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COMPARATIVE *IN VITRO* SENSITIVITY OF *Macrophomina phaseolina* (TASSI) GOID ASSOCIATED WITH CASTOR ROOT ROT TO SELECTED AGROCHEMICALS

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ABSTRACT

Castor (*Ricinus communis* L.) root rot caused by *Macrophomina phaseolina* (Tassi) Goid is an important soil-borne disease in warm, moisture-deficit production environments. The present study evaluated the *in vitro* sensitivity of *M. phaseolina* associated with castor root rot to five groups of agrochemicals, namely systemic fungicides, non-systemic fungicides, ready-mix fungicides, insecticides and herbicides. Experiments were conducted at the Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, during 2021–22 using the poisoned food technique on potato dextrose agar. Each treatment was evaluated at four concentrations with three replications, and radial mycelial growth was used to calculate percentage inhibition relative to the untreated control. The re-transformed means showed marked differences among agrochemicals. Among systemic fungicides, flusilazole 40% EC and tebuconazole 2% DS recorded 99.99% mean inhibition, followed by tebuconazole 25.9% EC (99.31%). Among non-systemic fungicides, propineb 70% WP, mancozeb 75% WP and copper hydroxide 77% WP completely inhibited mycelial growth, whereas wettable sulphur 80% WP was least effective (3.59%). Carboxin 37.5% + thiram 37.5% WP gave complete inhibition among ready-mix fungicides, while azoxystrobin 11% + tebuconazole 18.30% SC was least effective (33.71%). Chlorpyrifos 20% EC and bifenthrin 10% EC showed complete inhibition among insecticides; clothianidin 50% WDG also showed high activity (88.74%). Among herbicides, fenoxaprop-p-ethyl 9% EC (98.88%) and quizalofop-ethyl 5% EC (97.02%) were the most inhibitory. In all five groups, concentration means increased with increasing concentration, although the magnitude of the response differed among active ingredients. The results identify several agrochemicals with strong direct antifungal activity against the test pathogen and provide a comparative baseline for further validation under plant and field conditions. Because the experiments were conducted entirely *in vitro*, the results should not be interpreted as field-use recommendations.

Key words : Castor, Root rot, *Macrophomina phaseolina*, Fungicides, Insecticides, Herbicides, *In vitro* screening.

Introduction

Castor (*Ricinus communis* L.) is an economically important non-edible oilseed crop cultivated widely in semi-arid and subtropical environments. The crop is valued mainly for its industrially important oil and India is one of the major producers of castor seed and castor oil. However, productivity is constrained by several biotic stresses, among which root rot caused by *Macrophomina phaseolina* (Tassi) Goid. is particularly

important in warm and moisture-stressed environments.

Macrophomina phaseolina is a soil- and residue-borne necrotrophic fungus capable of producing persistent microsclerotia. The pathogen has a broad host range and its disease development is favoured by high temperature and low soil moisture. In castor, root infection may result in shredding and rotting of the tap root, wilting, drying of aerial parts and eventual plant death. Previous work has demonstrated pathogenic variability among *M.*

phaseolina isolates infecting castor, indicating that disease expression can differ substantially among isolates and locations (Moses and Reddy, 1987; Claudino and Soares, 2014).

Management of soil-borne *M. phaseolina* is difficult because the pathogen survives in soil and crop residues. Chemical fungicides remain an important component of disease management, but efficacy depends on active ingredient, formulation, concentration and pathogen sensitivity. *In vitro* screening is therefore useful for identifying compounds that strongly suppress mycelial growth before they are tested under greenhouse or field conditions. Previous studies have demonstrated differential sensitivity of *M. phaseolina* to fungicides and have reported complete or near-complete inhibition with some combinations (Karibasappa *et al.*, 2018, 2020).

