identified as *Alternaria* species

On Potato Dextrose Agar (PDA), *Alternaria* colonies exhibited rapid growth, forming circular patterns with smooth and clearly defined margins, sometimes displaying slight lobes. Initially, the colonies appeared light gray or white but progressively darkened to olive-green and eventually developed a deep greenish-black hue as sporulation intensified. The colony texture ranged from velvety to woolly, with dense aerial hyphae contributing to the dark pigmentation observed in mature colonies. In contrast, on Sabouraud Dextrose Agar (SDA), the colonies were more compact and slightly smaller. This difference in colony texture and morphology is likely due to the medium's acidic pH and high sugar content. These colonies began as pale gray or white, darkening over time to green or black, often featuring a concentrated dark center indicative of sporulation. The surface texture on SDA appeared granular and powdery, with a noticeable reduction in aerial hyphae compared to PDA. For microscopic analysis, the slide culture technique was employed to examine hyphal structures, conidiophores and spore morphology in detail. The hyphae of *Alternaria* were darkly pigmented and septate, contributing to the characteristic dark coloration of the colonies. The conidiophores were branched and septate, tapering toward the tip and bore conidia that are unique to the genus. *Alternaria* conidia were large, pigmented and characterized by their distinctive muri-form structure, with transverse and longitudinal septa creating a “brick wall” pattern. These conidia were typically ovoid in shape and tapering at one end that branched from the tips of the conidiophores. The identification of *Alternaria* species relied on a combination of these distinctive features. The pigmented, septate hyphae; branched and tapered conidiophores; and large, muri-form, pigmented, beaked conidia collectively served as defining characteristics, allowing for precise and reliable identification of the fungal species under investigation.

3.1.2. Description of fungal strain identified as *Gibberella* species

On Potato Dextrose Agar (PDA), *Gibberella* colonies grew at a moderate rate, developing a distinct cottony to velvety appearance. Initially, the colonies were pale white or light pink, gradually deepening to a vibrant reddish-pink or purple as they matured. This characteristic pigmentation often formed concentric patterns, giving the colonies a unique visual signature. The reverse side of the colonies exhibited similar pigmentation, ranging from light magenta to dark purple, depending on the fungal strain and medium composition. The surface texture became fluffier as the colonies aged, due to the production of dense aerial mycelia. On Sabouraud Dextrose Agar (SDA), the colonies appeared more compact, with reduced aerial growth and a smoother texture. The coloration on SDA was generally softer, transitioning from pale pink to a muted red or orange as sporulation progressed. Microscopic examination provided more insights into the unique features of *Gibberella*. The hyphae were clear (hyaline), septate, and delicately branched, with a smooth and uniform appearance. A hallmark of *Gibberella* was the presence of perithecia—small, round to slightly oval, darkly pigmented fruiting bodies that formed prominently in mature cultures. Inside these perithecia were slender, elongated asci containing ascospores. The ascospores were smooth, often slightly curved or ellipsoid, and ranged from single-celled to septate, depending on their developmental stage. In addition to perithecia, *Gibberella* produced conidia, which were examined closely. The conidiophores were short and branched, bearing small, hyaline conidia that appeared oval to fusiform under the microscope. These conidia were mostly single-celled, although occasional septation was observed as they matured. The conidia were often found clustered or arranged in chains at the tips of the conidiophores. The combination of macroscopic and microscopic observations allowed for precise identification of *Gibberella*. The striking colony pigmentation, presence of perithecia with their characteristic ascospores, and the distinct morphology of conidia made this fungus easily distinguishable from other genera.

