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Molecular identification of Alternaria alternata causing brown leaf spot of potato and its management using novel fungicides

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Molecular identification of *Alternaria alternata* causing brown leaf spot of potato and its management using novel fungicides

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Abstract

Brown leaf spot incited by *Alternaria alternata* has become a major foliar disease that affects potato production and under favorable weather conditions, causes significant yield losses. The objective of current investigation was to determine the causative pathogen and to assess the effectiveness of certain fungicides against *A. alternata* in both laboratory and field settings. Based on morphological and cultural characteristics, the pathogen isolated from diseased potato leaves was identified as *A. alternata*. This identification was further supported by molecular characterization using ITS sequence analysis (GenBank accession no. PX062184). Six fungicides comprising contact, systemic and combination formulations were assessed using the Poisoned food technique under *in vitro* conditions. Mancozeb 75% WP completely suppressed mycelial growth starting from 50 ppm, whereas, chlorothalonil 78.12% WP suppressed mycelial growth from 100 ppm and above, respectively. Among the systemic and combination fungicides, fluxapyroxad 167 g L^{-1} + pyraclostrobin 333 g L^{-1} SC totally inhibited fungal growth at all tested concentrations (5-25 ppm), followed by propiconazole 25% EC and tebuconazole 50% + trifloxystrobin 25% WG. Field evaluation of fungicides under natural epiphytotic conditions revealed that chlorothalonil 78.12% WP was the most effective treatment, exhibiting the lowest disease severity (34.75%), highest disease reduction (46.11%) and minimum AUDPC (562.43). Additionally, it yielded the maximum tuber yield (28.06 t ha^{-1}), yield increase (82.56%) and yield gain over control (12.69 t ha^{-1}), all of which had higher yield attributes. Chlorothalonil produced the highest gross (₹2,80,600 ha^{-1}) and net returns (₹1,67,424 ha^{-1}), however, mancozeb 75% WP was less expensive to apply, it had the highest benefit:cost ratio (2.54) and incremental cost-benefit ratio (20.50). Mancozeb was the most cost-effective treatment, whereas chlorothalonil was the most successful fungicide for controlling potato brown leaf spot and increasing productivity.

Key words: Potato, Brown leaf spot, Mancozeb, Fungicides, *Alternaria,* Potato

Introduction

Potato (*Solanum tuberosum* L.) ranks third next to rice and wheat globally, is a significant cool season vegetable crop belongs to night shade (Solanaceae) family, are among the most consumed food crops worldwide (Birch et al. 2012). India is the second largest producer of potatoes after China with an average productivity of 26 t ha^{-1} and 47.77 million tonnes from 2.13 million hectares (Das et al. 2024). Potatoes are a staple diet in many industrialized and developing nations because they are a great source of proteins, carbohydrates, vitamins, minerals and antioxidants (Devaux et al. 2020). Despite being a nutritionally diverse crop, its production is severely constrained by several biotic stresses incited by fungi, bacteria, viruses and nematodes.

Alternaria spp. incite serious foliar infection in potato worldwide, being responsible for considerable yield and quality losses under conducive environmental conditions (Schmey et al. 2024; Vinogradova et al. 2026). Small brown dots scattered throughout the leaf surface are the initial symptoms of the disease (Droby et al. 1984). Lesions can have a width of up to 10 mm and are smaller than early blight lesions (Ding et al. 2019). Additionally, lesions do not form concentric rings (Bessadat et al. 2021). The spots may appear at any stage of vegetative growth of plant. Dried, senescent leaves may eventually result due to coalescence of lesions into bigger necrotic patches with brown edges as the disease progresses (Fairchild et al. 2013). The pathogen demonstrates considerable variability and survive on various means which serve as an important source of primary inoculum for subsequent cropping season (Rotem 1994; Thomma 2003). Temperature ranging from 12°C at night to 30°C during daytime and high humidity were reported to be congenial for the development of *A. alternata* induced leaf spot disease in potatoes where 60% or more infection was reported under cultivated conditions (Choi et al. 2023).