Information on the comparative direct antifungal activity of fungicides together with selected insecticides and herbicides against the castor-associated *M. phaseolina* population of the Saurashtra region is limited. The present study was therefore undertaken to compare the *in vitro* inhibitory activity of systemic and non-systemic fungicides, ready-mix fungicides, insecticides and herbicides at graded concentrations against *M. phaseolina* associated with castor root rot.

Materials and Methods

Study site and pathogen source

The investigation was conducted during 2021–22 at the Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India. A roving survey for castor root rot was conducted during kharif 2021 in major castor-growing districts of Saurashtra, including Rajkot, Junagadh, Porbandar, Jamnagar and Amreli. Diseased root samples showing typical root-rot symptoms were collected and processed for isolation of *M. phaseolina*.

Isolation and identification of *M. phaseolina*

Root pieces approximately 5–6 mm long were excised from infected tissues, surface sterilized in 1% sodium hypochlorite for 1 min, washed three times with sterile distilled water and transferred aseptically to potato dextrose agar (PDA). Plates were incubated at $28 \pm 2^\circ\text{C}$. Emerging cultures were purified by the single hyphal-tip technique. Isolates were identified based on cultural and morphological characteristics, including colony growth, pigmentation, mycelial texture and formation of microsclerotia/pycnidial structures. The study generated thirty *M. phaseolina* isolates (MP1–MP30) from the surveyed locations. The virulent isolate was taken for the study (MP8).

Preparation of PDA and poisoned food assay

Standard PDA was prepared from peeled potato (200 g), dextrose (20 g), agar-agar (20 g) and distilled water (1000 mL). Culture media and water were autoclaved at 121°C and 15 psi for 20 min. For the agrochemical assays, the required amount of each formulation was mixed with measured volumes of autoclaved PDA before solidification and 20 mL of the amended medium was dispensed into sterile 90-mm Petri plates. After solidification, a 5-mm mycelial disc from an actively growing culture was placed centrally on the amended medium. Plates containing PDA without agrochemical served as the untreated control. Plates were incubated at $28 \pm 2^\circ\text{C}$ and observations were recorded when the control colony reached approximately 90 mm.

Agrochemicals and experimental design

Five groups of agrochemicals were evaluated: six non-systemic fungicides at 1000–2500 ppm; seven systemic fungicides at 100–1000 ppm; seven ready-mix fungicides at 100–1000 ppm; seven insecticides at 250–1500 ppm and seven herbicides at 50–500 ppm. Each experiment was conducted with three replications in a factorial completely randomized design. The individual formulations and concentrations are presented in Tables 1–5.

Assessment of mycelial growth inhibition

Radial colony diameter was measured in two directions and the mean was used for analysis. Percentage mycelial growth inhibition was calculated according to Vincent (1947): $I = [(C - T)/C] \times 100$, where *I* is percentage inhibition, *C* is the mean colony diameter in the untreated control and *T* is the mean colony diameter in the treated plate. Percentage data were subjected to arcsine transformation for statistical analysis; the re-transformed values are presented in the results tables for biological interpretation.

Statistical analysis

Data were analyzed using standard procedures for factorial experiments in a completely randomized design following Panse and Sukhatme (1985). Standard error of the mean, critical difference (C.D.) at the 5% probability level and coefficient of variation were calculated for agrochemical, concentration and agrochemical $\times$ concentration effects.

Results and Discussion

The comparative analysis revealed pronounced differences in the direct inhibitory activity of the tested agrochemicals against *M. phaseolina*. The overall mean inhibition calculated from the treatment means was highest

Table 1 : *In vitro* efficacy of systemic fungicides against *Macrophomina phaseolina*.