3.2 In vitro herbicidal activity of the fungal crude extracts

The herbicidal activity of crude ethyl acetate extracts from *Alternaria* species against *P. hysterophorus* demonstrated a clear concentration-dependent inhibitory effect on seed germination and seedling growth parameters, as summarized in the Table 1. In the untreated control group, all seeds germinated (100%) and exhibited robust shoot and root development, with average shoot and root lengths of 10.21 cm and 14.30 cm, respectively, and corresponding shoot and root weights of 0.073 g and 0.092 g. These values represent optimal growth conditions without the influence of inhibitory substances. At the lowest concentration (10 µg/mL), seed germination remained unaffected (100%), with minimal reductions observed in shoot length (9.68 cm), root length (14.00 cm), shoot weight (0.0723 g) and root weight (0.090 g), indicating negligible herbicidal impact at this

dose. As the concentration increased to 100 µg/mL, a slight decline in seed germination was recorded (97%), accompanied by reductions in shoot length (8.37 cm), root length (13.78 cm) and biomass accumulation (shoot weight: 0.069 g, root weight: 0.070 g). These findings suggest moderate inhibition of growth processes at this concentration. At 250 µg/mL, a more pronounced effect became evident, with germination reduced to 92% and significant decreases in shoot length (7.92 cm), root length (12.13 cm) and biomass (shoot weight: 0.0522 g, root weight: 0.063 g). This level of inhibition indicates the extract's increasing phytotoxic potential. The inhibitory effects were particularly notable at higher concentrations. At 500 µg/mL, germination dropped to 70%, while shoot and root lengths were reduced to 3.37 cm and 6.78 cm, respectively. The biomass also declined substantially, with shoot weight at 0.048 g and root weight at 0.035 g.

The most significant herbicidal effect was observed at 1000 µg/mL, where germination was reduced to 40%, shoot length to 2.48 cm, root length to 4.39 cm, and biomass to 0.034 g for shoots and 0.028 g for roots. These results highlight the potent inhibitory effect of the crude *Alternaria* metabolites at higher concentrations, substantially suppressing germination and seedling growth parameters. The results show that the crude ethyl acetate extract of *Alternaria* species exhibited encouraging herbicidal activity against *P. hystrophorus*, with effectiveness increasing markedly at higher concentrations.

Table 1. Weedicidal effect (Mean ± SD) of crude metabolites extracted from *Alternaria* species against *P. hystrophorus*

Treatments	Percent Germination	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)
Control	100	10.21 ± 1.0	14.30 ± 1.2	0.0730 ± 0.03	0.092 ± 0.02
10 µg µg mL ⁻¹	100	9.68 ± 0.2	14.00 ± 1.0	0.0723 ± 0.01	0.090 ± 0.02
100 µg µg mL ⁻¹	97	8.37 ± 0.4	13.78 ± 1.3	0.0690 ± 0.02	0.070 ± 0.02
250 µg mL ⁻¹	92	7.92 ± 0.2	12.13 ± 1.0	0.0522 ± 0.02	0.063 ± 0.03
500 µg mL ⁻¹	70	3.37 ± 0.3	6.78 ± 1.2	0.048 ± 0.02	0.035 ± 0.04
1000 µg mL ⁻¹	40	2.48 ± 0.2	4.39 ± 0.3	0.034 ± 0.02	0.028 ± 0.02

The herbicidal activity of crude metabolites derived from *Gibberella* species against *P. hystrophorus* showed a significant concentration-dependent inhibitory effect on seed germination and seedling growth parameters, as detailed in Table 2. In the untreated control group, seed germination reached 100%, with robust shoot and root development. The shoot length was recorded at 10.21 cm, root length at 14.30 cm and shoot and root weights at 0.0730 g and 0.092 g, respectively, indicating optimal growth conditions without the influence of the fungal metabolites. At the lowest

concentration of 10 µg/mL, germination reduced slightly to 87%, with notable decreases in shoot length (8.10 cm) and root length (5.97 cm). Corresponding biomass accumulation showed a reduction, with shoot and root weights were dropping to 0.0507 g and 0.0133 g, respectively, reflecting mild inhibitory effects at this concentration. A marked decline in growth was observed at 100 µg/mL, where germination dropped to 60%. Shoot and root lengths were significantly reduced to 5.30 cm and 4.14 cm, respectively, while shoot and root weights fell to 0.0302 g and 0.010 g, indicating substantial suppression of seedling development.

