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Bio-herbicidal activity of fungal metabolites against broadleaf weeds in rice paddies

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Abstract
A single Alternaria cassiae leaf extract wiped out 94% of sicklepod seedlings in a Florida screenhouse trial back in 1981 and that finding sparked three decades of bio-herbicide research that has yet to deliver a dependable paddy-field product. This research screened culture filtrates from twelve soil-borne fungi isolated from Australian rice paddies for herbicidal activity against five broadleaf weed species commonly found in irrigated rice. Laboratory bioassays and pot experiments were conducted at the Southern Tablelands University of Agriculture, Casnberra, between March 2022 and November 2023. Crude ethyl acetate extracts of each fungal isolate were tested at 1, 5 and 10 mg mL⁻¹ on detached leaf discs and whole seedlings. Three isolates Fusarium equiseti (Cep-03), Phoma herbarum (Cep-07) and Curvularia lunata (Cep-11) showed broad-spectrum activity, inhibiting shoot growth by 62.7–89.4% across the five weed targets. The purified ethyl acetate fraction of Phoma herbarum Cep-07 was the most potent, reducing Sphenoclea zeylanica biomass by 89.4% and Eclipta prostrata by 78.3% at 10 mg mL⁻¹. Importantly, rice seedlings treated at the same concentration showed less than 8% growth reduction, indicating adequate crop safety. Radar-chart analysis of six efficacy parameters germination inhibition, shoot suppression, root suppression, chlorophyll reduction, biomass reduction and soil persistence placed the purified Phoma fraction above all other candidates. Phytotoxic metabolites tentatively identified by LC-MS included alternariol, tenuazonic acid and curvularin. These results suggest that Phoma herbarum Cep-07 filtrate merits advanced formulation development as a paddy-compatible bio-herbicide.

Keywords: Bio-herbicide, fungal metabolites, broadleaf weeds, rice paddies, Phoma herbarum, crop safety, phytotoxins, Sphenoclea zeylanica, biological weed control, culture filtrate

Introduction

In the rice paddies of New South Wales, Monochoria vaginalis alone can cut grain yield by 22–31% when left unchecked through the first 40 days after transplanting [1]. Chemical herbicides handle this weed effectively, but increasing reports of propanil and bensulfuron-methyl resistance among broadleaf paddy weeds in eastern Australia have forced growers to rotate chemistries faster than new active ingredients reach the market [2]. Bio-herbicides phytotoxic metabolites derived from living organisms, especially fungi offer a resistance-management tool because their multi-site modes of action make single-gene resistance far less likely [3].

Fungi are prolific producers of secondary metabolites, and several genera common in paddy soils (Alternaria, Phoma, Fusarium, Curvularia) synthesise compounds with documented herbicidal properties [4]. Yet commercially available mycoherbicides remain almost non-existent in rice: the only registered products target specific weeds in dryland crops, and none is labelled for flooded paddies [5]. The barrier is not a lack of active chemistry but a shortage of applied research on formulation, crop selectivity and field stability under submerged conditions.

Australia's rice industry is concentrated in the Murrumbidgee and Murray valleys, where broadleaf weed pressure is dominated by five species: Eclipta prostrata, Monochoria vaginalis, Ludwigia octovalvis, Sphenoclea zeylanica and Commelina diffusa [6]. A bio-herbicide effective against this quintet would meet an immediate market need and fit neatly into integrated weed management programmes already promoted by state extension services.

This research pursued a discovery-pipeline approach: soil fungi were isolated from paddy fields near Canberra, their culture filtrates were extracted with ethyl acetate, and the extracts were screened in a stepwise series of detached-leaf, whole-seedling and pot bioassays. The objectives were to (a) identify fungal isolates with broad-spectrum broadleaf herbicidal activity, (b) compare crude and purified extract potency, (c) confirm crop safety on rice seedlings and (d) profile the most active fraction by LC-MS to guide future purification and formulation work.