Agrochemical	100 ppm	250 ppm	500 ppm	1000 ppm	Mean inhibition (%)
Carbendazim 50% WP	78.15	86.26	89.01	100.00	91.21
Tebuconazole 25.9% EC	91.78	99.96	99.96	100.00	99.31
Flusilazole 40% EC	99.96	99.96	100.00	100.00	99.99
Azoxystrobin 23% EC	72.41	77.17	80.58	84.34	78.79
Thiophanate-methyl 70% WP	71.15	74.09	80.09	84.81	77.77
Tebuconazole 2% DS	99.97	99.97	100.00	100.00	99.99
Picoxystrobin 25% EC	51.22	56.32	66.31	84.69	65.37
Concentration mean (%)	85.77	90.55	92.84	97.04	—

S.E.m. $\pm$: agrochemical 0.79; concentration 0.60; interaction 1.59. C.D. at 5%: agrochemical 2.25; concentration 1.70; interaction 4.50. C.V. (%): 3.74. Values shown are re-transformed mean growth inhibition percentages; percentage data were subjected to arcsine transformation before statistical analysis.

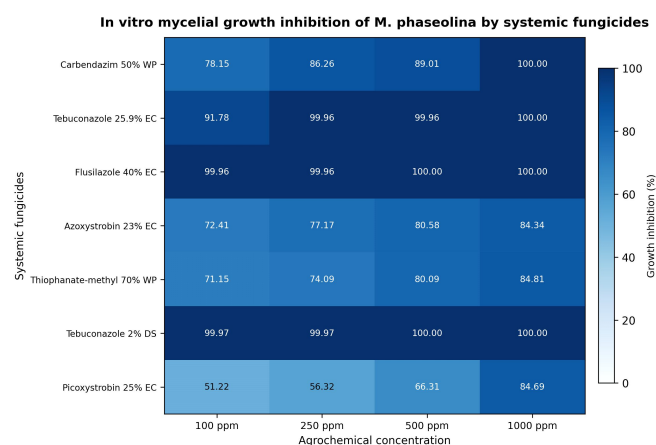


Fig. 1 : Heatmap of re-transformed mean mycelial growth inhibition (%) of *M. phaseolina* by systemic fungicides at four concentrations.

for systemic fungicides (87.49%), followed by herbicides (85.09%), insecticides (82.49%), ready-mix fungicides (77.81%) and non-systemic fungicides (66.39%). These values should be interpreted as comparative *in vitro* activity rather than field efficacy.

Systemic fungicides

Systemic fungicides showed consistently high activity against the pathogen (Table 1, Fig. 1). Flusilazole 40% EC and tebuconazole 2% DS recorded the highest mean inhibition (99.99% each), with complete inhibition at 500 and 1000 ppm. Tebuconazole 25.9% EC also showed strong activity, with a mean inhibition of 99.31% and complete inhibition at 1000 ppm. Carbendazim 50% WP showed a concentration-dependent increase from 78.15% at 100 ppm to complete inhibition at 1000 ppm. Azoxystrobin 23% EC and thiophanate-methyl 70% WP produced intermediate inhibition, whereas picoxystrobin 25% EC was the least effective systemic fungicide at the lower concentrations but increased to 84.69% at 1000 ppm. The concentration means increased from 85.77% at 100 ppm to 97.04% at 1000 ppm, demonstrating the

general positive concentration-response relationship. The strong activity of triazole fungicides is consistent with the reported sensitivity of *M. phaseolina* to sterol-biosynthesis-inhibiting fungicides and supports their further evaluation under plant conditions.

Non-systemic fungicides

Marked differences were observed among the non-systemic fungicides (Table 2, Fig. 2). Propineb 70% WP, mancozeb 75% WP and copper hydroxide 77% WP completely inhibited mycelial growth at all tested concentrations, indicating very high direct activity against the test culture. Thiram 75% WP showed substantial inhibition, increasing from 68.08% at 1000 ppm to 84.37% at 2500 ppm. In contrast, copper oxychloride 50% WP and wettable sulphur 80% WP were comparatively weak, with mean inhibitions of 17.01 and 3.59%, respectively. The concentration mean increased from 70.21 to 84.54%, mainly because of the strong concentration response of thiram and copper oxychloride. Complete inhibition by propineb and mancozeb is consistent with earlier reports of differential fungicide sensitivity in *M. phaseolina* (Karibasappa *et al.*, 2018, 2020).