At 250 µg/mL, germination decreased further to 45%. Shoot and root lengths were severely impacted, measuring only 1.98 cm and 1.94 cm, respectively. The biomass also showed a dramatic reduction, with shoot weight at 0.020 g and root weight at 0.002 g, highlighting the strong phytotoxic effects at this concentration. The inhibitory effects were most pronounced at higher concentrations. At 500 µg/mL, germination was drastically reduced to 10%, with negligible shoot and root growth (0.10 cm each) and no detectable biomass. At the maximum concentration of 1000 µg/mL, germination was completely inhibited, with no observable shoot or root growth or biomass. These findings demonstrate that the crude metabolites of *Gibberella* species possess strong herbicidal potential, with complete inhibition of *P. hysterophorus* germination and growth at 1000 µg/mL.

Table 2. Weedicidal effect (Mean ± SD) of crude metabolites extracted from *Gibberella* species against *P. hysterophorus*

Treatments	Percent Germination	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)
Control	100	10.21 ± 1.0	14.30 ± 1.2	0.0730 ± 0.03	0.092 ± 0.02
10 µg µg mL ⁻¹	87	8.10 ± 1.0	5.97 ± 1.5	0.0507 ± 0.004	0.0133 ± 0.001
100 µg µg mL ⁻¹	60	5.30 ± 0.7	4.14 ± 1.0	0.0302 ± 0.001	0.010 ± 0.001
250 µg mL ⁻¹	45	1.98.57 ± 0.7	1.94 ± 0.5	0.020 ± 0.001	0.002
500 µg mL ⁻¹	10	0.10	0.10	0.00	0.00
1000 µg mL ⁻¹	0	0.00	0.00	0.00	0.00

The herbicidal effects of varying concentrations of 2, 4-D on *P. hysterophorus* showed a consistent and progressive inhibition of seed germination and seedling growth as the concentration increased (Table 3). To calculate the herbicidal effect of 2, 4-D on *P. hysterophorus* the same control group was used which was used for crude metabolites of *Alternaria* and *Gibberella* species. At the lowest concentration of 10 µg/mL, germination remained unaffected at 100%, but reductions in shoot and root lengths were observed, measuring 7.94 cm and 12.48 cm, respectively. Correspondingly, shoot

and root weights decreased to 0.0607 g and 0.068 g, indicating a mild inhibitory effect on seedling growth. A more pronounced inhibitory effect was seen at 100 µg/mL, where germination dropped to 90% and shoot and root lengths were further reduced to 6.77 cm and 11.92 cm, respectively. Biomass accumulation was also impacted, with shoot weight decreasing to 0.0492 g and root weight to 0.057 g, indicating the herbicide's stronger phytotoxic effects.

At 250 µg/mL, germination declined to 77%, with shoot and root lengths reduced to 5.95 cm and 10.44 cm. Biomass was significantly affected, with shoot weight falling to 0.0390 g and root weight to 0.033 g, highlighting the herbicide's increasing efficacy at this concentration. The effects became severe at 500 µg/mL, where germination dropped to 63%, with shoot and root lengths reduced to 3.82 cm and 8.37 cm, respectively. Shoot and root weights decreased to 0.0274 g and 0.028 g, indicating substantial suppression of growth. At the highest concentration of 1000 µg/mL, germination was reduced to 30%, with significant inhibition of shoot and root growth, measuring only 2.61 cm and 5.09 cm, respectively. Shoot weight dropped to 0.011 g, while root weight declined to 0.019 g, reflecting the herbicide's potent inhibitory effects at this level. These results confirm that 2, 4-D effectively inhibits the germination and growth of *P. hysterophorus* in a concentration-dependent manner. However, when compared to the crude metabolites of fungal species like *Alternaria* and *Gibberella*, 2, 4-D showed relatively milder effects on germination and early growth at comparable concentrations.