Fungicides, resistant cultivars and cultural practices are the main components of integrated approaches used to manage *A. alternata* induced potato leaf spot disease. However, durable host resistance to *Alternaria* spp., is limited due to its high pathogenic variability (Schmey et al. 2024). As a result, fungicides continue to be the best way to manage disease in the field. *Alternaria* infections in potatoes have been effectively suppressed by fungicides from the triazole, strobilurin, SDHI and dithiocarbamate families (Park et al. 2024). However, persistent and careless fungicide application might lead to fungicide resistance and environmental issues (Pasche and Gudmestad 2008). For sustainable potato production, fungicides must be evaluated based on both economic viability and disease control effectiveness. Therefore, the current study was conducted to compare

the effectiveness of several fungicides against *A. alternata*, which causes leaf spot disease and to determine their effect on disease suppression, yield enhancement and economic viability in field settings.

Materials and Methods

Isolation and identification of *A. alternata* causing brown leaf spot disease in potatoes

During the *Rabi* season of 2024, infected potato leaves (5 x 5 mm) were collected and surface sterilized for one minute each using 70% ethanol and 1.5% NaOCl, followed by three rinses with sterile distilled water. The infected leaves were cultured for seven days at 25°C in the dark on water agar supplemented with 100 µg mL^{-1} streptomycin. On potato dextrose agar (PDA), emergent fungal growth was purified using the single spore isolation method (Hansen 1926). Using the CTAB method described by Murray and Thompson (1980), genomic DNA was extracted from fungal mycelia of the seven day old culture. The internal transcribed spacer (ITS) region of ribosomal DNA was amplified using the universal primers ITS1 and ITS4. The amplified products were sequenced and the resulting sequences were compared with reference sequences available in the NCBI GenBank database for molecular identification.

In vitro efficacy of fungicides against *A. alternata*

Two contact fungicides, Chlorothalonil 78.12% WP and Mancozeb 75% WP and four systemic fungicides, Azoxystrobin 11% + Tebuconazole 18.3% SC, Fluxapyroxad 167 g L^{-1} + Pyraclostrobin 333 g L^{-1} SC, Propiconazole 25% EC and Tebuconazole 50% + Trifloxystrobin 25% WG were evaluated against *A. alternata* under *in vitro* assays to investigate the inhibitory effect of these fungicides against test fungus using Poison food technique outlined by Nene and Thapliyal (1982). A double strength potato dextrose agar (PDA) medium was used in the Poisoned food technique. Double strength PDA medium was prepared by doubling the amount of potato infusion (400 g peeled potato), agar (40 g) and dextrose (40 g), while maintaining the final volume of distilled water at 1 liter. After sterilizing the medium for 20 minutes at 15 psi (121.6°C) in an autoclave, it was refrigerated for later use. The dilution equation ($C_1V_1=C_2V_2$) was used to determine the amount of each fungicide formulation needed to achieve the intended concentrations. To make a double strength fungicide solution (2X), the calculated amount of fungicide was aseptically transferred into a sterile 100 mL Erlenmeyer flask and filled to a final

capacity of 30 mL with sterile distilled water. Subsequently, 30 mL of the double strength fungicide solutions was combined with 30 mL of sterilized molten double strength potato dextrose agar (PDA) maintained at 45-50°C yielding 60 ml of poisoned medium with the intended final fungicide concentration. After thorough agitation, the mixture was aseptically transferred into sterilized Petri dishes. In each 90 mm sterilized Petri dish, 20 mL of the poisoned medium was added, with three replicates for each treatment and allowed to solidify. Once the medium had set, 5 mm sections of a seven day old culture were placed in the center of the Petri dishes. The mycelial disc was inverted to facilitate direct contact between the fungus and the medium. Following inoculation, the Petri plates were sealed with paraffin wax strips and incubated in a B.O.D. incubator at 25 ± 2°C. PDA without fungicides served as control. After a period of seven days, radial colony diameter was measured in two perpendicular directions and averaged. Growth inhibition was calculated in relation to the control using the formula outlined by Vincent (1927).

$$Percent\ Growth\ Inhibition = \frac{C - T}{C} \times 100$$

Where;

C = Average diameter of mycelial colony in control plates (mm)

T = Average diameter of mycelial colony in treated plates (mm)

