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ASSESSMENT OF CRUDE METABOLITE PRODUCTION BY WEED PATHOGENIC FUNGI CULTURED ON SYNTHETIC AND NATURAL MEDIA: IMPLICATIONS FOR MYCOHERBICIDE DEVELOPMENT

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ABSTRACT

The increasing prevalence of herbicide-resistant weeds, coupled with growing environmental and regulatory concerns associated with synthetic herbicides, has intensified the search for sustainable alternatives for weed management. Weed pathogenic fungi represent a promising source of biologically active phytotoxins that can be exploited for the development of metabolite-based mycoherbicide. The present study investigated the production of herbicidal secondary metabolites by four weed pathogenic fungi, *Alternaria alternata*, *Fusarium oxysporum*, *Curvularia lunata*, and *Phoma herbarum*, cultured on various synthetic and natural substrates. Fungal isolates were grown on Potato Dextrose Broth (PDB), Czapek Dox Broth (CDB), Richard's Broth (RB), selected cereal grains, and natural liquid media supplemented with either yeast extract or weed tissue homogenate. Following incubation, crude metabolites were extracted using ethyl acetate and quantified gravimetrically. Significant differences in metabolite production were observed among fungal species, culture media, and supplementation treatments. Among the synthetic media, Richard's Broth supported the highest metabolite production, whereas among natural substrates, red rice, broken sorghum, and sugarcane juice proved particularly favorable. The highest crude metabolite yield was obtained from *Fusarium oxysporum* cultured in sugarcane juice supplemented with weed tissue homogenate. Preliminary phytotoxicity assays demonstrated that the crude extracts induced chlorosis, necrosis, and tissue damage in target weed species, indicating the presence of biologically active herbicidal compounds. The findings suggest

that inexpensive agricultural substrates can substantially enhance fungal metabolite production and provide a cost-effective platform for large-scale production of phytotoxins. These results highlight the potential of weed pathogenic fungi as sources of natural herbicidal compounds and support further research on metabolite characterization, formulation development, and field evaluation for sustainable mycoherbicide development.

KEYWORDS: Fungal phytotoxins, herbicidal metabolites, Mycoherbicide, secondary metabolites, weed pathogenic fungi.

1.INTRODUCTION

Weeds are among the most significant biological constraints limiting agricultural productivity worldwide. It has been estimated that uncontrolled weeds can reduce crop yields by 30–45%, depending on crop species, weed flora, and environmental conditions (Oerke 2006). Chemical herbicides have traditionally been the primary means of weed control because of their effectiveness and ease of application. However, excessive dependence on herbicides has resulted in the evolution of herbicide-resistant weeds, environmental contamination, non-target effects, and concerns regarding human and animal health (Heap 2024).

Biological weed control offers a sustainable alternative to chemical weed management. Among biological approaches, weed pathogenic fungi have received considerable attention because of their ability to infect, colonize, and suppress target weeds under natural conditions (Charudattan 2001). Several fungal genera, including *Alternaria, Fusarium, Curvularia, Phoma, Colletotrichum, Myrothecium*, and *Bipolaris*, have demonstrated potential as biological control agents against economically important weeds (Boyette et al. 2018).

Apart from direct infection, many weed pathogenic fungi produce a wide array of secondary metabolites that exhibit phytotoxic activity. These metabolites can induce chlorosis, necrosis, inhibition of photosynthesis, membrane disruption, oxidative stress, and growth suppression in susceptible plants (Evidente et al. 2006; Dayan and Duke 2014). Such compounds have attracted considerable interest as potential sources of natural herbicides because they often possess unique modes of action and favorable environmental characteristics.

Several fungal phytotoxins including tentoxin from *Alternaria alternata*, fusaric acid from *Fusarium* spp., curvularin from *Curvularia lunata*, and various polyketides produced by *Phoma* species have demonstrated herbicidal activity against broadleaf and grassy weeds (Abbas and Duke 1995; Evidente et al. 2006). Commercial exploitation of these compounds

requires efficient production systems capable of generating high metabolite yields at low cost.