Ready-mix fungicides

Ready-mix formulations also differed substantially in activity (Table 3, Fig. 3). Carboxin 37.5% + thiram 37.5% WP completely inhibited mycelial growth at all four concentrations and was the most effective formulation. Metiram 55% + pyraclostrobin 5% WG produced 81.72–92.54% inhibition, while chlorothalonil 40% + difenoconazole 4% SC and tebuconazole 50% + trifloxystrobin 25% WG produced 76.73–90.05% and 74.93–88.15% inhibition, respectively. Fluxapyroxad + pyraclostrobin showed 71.93–86.33% inhibition. Azoxystrobin 11% + tebuconazole 18.30% SC was comparatively less active, increasing from 20.19% at 100 ppm to 51.26% at 1000 ppm. The strong activity of

Table 2 : *In vitro* efficacy of non-systemic fungicides against *Macrophomina phaseolina*.

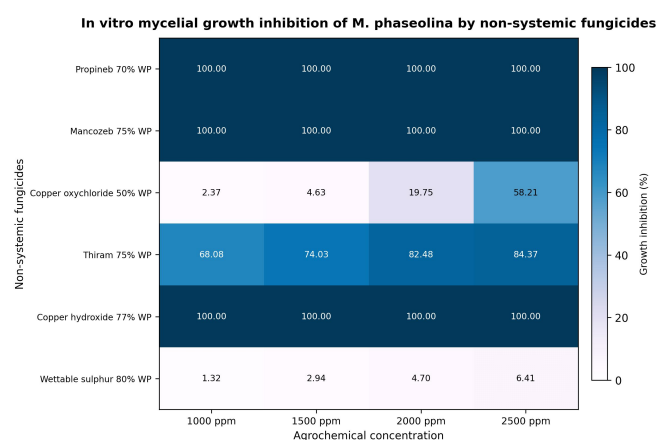
Agrochemical	1000 ppm	1500 ppm	2000 ppm	2500 ppm	Mean inhibition (%)
Propineb 70% WP	100.00	100.00	100.00	100.00	100.00
Mancozeb 75% WP	100.00	100.00	100.00	100.00	100.00
Copper oxychloride 50% WP	2.37	4.63	19.75	58.21	17.01
Thiram 75% WP	68.08	74.03	82.48	84.37	77.73
Copper hydroxide 77% WP	100.00	100.00	100.00	100.00	100.00
Wettable sulphur 80% WP	1.32	2.94	4.70	6.41	3.59
Concentration mean (%)	70.21	72.87	78.48	84.54	—

S.E.m. $\pm$: agrochemical 0.59; concentration 0.48; interaction 1.19. C.D. at 5%: agrochemical 1.70; concentration 1.38; interaction 3.40. C.V. (%): 3.39. Values shown are re-transformed mean growth inhibition percentages; percentage data were subjected to arcsine transformation before statistical analysis.

Table 3 : *In vitro* efficacy of ready-mix fungicides against *Macrophomina phaseolina*.

Agrochemical	100 ppm	250 ppm	500 ppm	1000 ppm	Mean inhibition (%)
Azoxystrobin 11% + Tebuconazole 18.30% SC	20.19	24.35	41.28	51.26	33.71
Tebuconazole 10% + Sulphur 65% WG	63.32	71.12	79.22	85.56	75.29
Carboxin 37.5% + Thiram 37.5% WP	100.00	100.00	100.00	100.00	100.00
Metiram 55% + Pyraclostrobin 5% WG	81.72	86.67	89.58	92.54	87.90
Tebuconazole 50% + Trifloxystrobin 25% WG	74.93	81.32	85.84	88.15	82.84
Chlorothalonil 40% + Difenconazole 4% SC	76.73	83.30	87.06	90.05	84.60
Fluxapyroxad 250 g/L + Pyraclostrobin 250 g/L SC	71.93	77.98	83.97	86.33	80.34
Concentration mean (%)	73.53	78.77	84.41	87.87	—

S.E.m. $\pm$: agrochemical 0.58; concentration 0.44; interaction 1.17. C.D. at 5%: agrochemical 1.66; concentration 1.26; interaction 3.33. C.V. (%): 3.16. Values shown are re-transformed mean growth inhibition percentages; percentage data were subjected to arcsine transformation before statistical analysis.