Field evaluation of fungicides for the management of potato leaf spot disease

Following the harvest of the previous crop, the field was readied with tractor-mounted equipment. It was ploughed once using a disc plough, succeeded by two passes of cross-disc harrowing and levelling with a plank. The field was divided into separate plots with bunds and irrigation channels were installed to enable a consistent flow of water. Potato cv. Kufri Bahar was planted in plots measuring 2.4 m × 2.5 m. The sowing of seed potato was done using recommended agronomic practices. Sowing was done on 25th October, 2024. A randomized block design with three replications was used for the field experimental trail. Different fungicidal treatments were given as foliar sprays at the recommended doses (per hectare) and three consecutive sprays were administered ten to twelve days after the onset of disease symptoms. The dates of the first, second and third sprays were November 21, 2024, December 1, 2024 and December 11, 2024, respectively. Prior to application, fungicides were dissolved in water and evenly sprayed with a

knapsack sprayer. Disease severity was recorded using a 0-9 disease severity scale outlined by Mayee and Datar (1986). Furthermore, disease severity ratings were converted into percent disease index (PDI) using formula outlined by Wheeler, 1969.

$$Percent\ Disease\ Index = \frac{Sum\ of\ all\ ratings}{Total\ number\ of\ samples\ examined \times Maximum\ rating} \times 100$$

Yield attributes and economics

At physiological maturity, in every experimental plot, representative plants were observed for yield and yield contributing traits. Tuber yield (t ha^{-1}), average tuber size (mm), tuber weight per plant (g) and tuber weight per plot (kg) were all measured. By calculating yield gain over the untreated control, yield response ratio, percentage increase in yield over control and relative yield index, the impact of fungicidal treatments on productivity was further evaluated. The yield response ratio was computed as the ratio of the yield under fungicidal treatment to that of the untreated control, while the yield gain over control was determined by deducting the yield of the untreated control from the yield of each treatment. The relative yield index was calculated by dividing each treatment's yield by the total mean yield across all treatments.

To ascertain the profitability of fungicidal treatments in the field, an economic analysis was conducted. Seed tubers, fertilizers, irrigation, manpower, plant protection measures and other cultural procedures were all included in the total cost of cultivation. Labour requirements were estimated at 120 man days ha^{-1} on average. The marketable tuber yield and the current potato tuber price (₹10,000 t^{-1}) were used to compute the gross return. The net return was calculated by deducting the entire cultivation cost from the gross return. The ratio of gross return to total cultivation costs was used to compute the benefit:cost (B:C) ratio. The difference in gross return between treated and untreated plots was used to estimate additional return over the untreated control. The ratio of the enhanced return above the untreated control to the fungicide costs paid in each treatment was used to compute the incremental cost-benefit ratio (ICBR). Based on how various fungicides affected yield and profitability, their economic viability was evaluated.

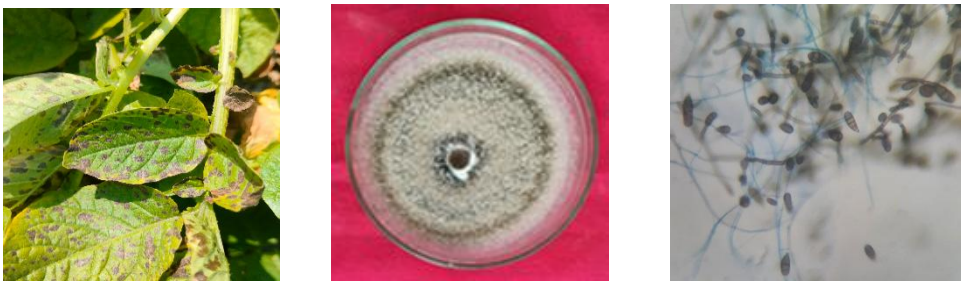
Data analysis

Data were subjected to analysis of variance (ANOVA) using R software (R Foundation for Statistical Computing, Vienna, Austria; version 4.6.0). Treatment means were separated employing the critical difference (CD) test at $P \leq 0.05$.

Results

Isolation and identification of *A. alternata* causing brown leaf spot disease in potatoes

The isolated fungi produce rapidly growing, grey to dark olivaceous colonies with irregular margins, raised elevation and no zonation (Fig. 1b). Microscopic examination revealed that the conidia were found to be obclavate to pyriform, light brown to olivaceous, measuring 23-61 × 10-17 µm, with two-three transverse septa and a short tapering beak (Fig. 1c). The pathogen was identified as *A. alternata* based on morphological and cultural traits. ITS sequence analysis further confirmed the identity of an isolate as *A. alternata* and the sequence was added to GenBank with accession number PX062184.