The nutritional composition of culture media is one of the most important factors influencing fungal growth and secondary metabolite biosynthesis. Carbon sources, nitrogen sources, micronutrients, host-derived compounds, and environmental conditions can profoundly affect metabolite production (Keller et al. 2005). Identification of inexpensive and readily available substrates capable of supporting high metabolite yields is therefore essential for commercial-scale production of fungal phytotoxins.

Natural agricultural substrates such as cereal grains, sugarcane juice, and plant extracts offer attractive alternatives to expensive synthetic media because of their low cost and nutrient richness. Furthermore, supplementation with weed tissue homogenates may mimic host–pathogen interactions and stimulate production of phytotoxic metabolites.

The present investigation was undertaken to evaluate crude metabolite extract yield by selected weed pathogenic fungi cultured on various synthetic and natural media. The specific objectives were to identify suitable low-cost substrates for metabolite production and to assess the influence of nutritional supplements on crude metabolite recovery for future mycoherbicide development.

2. MATERIALS AND METHODS

2.1 Study Location

The study was conducted during 2002–2006 in the Mycological Research Laboratory, Department of Biological Sciences, Rani Durgawati University, Jabalpur, Madhya Pradesh, India. All experiments were performed under controlled laboratory conditions at 25 ± 2°C with a relative humidity of 70–80%.

2.2 Fungal Cultures

Four weed pathogenic fungi known for their phytopathogenic and phytotoxic potential were used in the present investigation:*Alternaria alternata, Fusarium oxysporum, Curvularia lunata* and *Phoma herbarum*. Pure cultures were obtained from the culture collection of the Mycological Research Laboratory and maintained on Potato Dextrose Agar (PDA) slants at 4°C. Active cultures were sub-cultured periodically on fresh PDA plates and incubated at 25 ± 2°C for 7–10 days before experimental use following standard mycological procedures (Booth 1971; Barnett and Hunter 1998).

2.3 Preparation of Synthetic Culture Media

Four synthetic liquid media commonly employed for fungal growth and crude metabolite extract yield were evaluated.

2.3.1 Potato Dextrose Broth (PDB)

Potato Dextrose Broth was prepared by boiling 200 g peeled potato tubers in 1 L distilled waterfor 30 min. The extract was filtered through muslin cloth and supplemented with 20 g dextrose. The final volume was adjusted to 1 L and pH was maintained at 6.5.

2.3.2 Czapek Dox Broth (CDB)

Czapek Dox Broth was prepared using Sucrose – 30 g; Sodium nitrate – 2 g; Dipotassium phosphate – 1 g; Magnesium sulphate – 0.5 g; Potassium chloride – 0.5 g; Ferrous sulphate – 0.01 g and Distilled water – 1000 ml

2.3.3 Richard's Broth (RB)

Richard's Broth was prepared using Sucrose – 50 g; Potassium nitrate – 10 g; Potassium dihydrogen phosphate – 5 g; Magnesium sulphate – 2.5 g; Ferric chloride – 0.02 g and Distilled water – 1000 ml.

2.4 Preparation of Natural Solid Substrates

2.4.1 Cereal Grain Substrates

Five cereal substrates were selected Whole rice; Broken rice; Red rice; Broken sorghum; Broken maize. Fifty grams of each substrate were soaked overnight, drained, and transferred to autoclavable polypropylene bags.

2.5 Preparation of Natural Liquid Media

Three natural liquid media were evaluated:

2.5.1 Sugarcane Juice

Fresh sugarcane juice was extracted, filtered through muslin cloth and sterilized.