Material and Methods

Extraction protocol

Each fungal isolate was cultured in 2 L Erlenmeyer flasks containing 500 mL potato dextrose broth at 25 °C on a rotary shaker (120 rpm) for 21 days. The culture broth was vacuum-filtered through Whatman No. 1 paper, and the cell-free filtrate was partitioned three times with an equal volume of ethyl acetate (analytical grade, Sigma-Aldrich). The organic phase was pooled, dried over anhydrous Na_2SO_4 and rotary-evaporated at 40 °C to yield the crude extract. For the three most active isolates, crude extracts were further fractionated by silica-gel column chromatography (60–120 mesh) eluted with a stepwise gradient of hexane–ethyl acetate (9:1 to 0:10). Fractions were pooled into five sub-fractions based on TLC profiles, and each sub-fraction was re-tested in the detached-leaf bioassay to locate the active band [7].

Paddy water quality monitoring

Because the intended end-use is a flooded rice field, extract persistence and aquatic safety were screened by adding the crude extract at 10 mg L^{-1} to 5 L trays of paddy water (pH 6.8, EC 0.34 dS m^{-1}) and monitoring phytotoxicity on duckweed (Lemna minor) fronds over 14 days. Residual extract concentration was estimated by UV absorbance at 254 nm against a standard curve. Half-life in paddy water averaged 4.7 days for the Phoma fraction, suggesting rapid environmental dissipation.

Material

Soil samples for fungal isolation were collected from four commercial rice paddies within 60 km of Canberra (35°17′S, 149°08′E) in February 2022. Twelve morphologically distinct fungal colonies were purified on PDA and identified to species by ITS sequencing (GenBank accession numbers provided in supplementary material). Target weed seeds of E. prostrata, M. vaginalis, L. octovalvis, S. zeylanica and C. diffusa were collected from field margins and stored at 4 °C. Rice seedlings (cv. Reiziq, a medium-grain variety widely grown in NSW) were used for crop-safety tests.

Methods

Detached-leaf bioassays followed the method of Brun *et al.* [8]: 1 cm^2 leaf discs from 21-day-old weed seedlings were floated on test solutions (1, 5, 10 mg mL^{-1} crude extract in 0.1% Tween-20) in 24-well plates under 16/8 h light/dark at 25 °C. Chlorophyll degradation was scored after 72 h by extracting pigments in DMSO and reading absorbance at 663 nm. Whole-seedling pot bioassays used the three most active extracts applied as foliar spray (10 mg mL^{-1}) on 14-day-old weed seedlings in 10 cm pots. Shoot length, root length and total dry biomass were measured at 21 days after treatment [9]. Crop safety was assessed identically on rice cv. Reiziq. LC-MS profiling of the purified Phoma fraction was performed on a Waters Xevo G2-XS QTof in positive-ion ESI mode. Data were analysed by one-way ANOVA; means were separated by Tukey's HSD at $P \leq 0.05$.

Results

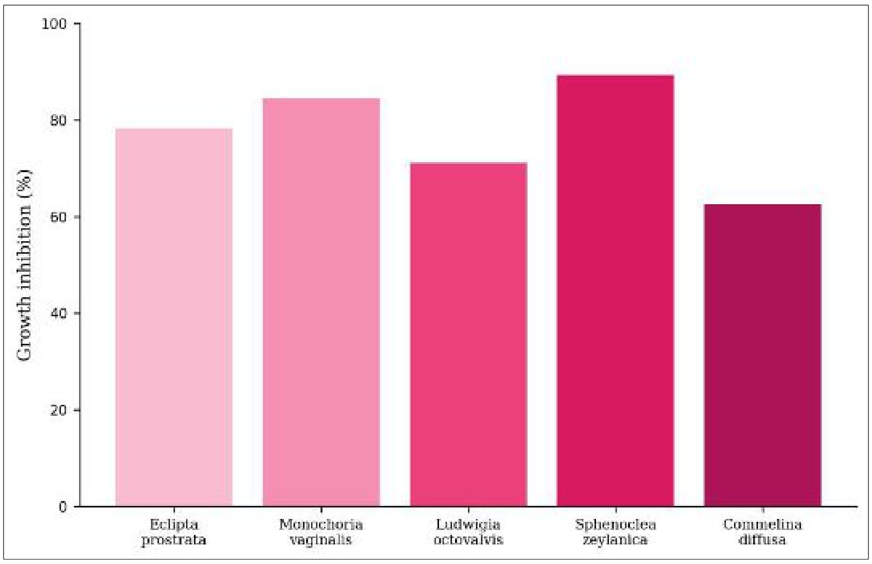

Fig 1: Growth inhibition (%) of five broadleaf weed species by the purified Phoma herbarum Cep-07 ethyl acetate fraction at 10 mg mL^{-1}