(a) **(b)** **(c)**

Fig. 1 a Symptomatology of brown leaf spot disease of potato, **b** Pure culture of *A. alternata* and **c** Conidial morphology of *A. alternata*

In vitro efficacy of contact and systemic fungicides against *A. alternata*

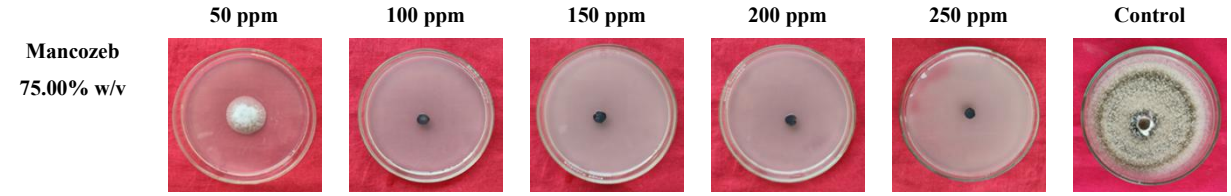
The *in vitro* efficacy of six fungicides was evaluated at different concentrations against *A. alternata* using the Poisoned food technique (Fig. 2 and 3). Out of contact fungicides (Table 1) (Fig. 4), mancozeb 75% WP completely inhibited the mycelial growth, from 50 ppm onwards, leading to 100% inhibition at 100, 150 and 200 ppm. At the lowest concentration (25 ppm), mancozeb recorded 18.83 mm mycelial growth of test pathogen, which is equivalent to 79.07% inhibition over the untreated control. In contrast, chlorothalonil 78.12% WP was less effective at lower concentrations, recording mycelial growth of 22.23 mm and 12.97 mm at 25 and 50 ppm with corresponding inhibitions of 75.30% and 85.59% respectively. However, complete inhibition

(100%) was recorded starting from 100 ppm, whereas, untreated control exhibiting maximum mycelial growth (90 mm).

Systemic and combination fungicides were more effective than contact fungicides under *in vitro* conditions (Table 2) (Fig. 4). At all tested concentrations (5 to 25 ppm), fluxapyroxad 167 g L^{-1} + pyraclostrobin 333 g L^{-1} SC effectively inhibited the mycelial growth of test pathogen, resulting in 100% mycelial inhibition and no discernible fungal growth. Tebuconazole 50% + trifloxystrobin 25% WG was the next most efficient fungicide, exhibiting total inhibition (100%) at 10 ppm and 86.89% inhibition at 5 ppm with mycelial growth of 11.80 mm. Azoxystrobin 11% + tebuconazole 18.3% SC recorded 19.13 mm radial growth at 5 ppm or 78.74% inhibition of test pathogen and from 10 ppm onward, fungal growth was totally suppressed. Propiconazole 25% EC, an individual systemic fungicide among all other combination fungicides exhibited highest efficacy, limiting mycelial growth to 7.00 mm and 5.67 mm at 5 and 10 ppm, equating to 92.22% and 93.70% inhibition and achieving 100% inhibition starting at 15 ppm respectively.

Table 1 *In vitro* evaluation of contact fungicides against *A. alternata*

Fungicides	Mycelial growth (mm)					Mycelial inhibition (%)				
	25 ppm	50 ppm	100 ppm	150 ppm	200 ppm	25 ppm	50 ppm	100 ppm	150 ppm	200 ppm
Mancozeb 75% WP	18.83	0.00	0.00	0.00	0.00	79.07	100.00	100.00	100.00	100.00
Chlorothalonil 78.12% WP	22.23	12.97	0.00	0.00	0.00	75.30	85.59	100.00	100.00	100.00
Control (Untreated)	90.00	90.00	90.00	90.00	90.00	-	-	-	-	-
CD at 5% A (Fungicides) B (concentrations) A×B (Interaction)	0.07 0.09 0.16					-	-	-	-	-
S.Em. A (Fungicides) B (concentrations) A×B (Interaction)	0.024 0.031 0.76					-	-	-	-	-
CV	0.28					-	-			-