2.6 Preparation of Weed Tissue Homogenate

Fresh tissues of Lantana (*Lantana camara*) and parthenium (*Partheniumhysterophorus*) were collected from naturally infested areas.The plant material was washed thoroughly and surface sterilized using 1% sodium hypochlorite for 2 min followed by three rinses with sterile distilled water.Fifty grams of fresh weed tissue were homogenized with 50 ml sterile distilled water using a laboratory blender. The homogenate was filtered through double-layered muslin cloth and stored at 4°C until use.Host-derived tissue homogenates have been reported to stimulate production of phytotoxic metabolites in plant pathogenic fungi (Abbas and Duke 1995; Evidente et al. 2006).

2.7 Supplementation Treatments

Each medium received one of the following treatments:Control (without supplementation); Yeast extract (5% v/v); Weed tissue homogenate (5% v/v).

The supplements were added aseptically after autoclaving and cooling.Nutritional factors and host-derived compounds are known to regulate fungal secondary metabolism and toxin biosynthesis (Calvo et al. 2002; Keller et al. 2005).

2.8 Experimental Design and Inoculation

Twenty-five millilitres of liquid media or fifty grams of solid substrates were transferred intosterile culture bottles or polypropylene bags and sterilized at 121°C under 15 psi pressure for 20 min.

Each treatment was inoculated with 1 ml conidial suspension containing approximately 1×10^7spores/ml obtained from actively growing fungal cultures.The inoculated cultures were incubated at Temperature: 25 ± 2°C; Relative humidity: 70–80%; Photoperiod: 12 h light: 12 h dark. The experiment was conducted in a Completely Randomized Design (CRD) with three replications.

2.9 Extraction of Secondary Metabolites

After 7 days of incubation, fungal biomass together with the culture substrate was subjected tosolvent extraction.

The extraction procedure consisted ofMixing fungal biomass and substrate with ethyl acetate in a ratio of 1:2.5 (w/v); Incubation at room temperature for 24 h; Filtration through Whatman No. 1 filter paper; Collection of filtrates in sterile conical flasks; Solvent evaporation under reduced pressure using a rotary evaporator at 40°C; Recovery of crude metabolite residue.

Ethyl acetate was selected because of its efficiency in extracting a broad spectrum of fungalsecondary metabolites including phytotoxins, polyketides, and organic acids (Strobel and Daisy 2003).

2.10Determination of Crude Metabolite Yield

The crude metabolite yield was determined gravimetrically.The quantity of metabolite obtained was calculated as:

Crude metabolite yield (g) = Final weight of dried extract − Weight of empty collection vessel

Results were expressed as:g metabolite per 25 ml liquid medium; g metabolite per 50 g solid substrate. The present study quantified crude metabolite extracts and did not identify individual phytotoxic compounds.

2.11 Preliminary Phytotoxicity Screening

The herbicidal potential of crude fungal metabolites was evaluated using detached leaf bioassays against three economically important invasive weeds, namely *Parthenium hysterophorus* L., *Lantana camara* L., and *Hyptis suaveolens* (L.) Poit. Healthy, fully expanded young leaves were collected from greenhouse-grown plants and thoroughly washed with sterile distilled water prior to bioassay.

Crude metabolite extracts obtained from each fungal treatment were dissolved in methanol to prepare a stock solution of 10 mg/ml. Twenty microlitres of the extract were applied uniformly into the adaxial surface of detached leaves using a micropipette. Leaves treated with methanol alone served as the control. The treated leaves were placed in sterile Petri dishes lined with moistened filter paper and incubated in moist chambers at 25 ± 2°C under a 12 h light:12 h dark photoperiod.

Observations were recorded at 24, 48, and 72 h after treatment for the development of phytotoxic symptoms. The following parameters were assessedChlorosis (%) indicates the proportion of leaf area showing yellowing; Necrosis (%) indicates the proportion of leaf area showing dead tissue); Lesion diameter (mm); Tissue collapse index; Visual phytotoxicity rating (0–10 scale), where 0 = no visible injury and 10 = complete tissue death. Phytotoxicity ratings were assigned based on symptom severity, including chlorosis, necrosis, tissue desiccation, and lesion expansion. Percentage chlorosis and necrosis were estimated visually using a standardized injury assessment scale. The assay was conducted with three replications per treatment.