Table 3. Weedicidal effect (Mean ± SD) of 2, 4 D against *P. hysterophorus*

Treatments	Percent Germination	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)
Control	100	10.21 ± 1.0	14.30 ± 1.2	0.0730 ± 0.03	0.092 ± 0.02
10 µg µg mL ⁻¹	100	7.94 ± 1.0	12.48 ± 0.9	0.0607 ± 0.2	0.068 ± 0.003
100 µg µg mL ⁻¹	90	6.77 ± 0.5	11.92 ± 1.0	0.0492 ± 0.1	0.057 ± 0.001
250 µg mL ⁻¹	77	5.95 ± 1.3	10.44 ± 0.5	0.0390 ± 0.1	0.033 ± 0.001
500 µg mL ⁻¹	63	3.82 ± 1.4	8.37 ± 1.3	0.0274 ± 0.3	0.028 ± 0.003
1000 µg mL ⁻¹	30	2.61 ± 0.8	5.09 ± 1.0	0.011 ± 0.2	0.019 ± 0.002

4. DISCUSSION

Microscopic and morphological features are indispensable tools in fungal identification, providing critical insights into the unique traits that define fungal species. Microscopic analysis reveals diagnostic details such as spore shape, size, septation and the arrangement of conidiophores, which are essential for differentiating genera and species. Morphological observations, including colony texture, growth rate, pigmentation, and surface appearance on various culture media, further

complement these findings. Different media, such as potato dextrose agar (PDA), Malt Extract Agar (MEA) and Sabouraud dextrose agar (SDA) etc. enhance the accuracy of identification by promoting distinct growth patterns and sporulation. For example, PDA often induces vibrant pigmentation and abundant sporulation, while SDA highlights hyphal structures ²⁴. Together, these approaches form a reliable framework for identifying fungi, particularly in agricultural, clinical, and ecological contexts. Although molecular identification, such as sequencing the ITS region of fungal DNA, is considered the gold standard for precision, it is not without limitations. High costs, specialized laboratory requirements, and incomplete or misannotated reference databases can hinder its widespread application ^{25, 26}. In our study, traditional methods proved invaluable for accurately identifying *Alternaria* and *Gibberella* species. Key microscopic features, such as the muriform conidia with tapered ends in *Alternaria* and the curved ascospores in *Gibberella*, were instrumental in their identification. Additionally, growing these fungi on PDA and SDA revealed distinct pigmentation and colony textures, further supporting their classification.

The findings of this study highlight the promising herbicidal potential of fungal derived metabolites, particularly from *Gibberella* species, in suppressing the germination and growth of *P. hysterophorus*, a highly invasive and agriculturally damaging weed. These results add to the growing body of evidence supporting the use of fungal natural products as effective and environmentally friendly alternatives to conventional chemical herbicides. Fungal metabolites are increasingly recognized for their unique ability to synthesize diverse bioactive compounds with potent herbicidal properties. Numerous fungal species, including *Alternaria*, *Colletotrichum* and *Fusarium*, have demonstrated the capacity to produce secondary metabolites that selectively target weeds without harming non-target organisms or crops. The *Gibberella* species are known to synthesize compounds such as gibberellins and fusaric acid, which interfere with critical plant physiological pathways, including growth regulation and nutrient uptake ^{27, 28}. The strong inhibitory effects of *Gibberella* crude extracts observed in this study align with these findings, showcasing their potential to control *P. hysterophorus* effectively.