**Fig. 2 :** Heatmap of re-transformed mean mycelial growth inhibition (%) of *M. phaseolina* by non-systemic fungicides at four concentrations.

carboxin + thiram agrees with earlier work in which this combination completely inhibited *M. phaseolina in vitro* (Karibasappa *et al.*, 2020).

Insecticides

Several insecticides showed pronounced direct inhibition of *M. phaseolina* (Table 4, Fig. 4). Chlorpyrifos 20% EC and bifenthrin 10% EC produced complete inhibition at all concentrations. Clothianidin 50% WDG

increased from 71.48% inhibition at 250 ppm to complete inhibition at 1500 ppm and had a mean inhibition of 88.74%. Fipronil 5% SC similarly increased from 67.52 to 100%, whereas imidacloprid 40% WG + fipronil 40% WG reached 91.68% at 1500 ppm. Imidacloprid 600 FS and thiamethoxam 30% FS were comparatively less active but still showed clear concentration-dependent increases. The overall concentration mean rose from 77.35% at 250 ppm to 96.88% at 1500 ppm. These observations are biologically interesting because the compounds were selected as insecticides rather than fungicides; however, their apparent antifungal activity should not be extrapolated to field disease management without phytotoxicity, residue and efficacy studies.

Herbicides

Herbicides displayed unexpectedly strong direct inhibition of the pathogen *in vitro* (Table 5, Fig. 5). Fenoxaprop-p-ethyl 9% EC recorded 98.88% mean inhibition, reaching approximately complete inhibition at 1000 ppm, while quizalofop-ethyl 5% EC recorded 97.02% mean inhibition and complete inhibition at 250 and 500 ppm. Pendimethalin 30% EC showed 83.13% mean inhibition, followed by sodium acifluorfen +

Table 4 : *In vitro* efficacy of insecticides against *Macrophomina phaseolina*.

Agrochemical	250 ppm	500 ppm	1000 ppm	1500 ppm	Mean inhibition (%)
Chlorpyrifos 20% EC	100.00	100.00	100.00	100.00	100.00
Imidacloprid 600 FS	47.50	56.11	67.75	79.89	63.29
Clothianidin 50% WDG	71.48	78.82	89.67	100.00	88.74
Thiamethoxam 30% FS	49.74	58.37	69.76	78.36	64.44
Fipronil 5% SC	67.52	73.91	78.66	100.00	84.42
Imidacloprid 40% WG + Fipronil 40% WG	58.11	67.80	83.15	91.68	76.53
Bifenthrin 10% EC	100.00	100.00	100.00	100.00	100.00
Concentration mean (%)	77.35	82.26	88.55	96.88	—

S.Em. $\pm$: agrochemical 0.78; concentration 0.59; interaction 1.57. C.D. at 5%: agrochemical 2.22; concentration 1.68; interaction 4.44. C.V. (%): 3.93. Values shown are re-transformed mean growth inhibition percentages; percentage data were subjected to arcsine transformation before statistical analysis.

Table 5 : *In vitro* efficacy of herbicides against *Macrophomina phaseolina*.