Detached leaf bioassays were performed following standard procedures used for evaluating phytotoxic fungal metabolites and bioherbicidal compounds (Dayan et al. 2000; Duke et al. 2002; Abbas and Duke 1995). The results were used to identify metabolite extracts with the greatest herbicidal potential for subsequent characterization and bioherbicide development studies.

2.12Experimental Workflow

Isolation and Maintenance of Weed Pathogenic Fungi→Preparation of Synthetic and Natural Media→Addition of Yeast Extract or Weed Tissue Homogenate→Fungal Inoculation →Incubation for 7 Days→Ethyl Acetate Extraction→Solvent Evaporation→Crude Metabolite Recovery→Yield Determination→Phytotoxicity Screening→Analysis→

3. RESULTS

3.1 Crude metabolite extract yield on Synthetic Liquid Media

Significant differences were observed in crude metabolite production among the four weed pathogenic fungi cultured on different synthetic liquid media (Table 1). Among the tested media, Richard's Broth (RB) supported comparatively higher metabolite production than Potato Dextrose Broth (PDB) and Czapek Dox Broth (CDB).*Fusarium oxysporum* produced the highest metabolite yield in Richard's Broth (0.418 g/25 ml). *Curvularia lunata* and *Phoma herbarum* produced comparatively lower quantities of metabolites across all synthetic media.Overall, broth media rich in carbohydrates and complex nutrients promoted greater metaboliteaccumulation than chemically defined media.

Table 1. Crude metabolite extract yield of weed pathogenic fungi cultured on syntheticliquid media.

Medium	**Weed pathogenic Fungal strains**			
	Alternaria alternata	***Fusarium oxysporum***	***Curvularia lunata***	***Phoma herbarum***
PDB	0.215	0.286	0.154	0.132
CDB	0.184	0.248	0.139	0.118
Richard's Broth	0.354	0.418	0.281	0.236

Values expressed as g crude metabolite per 25 ml medium.

3.2 Influence of Cereal Grain Substrates on Metabolite Production

Among cereal grain substrates, red rice and broken sorghum significantly enhanced metabolite production (Table 2). Red rice supported the highest metabolite yield for *Alternaria alternata* (1.412 g/50 g), whereas broken sorghum supported maximum metabolite production by *Fusarium oxysporum* (1.685 g/50 g).

Supplementation with yeast extract significantly improved metabolite recovery compared with untreated controls.

Table 2. Influence of cereal grain substrates on crude metabolite production.

Substrate	**Weed pathogenic Fungal strains**			
	Alternaria alternata	***Fusarium oxysporum***	***Curvularia lunata***	***Phoma herbarum***
Whole rice	0.786	0.924	0.588	0.465
Broken rice	0.932	1.086	0.713	0.548
Red rice	1.412	1.521	0.998	0.815
Broken maize	0.765	0.882	0.632	0.494
Broken sorghum	1.325	1.685	1.045	0.864

Values expressed as g crude metabolite per 50 g substrate.

3.3 Influence of Natural Liquid Media on Metabolite Production

Natural liquid media significantly influenced metabolite production (Table 3). Sugarcane juice proved to be the most productive medium for all fungal species.

The highest metabolite yield was recorded in *Fusarium oxysporum* grown in sugarcane juice supplemented with weed tissue homogenate (3.842 g/25 ml), followed by *Alternaria alternata* (3.465 g/25 ml).

Table 3. Influence of natural liquid media on crude metabolite production.

Medium	Weed pathogenic Fungal strains			
	Alternaria alternata	***Fusarium oxysporum***	***Curvularia lunata***	***Phoma herbarum***
Sugarcane juice	3.465	3.842	2.984	2.214

Values expressed as g crude metabolite per 25 ml medium.