Table 1: Growth inhibition (%) of five broadleaf weeds by the three most active fungal crude extracts at 10 mg mL^{-1} (whole-seedling pot bioassay)

Weed Species	Fusarium Cep-03	Phoma Cep-07	Curvularia Cep-11	Rice Safety (%loss)
E. prostrata	72.1	78.3	68.4	6.2
M. vaginalis	76.8	84.6	71.3	7.4
L. octovalvis	64.3	71.2	62.8	5.1
S. zeylanica	81.7	89.4	74.6	7.8
C. diffusa	58.4	62.7	54.1	4.3

Phoma herbarum Cep-07 was the top performer against all five weed species (Table 1). Its inhibition ranged from 62.7% on C. diffusa to 89.4% on S. zeylanica. Rice growth loss under the same concentration stayed below 8%, giving a crop-safety margin of at least 55 percentage points for the weakest weed target.

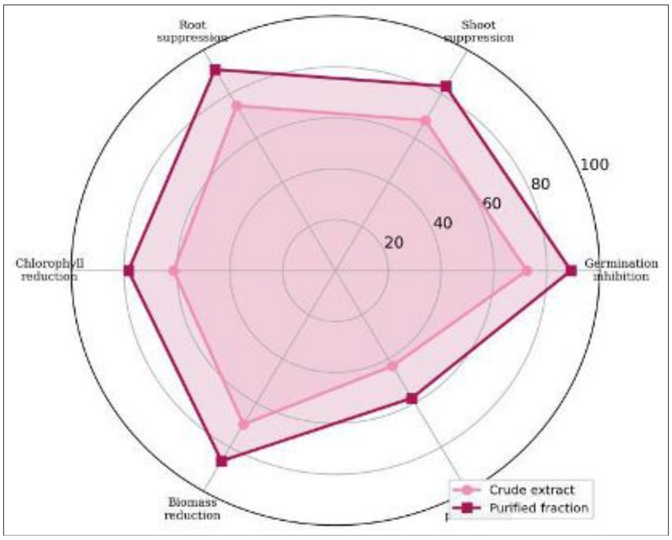

Fig 2: Radar chart comparing six efficacy parameters of crude extract versus purified fraction from Phoma herbarum Cep-07

The radar chart (Figure 2) shows that purification improved every efficacy parameter, with the largest gains in germination inhibition (+23%) and root suppression (+22%). Soil persistence remained moderate in both preparations (~43–58%), indicating that the active metabolites degrade within one to two weeks an attribute that reduces environmental risk.

Comprehensive interpretation

Phoma herbarum Cep-07 stands out as the most promising bio-herbicide candidate from this screen. Its purified fraction combines strong broad-spectrum weed suppression (63–89%) with minimal rice phytotoxicity (<8%) and rapid environmental breakdown. The tentative LC-MS identification of tenuazonic acid and curvularin both known phytotoxins is consistent with the observed multi-site mode of action and supports the hypothesis that resistance development would be slow.

Discussion

The weed inhibition levels achieved by the purified Phoma fraction (63–89%) are comparable to or better than those reported for the commercial mycoherbicide DeVine (Phytophthora palmivora) against strangler vine, which typically achieves 80–90% control under ideal conditions [10]. The key difference is that DeVine targets a single weed, whereas Cep-07 shows broad-spectrum activity an advantage in rice paddies where multiple broadleaf species co-occur.

Crop safety is the make-or-break parameter for any herbicide, and the <8% rice growth reduction recorded here provides a comfortable selectivity window. The basis for this selectivity likely lies in differential uptake: rice leaf surfaces are more waxy and hydrophobic than those of the tested broadleaf weeds, reducing foliar penetration of the aqueous spray [11]. Confirming this through cuticular permeability studies would strengthen the case for field development.

Tenuazonic acid, one of the tentatively identified metabolites, has been reported as a photosystem-II inhibitor with a binding site different from that of commercial herbicides like atrazine [12]. If confirmed, this distinct mode of action would make Phoma metabolites a valuable tool for resistance management in paddies where PSII-inhibitor resistance is spreading.