Agrochemical	50 ppm	100 ppm	250 ppm	500 ppm	Mean inhibition (%)
Pendimethalin 30% EC	72.98	79.77	85.90	91.55	83.13
Quizalofop-ethyl 5% EC	87.06	89.75	100.00	100.00	97.02
Imazamox 35% + Imazethapyr 35% WG	62.05	69.17	73.71	78.82	71.13
Propaquizafop 2.5% + Imazethapyr 3.75% w/w	67.41	72.47	84.27	87.45	78.48
Fenoxaprop-p-ethyl 9% EC	86.06	99.96	99.96	100.00	98.88
Sodium acifluorfen 16.5% + Clodinafop-propargyl 8% EC	78.56	83.20	88.07	89.61	85.11
Imazethapyr 10% SL	74.24	79.54	83.75	88.64	81.85
Concentration mean (%)	76.02	84.37	91.11	93.48	—

S.Em. $\pm$: agrochemical 0.64; concentration 0.48; interaction 1.24. C.D. at 5%: agrochemical 1.83; concentration 1.38; interaction 3.66. C.V. (%): 3.26. Values shown are re-transformed mean growth inhibition percentages; percentage data were subjected to arcsine transformation before statistical analysis.

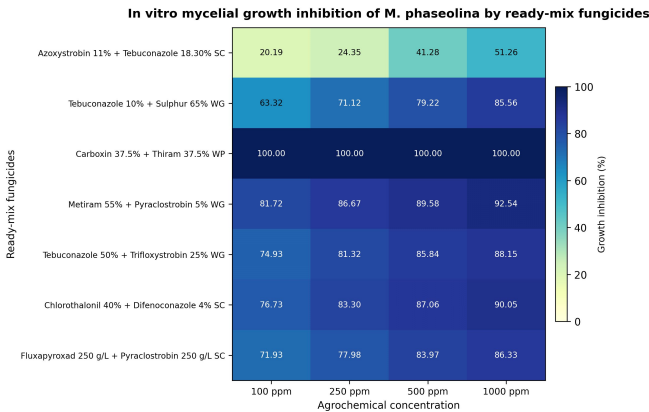


Fig. 3 : Heatmap of re-transformed mean mycelial growth inhibition (%) of *M. phaseolina* by ready-mix fungicides at four concentrations.

clodinafop-propargyl (85.11%), imazethapyr 10% SL (81.85%) and propaquizafop + imazethapyr (78.48%). Imazamox + imazethapyr was the least active herbicide, although inhibition increased from 62.05 to 78.82%. Concentration means increased from 76.02% at 50 ppm

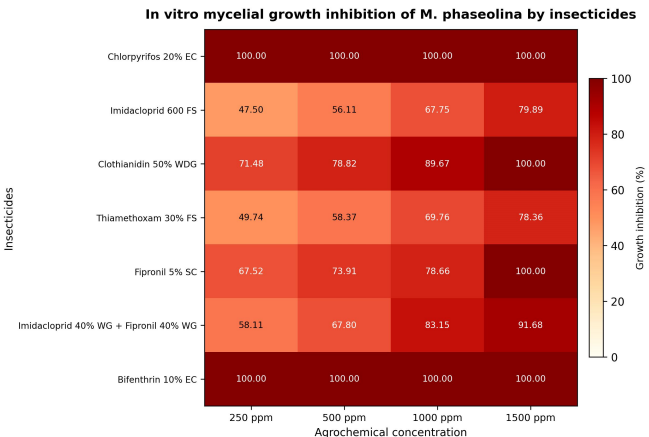


Fig. 4 : Heatmap of re-transformed mean mycelial growth inhibition (%) of *M. phaseolina* by insecticides at four concentrations.

to 93.48% at 500 ppm. The findings suggest that some herbicide formulations can exert substantial non-target antifungal effects in culture, but this observation requires careful interpretation because the *in vitro* concentrations