3.4 Preliminary Phytotoxic Activity of Crude Metabolites

Detached leaf bioassays revealed marked differences in phytotoxic activity among fungal extracts (Table 4). Metabolites obtained from *Fusarium oxysporum* cultured in sugarcane juice supplemented with weed tissue homogenate caused the highest necrosis (86.4%) and chlorosis (92.8%) in *Parthenium hysterophorus* leaves after 72 h.

Similarly, metabolites from *Alternaria alternata* induced extensive chlorosis and tissue collapse in *Lantana camara*leaves. Extracts obtained from *Curvularia lunata* and *Phoma herbarum* showed moderate phytotoxic effects.

Table 4. Phytotoxicity of crude fungal metabolites against target weeds.

Fungal species	Target weed	Chlorosis (%)	Necrosis (%)	Lesion diameter (mm)
Alternaria alternata	*Lantana camara*	88.2	79.5	14.6
Alternaria alternata	*Parthenium hysterophorus*	84.6	76.8	13.8
Fusarium oxysporum	*Parthenium hysterophorus*	92.8	86.4	17.2
Fusarium oxysporum	*Hyptis suaveolens*	87.6	82.3	15.4
Curvularia lunata	*Parthenium hysterophorus*	68.4	61.8	10.2
Phoma herbarum	*Lantana camara*	63.5	58.6	9.4

Summary of Major Findings

1. Richard's Broth supported superior metabolite production among synthetic media.
2. Red rice and broken sorghum were the most effective cereal substrates.
3. Sugarcane juice was the most productive natural liquid medium.
4. *Fusarium oxysporum* produced the highest quantity of phytotoxic metabolites.
5. Crude metabolites exhibited strong phytotoxic activity against *Parthenium hysterophorus*and *Lantana camara* indicating potential for bioherbicide development.

4. DISCUSSION

The present study demonstrated that culture medium composition exerts a profound influence on crude metabolite extract yield by weed pathogenic fungi. Significant variation in crude metabolite yield was observed among fungal species, substrate types, and nutritional supplements, indicating that both fungal genetics and environmental factors regulate biosynthesis of phytotoxic metabolites. These findings are consistent with previous reports showing that carbon sources, nitrogen availability, micronutrients, and host-derived compounds are major determinants of fungal secondary metabolism (Calvo et al. 2002; Keller et al. 2005; Brakhage 2013).

Secondary metabolites produced by plant pathogenic fungi have attracted considerable attention as potential bioherbicides because many of these compounds possess unique modes of action and exhibit selective phytotoxicity against target weeds (Abbas and Duke 1995; Evidente et al. 2006). Unlike synthetic herbicides, fungal metabolites are biodegradable and generally pose lower environmental risks, making them attractive candidates for sustainable weed management (Dayan and Duke 2014). The present investigation therefore focused on identifying low-cost substrates capable of supporting enhanced metabolite production for future development of metabolite-based mycoherbicides.

4.1 Effect of Synthetic Media on Crude metabolite extract yield

Among the synthetic media evaluated, Richard's Broth supported significantly higher metabolite production than Potato Dextrose Broth and Czapek Dox Broth. These findings suggest that complex carbon and nitrogen sources promote secondary metabolism more effectively than chemically defined media.

Richard's Broth contains high concentrations of sucrose and nitrate nitrogen, which are known to influence fungal secondary metabolite biosynthesis. Carbon-rich media often stimulate accumulation of polyketides, non-ribosomal peptides, and host-specific toxins during the stationary growth phase (Keller et al. 2005).

Among the fungi evaluated, *Fusarium oxysporum* consistently produced the highest metabolite yield. Species of *Fusarium* are well known for producing diverse phytotoxic compounds including fusaric acid, beauvericin, moniliformin, fumonisins, and enniatins (Bacon et al. 1996; Desjardins 2006). Many of these compounds interfere with cellular metabolism, ion transport, and oxidative processes, ultimately causing plant injury and growth suppression.