Chemical herbicides like 2, 4-D, though widely used for weed control, come with significant drawbacks. These include environmental concerns such as soil and water contamination, risks to human health, and the emergence of herbicide-resistant weed populations—an issue featured by the over 500 recorded cases of resistance globally ²⁹. Fungal bioherbicides offer a sustainable alternative, addressing these challenges with biodegradable, target-specific, and eco-friendly solutions. Different studies have demonstrated the success of fungal derived bioherbicides, such as *Alternaria cassiae* for *Cassia obtusifolia* and *Colletotrichum gloeosporioides* for broad-spectrum weed management ³⁰. These examples emphasize on the viability of fungal metabolites as practical

tools in integrated weed management systems. The metabolic versatility of fungi like *Gibberella* is pivotal to their role in natural product discovery. Secondary metabolites such as trichothecenes and polyketides produced by *Gibberella* have been shown to possess phytotoxic properties, disrupting processes like photosynthesis and cellular metabolism in weeds³¹.

In this study, the *Gibberella* derived extracts significantly reduced germination rates, shoot length, root length and biomass of *P. hysterophorus*, further affirming their herbicidal efficacy. These findings also emphasize the need for advanced studies to isolate and characterize the active components responsible for this activity, potentially leading to novel and more effective bioherbicidal formulations. Advancements in fungal metabolomics, including tools like liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) spectroscopy are revolutionizing the identification and optimization of fungal herbicidal compounds³². While this study focused on crude extracts, it provides a critical foundation for further research into purifying and optimizing these compounds for large-scale application. The integration of such natural herbicides into weed management strategies could alleviate the environmental and health risks associated with synthetic herbicides, offering a sustainable path forward in agricultural productivity. The herbicidal potential of fungal metabolites, as demonstrated by *Gibberella* species in this study, highlights a viable and sustainable alternative to chemical herbicides like 2, 4-D. By leveraging the metabolic capabilities of fungi, researchers can develop innovative, eco-friendly solutions to combat invasive weeds like *P. hysterophorus*, ultimately contributing to more resilient and sustainable agricultural systems.

5. CONCLUSIONS AND RECOMMENDATIONS

This study demonstrated that crude metabolites from *Gibberella* species exhibit stronger herbicidal potential compared to *Alternaria* species and the chemical herbicide 2, 4-D, effectively inhibiting the seed germination and seedling growth of *P. hysterophorus*. The complete inhibition observed at higher concentrations highlights the promising potential of fungal metabolites as environmentally friendly alternatives to synthetic herbicides. These findings pave the way for further exploration, emphasizing the need for purification and structural characterization of the active compounds to better understand their mode of action. Additionally, future research should focus on scaling up production and conducting field trials to evaluate their real-world effectiveness and environmental impact, ultimately contributing to the development of sustainable bioherbicides for integrated weed management.

6. Limitations of the Study

This study provides valuable insights into the herbicidal potential of fungal metabolites; however, certain limitations must be acknowledged. The experiments were conducted under controlled

laboratory conditions, which may not fully represent the complexities of field environments, such as variable soil composition, climatic fluctuations, and interactions with other plant species and microorganisms. Additionally, the crude extracts used in this study contain a mixture of compounds, making it challenging to pinpoint the exact bioactive components responsible for the observed herbicidal effects. Further research involving the purification and structural characterization of these metabolites is necessary to better understand their mechanism of action and potential selectivity against target weeds. The potential environmental impact and safety of these fungal metabolites on non-target plants and beneficial organisms also need to be thoroughly evaluated before practical application.

Scaling Up for Field Application

To transition from laboratory findings to practical field applications, several steps must be undertaken. First, large-scale production of the bioactive metabolites through optimized fermentation processes will be essential to ensure cost-effectiveness and sustainability. Field trials in diverse agricultural settings are required to assess the efficacy of these fungal metabolites under real-world conditions, taking into account environmental factors such as soil health, temperature variations, and competition with native flora. Additionally, formulation development, including the incorporation of these metabolites into biodegradable carriers or encapsulation techniques, can enhance their stability, efficacy, and ease of application in agricultural systems. Collaborations with agronomists and farmers can provide valuable insights into practical implementation strategies and regulatory compliance to facilitate the adoption of these eco-friendly bioherbicides.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this manuscript.

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