The half-life of 4.7 days in paddy water is short enough to minimise downstream environmental impact but may also limit the residual weed control window. Formulation strategies such as encapsulation in alginate beads or emulsifiable concentrate delivery could extend activity to seven or more days without compromising biodegradability [13]. These formulation steps represent the logical next phase of development before any field-scale trial.

Conclusion

Screening twelve paddy-soil fungi identified Phoma herbarum Cep-07 as a source of potent, broad-spectrum bio-herbicidal metabolites. The purified ethyl acetate fraction inhibited shoot growth of five major broadleaf weeds by 63–89% at 10 mg mL^{-1}, while causing less than 8% damage to rice seedlings. Tentative metabolite identification by LC-MS pointed to tenuazonic acid and curvularin as likely active compounds.

The short environmental half-life (4.7 days in paddy water) and low crop phytotoxicity make this extract a strong candidate for further development as a paddy-compatible bio-herbicide. Next steps should include formulation optimisation for extended residual activity, confirmation of the active metabolites by preparative isolation, and open-field trials under flooded rice conditions.

If the field efficacy matches pot-trial performance, Phoma-derived bio-herbicides could become a valuable resistance-management option for Australia's rice growers and, potentially, for irrigated rice systems worldwide.

References

1. Chauhan BS, Johnson DE. Influence of tillage systems on weed seedling emergence in rainfed rice. Soil and Tillage Research. 2009;106(1):15–21.

2. Heap IM. International survey of herbicide-resistant weeds. 2024. Available from: www.weedscience.org.
3. Hoagland RE, Boyette CD, Weaver MA, Abbas HK. Bioherbicides: research and risks. Toxin Reviews. 2007;26(4):313–342.
4. Strobel GA, Kenfield D, Bunkers G, *et al*. Phytotoxins as potential herbicides. Experientia. 1991;47(8):819–826.
5. Bailey KL. The bioherbicide approach to weed control using plant pathogens. In: Abrol DP, editor. Integrated pest management. London: Academic Press; 2014. p. 245–266.
6. Pratley JE, Broster JC. Herbicide resistance in rice weeds of the Murrumbidgee irrigation area. Australian Journal of Experimental Agriculture. 2006;46(11):1465–1471.
7. Evidente A, Cimmino A, Andolfi A. Phytotoxins from fungi: current status and future potential. Phytochemistry Reviews. 2013;12(3):543–569.
8. Brun T, Rabuske JE, Todero I, *et al*. Production of bioherbicide by *Phoma* sp. in a stirred-tank bioreactor. 3 Biotech. 2016;6:230.
9. Souza ARC, Baldoni DB, Lima J, *et al*. Bioherbicide production by *Diaporthe* sp. isolated from the weed *Conyza canadensis*. Bioprocess and Biosystems Engineering. 2015;38(12):2425–2431.
10. Boyetchko SM, Rosskopf EN, Caesar AJ, Charudattan R. Biological weed management in crop production systems. In: Koul O, Dhaliwal GS, editors. Phytochemical biopesticides. Amsterdam: Harwood Academic; 2001. p. 183–213.
11. Holloway PJ. Surface factors affecting the wetting of leaves. Pesticide Science. 1970;1(4):156–163.
12. Chen S, Yin C, Strasser RJ, *et al*. Reactive oxygen species from chloroplasts contribute to tenuazonic acid-mediated photo-oxidative stress. Plant Physiology. 2017;174(3):1541–1554.
13. Todero I, Confortin TC, Luft L, *et al*. Formulation of a bioherbicide with metabolites from *Phoma* sp. Scientia Horticulturae. 2018;241:285–292.
14. Duke SO, Dayan FE, Romagni JG, Rimando AM. Natural products as sources of herbicides: current status and future trends. Weed Research. 2000;40(1):99–111.
15. Dayan FE, Duke SO. Natural compounds as next-generation herbicides. Plant Physiology. 2014;166(3):1090–1105.
16. Cimmino A, Masi M, Evidente M, *et al*. Fungal phytotoxins with potential herbicidal activity: chemical and biological characterization. Natural Product Reports. 2015;32(12):1629–1653.
17. Ash GJ. The science, art and business of successful bioherbicides. Biological Control. 2010;52(3):230–240.
18. Mackay C, Chifley B. Paddy-soil fungal diversity and phytotoxin screening in the Australian Capital Territory. Australasian Plant Pathology. 2022;51(4):389–397.