Previous studies have shown that *Alternaria* species produce a variety of phytotoxins including tentoxin, tenuazonic acid, alternariol, and alternariol monomethyl ether, which contribute significantly to pathogenicity and host damage (Tsuge et al. 2013; Meena and Samal 2019).

4.2 Influence of Cereal Grain Substrates

Among cereal substrates, red rice and broken sorghum supported significantly greater metabolite production than other grains. These substrates are rich in carbohydrates, proteins, minerals, and vitamins, which may provide favorable nutritional conditions for fungal growth and secondary metabolism.

Secondary metabolite biosynthesis is often triggered under nutrient-rich conditions followed by nutrient limitation during the stationary phase of fungal growth (Calvo et al. 2002). The high starch content of red rice and sorghum likely supported rapid biomass accumulation, thereby creating physiological conditions favorable for metabolite production.

Agricultural grains have been widely used for production of fungal inoculum and metabolites because of their low cost and high nutrient content (Pandey and Soccol 2000). Similar observations have been reported for production of destruxins, cytochalasins, and other fungal metabolites on cereal-based substrates (Jackson and Schisler 1995).

The superior performance of red rice may also be attributed to its higher levels of micronutrients and phenolic compounds, which can influence fungal metabolic pathways. Likewise, sorghum contains substantial quantities of carbohydrates and trace elements that may serve as precursors for biosynthesis of secondary metabolites.

From a commercialization perspective, cereal grains offer significant advantages because they are inexpensive, readily available, and suitable for large-scale solid-state fermentation systems. Their use could substantially reduce production costs associated with mycoherbicide manufacturing.

4.3 Influence of Natural Liquid Media

Natural liquid media exhibited marked effects on metabolite production. Sugarcane juice emerged as the most productive substrate for all fungal species evaluated. This medium

supported metabolite yields several-fold higher than those obtained with synthetic media.Sugarcane juice contains high concentrations of sucrose, glucose, fructose, minerals, vitamins, and amino acids that collectively support vigorous fungal growth and secondary metabolism. The high metabolite production observed in sugarcane juice agrees with previous studies reporting enhanced microbial metabolite production in sugar-rich natural substrates (Pandey et al. 2000).

The exceptionally high metabolite yield recorded for *Fusarium oxysporum* in sugarcane juice supplemented with weed tissue homogenate suggests a synergistic effect between nutrient availability and host-derived signals. Such interactions may activate silent biosynthetic gene clusters responsible for phytotoxin production.

4.4 Phytotoxic Activity of Crude Metabolites

The detached leaf bioassays clearly demonstrated that crude metabolites obtained from weed pathogenic fungi possess significant phytotoxic activity. Extracts from *Fusarium oxysporum* produced the highest levels of chlorosis, necrosis, and tissue collapse in *Parthenium hysterophorus*, whereas metabolites from *Alternaria alternata* showed strong activity against *Lantana camara*.The phytotoxic symptoms observed are characteristic of fungal toxins that interfere with photosynthesis, membrane integrity, and cellular respiration (Abbas and Duke 1995; Dayan and Duke 2014). Chlorosis generally results from degradation of chlorophyll and disruption of photosynthetic pathways, while necrosis indicates irreversible cellular damage and tissue death.

Several metabolites produced by *Fusarium* species, including fusaric acid and beauvericin, are known to disrupt ion transport, oxidative metabolism, and membrane function (Bacon et al. 1996). Similarly, tentoxin and tenuazonic acid produced by *Alternaria* species inhibit chloroplast function and protein synthesis, resulting in chlorosis and growth inhibition (Tsuge et al. 2013).

The substantial phytotoxicity observed in the present study supports the hypothesis that secondary metabolites contribute significantly to the weed suppressive potential of plant pathogenic fungi and may represent promising candidates for development of metabolite-based bioherbicides.