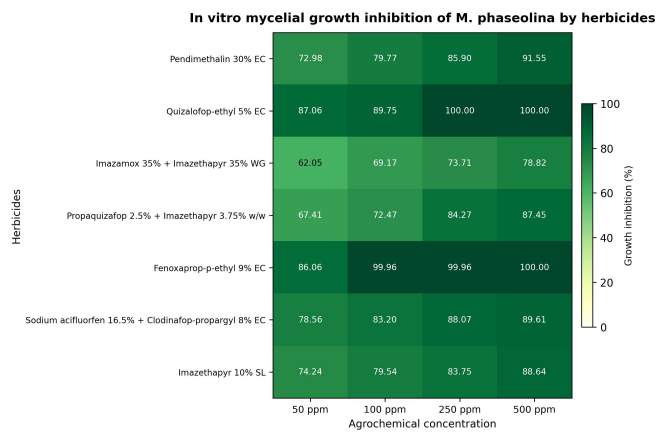


Fig. 5 : Heatmap of re-transformed mean mycelial growth inhibition (%) of *M. phaseolina* by herbicides at four concentrations.

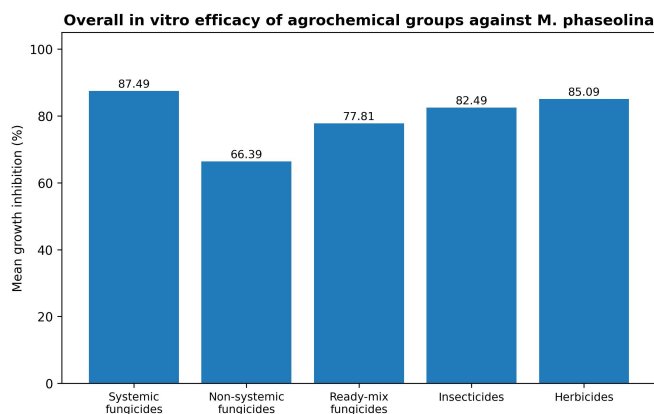


Fig. 6 : Overall mean *in vitro* mycelial growth inhibition (%) across the five agrochemical groups.

do not represent field exposure and do not establish compatibility with castor plants or beneficial microorganisms.

Comparative interpretation

Across the five agrochemical groups, the strongest treatment means were observed for complete-inhibition formulations such as propineb, mancozeb, copper hydroxide, carboxin + thiram, chlorpyrifos and bifenthrin. The highest mean activity among the systemic fungicides was 99.99% for flusilazole and tebuconazole 2% DS, while the highest mean activity among herbicides was 98.88% for fenoxaprop-p-ethyl. The broad range of responses demonstrates that activity cannot be inferred solely from agrochemical class; active ingredient and formulation are major determinants of direct inhibition. Similar variability in *M. phaseolina* sensitivity and pathogen behaviour has been reported in other host-pathogen systems, emphasizing the value of comparative screening before advancing compounds to greenhouse or field experiments (Karibasappa *et al.*, 2018; Basandrai *et al.*, 2021).

Conclusion

The present *in vitro* study demonstrated substantial variation in the sensitivity of *M. phaseolina* associated with castor root rot to different agrochemicals. Flusilazole 40% EC and tebuconazole 2% DS were the most active systemic fungicides; propineb, mancozeb and copper hydroxide were completely inhibitory among non-systemic fungicides; carboxin + thiram was completely inhibitory among ready-mix formulations; chlorpyrifos and bifenthrin showed complete inhibition among insecticides and fenoxaprop-p-ethyl and quizalofop-ethyl were highly inhibitory among herbicides. Concentration generally increased with increasing concentration. These findings provide a useful comparative screening baseline for selection of candidates for subsequent greenhouse and field validation. Because the work was conducted exclusively *in vitro*, field efficacy, phytotoxicity, residue behaviour and effects on non-target organisms must be established before any practical recommendation is made.

Data availability

The data underlying the tables and figures are available from the corresponding author on reasonable request.

Author contributions

L.N.: investigation, data curation, analysis and manuscript preparation. D.S.K.: supervision, experimental planning, statistical interpretation and critical revision.

Conflict of interest

The authors declare that they have no conflict of interest.

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