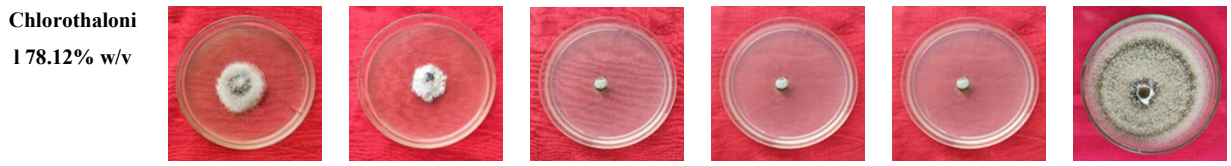
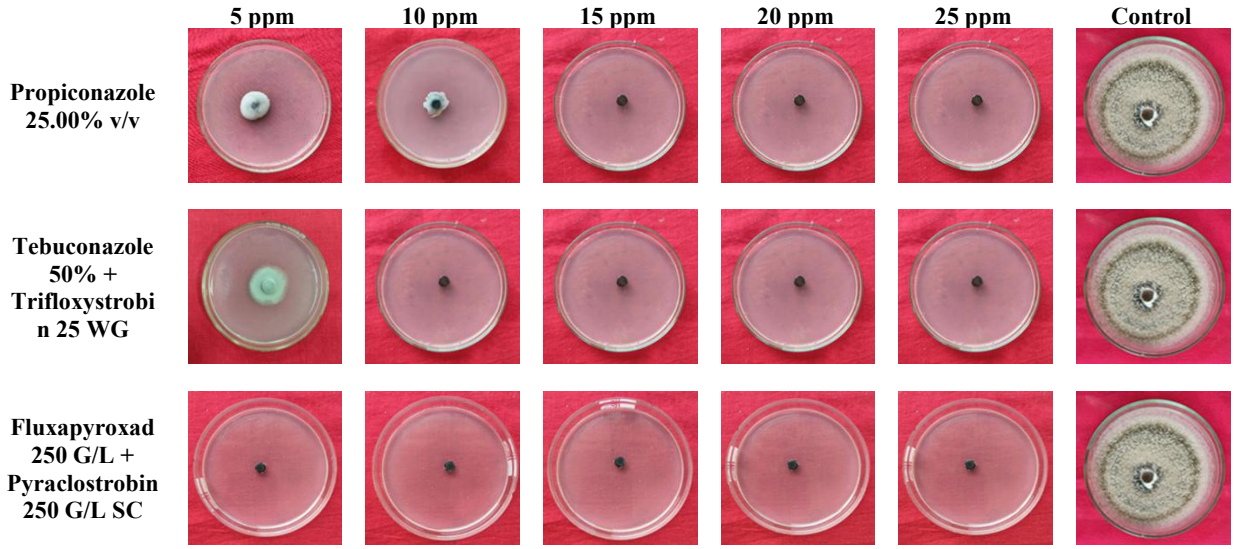


Fig. 2 *In vitro* evaluation of contact fungicides against *A. alternata*

Table 2 *In vitro* evaluation of systemic fungicides against *A. alternata*

Fungicides	Mycelial growth (mm)					Mycelial inhibition (%)				
	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
Tebuconazole 50% + Trifloxystrobin 25 WG	11.80	0.00	0.00	0.00	0.00	86.89	100.00	100.00	100.00	100.00
Fluxapyroxad 167 g L^{-1} + Pyraclostrobin 333 g L^{-1} SC	0.00	0.00	0.00	0.00	0.00	100.00	100.00	100.00	100.00	100.00
Azoxystrobin 11% + Tebuconazole 18.3% SC	19.13	0.00	0.00	0.00	0.00	78.74	100.00	100.00	100.00	100.00
Propiconazole 25% EC	7.00	5.67	0.00	0.00	0.00	92.22	93.70	100.00	100.00	100.00
Control (untreated)	90.00	90.00	90.00	90.00	90.00	-	-	-	-	-
CD at 5% A (Fungicides) B (Concentrations) A×B (Interaction)	0.284 0.284 0.635					-	-	-	-	-
SEm ± A (Fungicides) B (Concentrations) A×B (Interaction)	0.10 0.10 0.22					-	-	-	-	-
CV	1.96					-	-			-