4.5 Implications for Mycoherbicide Development

The growing incidence of herbicide-resistant weeds has intensified the search for alternative weed management technologies. Fungal metabolites offer several advantages over conventional herbicides, including novel modes of action, biodegradability, environmental compatibility, and potential species specificity (Dayan and Duke 2014).

The present findings demonstrate that inexpensive agricultural substrates can support substantial metabolite production by weed pathogenic fungi. Sugarcane juice, red rice, and sorghum appear particularly promising for commercial-scale production systems.

Future investigations should focus on purification and structural characterization of the active metabolites using chromatographic and spectroscopic techniques such as HPLC, LC-MS/MS, GC-MS, FTIR, and NMR spectroscopy. Identification of the compounds responsible for phytotoxic activity will facilitate optimization of production systems and formulation development.

Furthermore, greenhouse and field studies are necessary to determine efficacy against target weeds, crop selectivity, environmental fate, and integration into weed management programs. Advances in formulation technology, nanodelivery systems, and fermentation engineering may further enhance the commercial potential of fungal metabolite-based bioherbicides.

Overall, the results indicate that weed pathogenic fungi represent valuable sources of bioactive metabolites and that optimization of culture media offers an effective strategy for increasing production of phytotoxic compounds intended for sustainable weed management.

5. CONCLUSION

Weed pathogenic fungi represent an important and underexploited source of phytotoxic secondary metabolites with considerable potential for sustainable weed management. The present study demonstrated that culture medium composition significantly influences metabolite production by *Alternaria alternata*, *Fusarium oxysporum*, *Curvularia lunata*, and *Phoma herbarum*. Both fungal species and substrate type played crucial roles in determining metabolite yield and phytotoxic activity.

Among the synthetic media evaluated, Richard's Broth supported superior metabolite production. However, natural substrates proved more effective and economically attractive for metabolite biosynthesis. Red rice and broken sorghum among cereal substrates, and sugarcane juice among natural liquid media supported significantly greater metabolite production. Supplementation with weed tissue homogenate further enhanced metabolite yield, indicating that host-derived compounds may function as elicitors of phytotoxin biosynthesis.Among the fungal species studied, *Fusarium oxysporum* consistently produced the highest quantity of crude metabolites and exhibited the greatest phytotoxic activity against *Parthenium hysterophorus*, while *Alternaria alternata* demonstrated strong activity against *Lantana camara*. Crude extracts induced pronounced chlorosis, necrosis, tissue

collapse, and lesion formation, confirming the presence of biologically active phytotoxic compounds.

The findings clearly demonstrate that inexpensive agricultural substrates can be successfully utilized for cost-effective production of fungal secondary metabolites. Such substrates offer practical advantages for large-scale fermentation and may substantially reduce production costs associated with metabolite-based bioherbicides.

Although the present investigation focused on crude metabolite production and preliminary phytotoxicity assessment, the results provide a strong foundation for future development of fungal metabolite-based weed management technologies.

Future research should focus on:

1. Isolation and purification of active phytotoxic compounds.
2. Chemical characterization using HPLC, LC-MS/MS, GC-MS, FTIR, and NMR techniques.
3. Elucidation of mechanisms of action and molecular targets in weeds.
4. Development of stable formulations for field application.
5. Greenhouse and field evaluation against invasive and problematic weeds.
6. Assessment of crop selectivity, environmental fate, and non-target safety.
7. Scale-up fermentation and commercialization studies.

In conclusion, the study confirms that weed pathogenic fungi are valuable producers ofphytotoxic secondary metabolites and that optimization of culture media represents an effectivestrategy for enhancing metabolite production. These findings support the development ofenvironmentally compatible metabolite-based mycoherbicides as promising alternatives toconventional chemical herbicides and contribute to the advancement of sustainable weedmanagement systems.

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