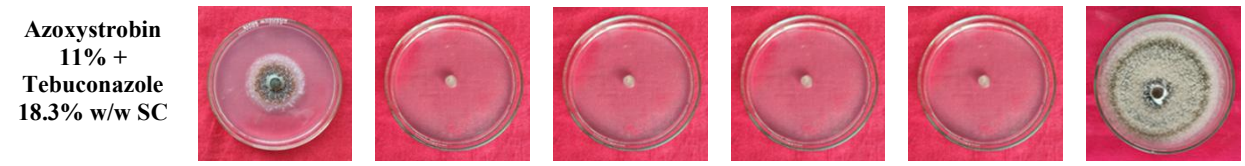


Fig. 3 *In vitro* evaluation of systemic fungicides against *A. alternata*

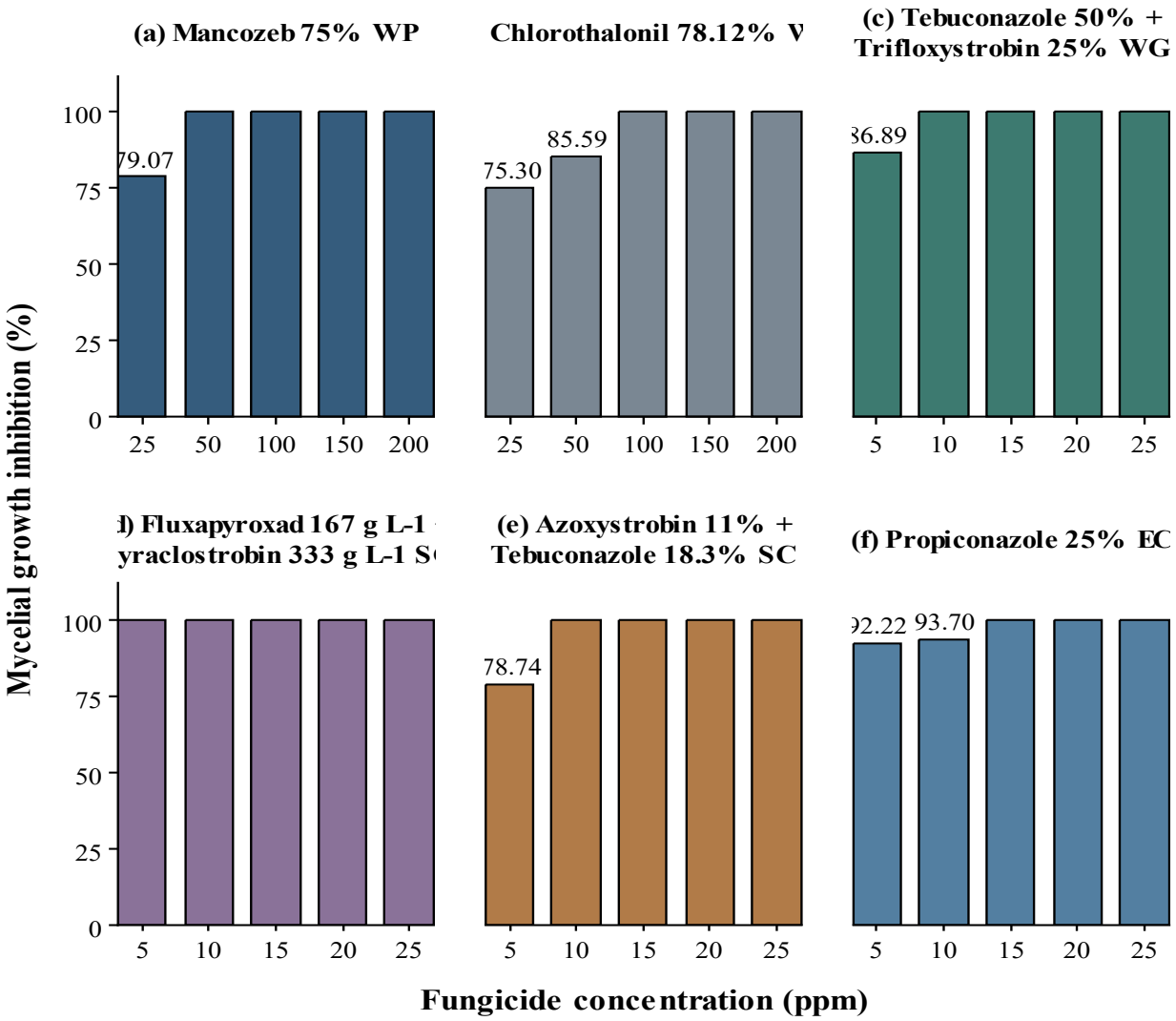


Fig. 4 Concentration dependent inhibition of mycelial growth of *A. alternata* by fungicides

Field evaluation of fungicides for the management of brown leaf spot disease

Following three successive foliar sprays, the field study showed notable variations in the ability of fungicide treatments to slow down the severity of brown leaf spot disease (Table 3) (Fig. 5). All treatments exhibited a gradual increase in disease severity following each spray, however all fungicidal treatments markedly decreased disease severity over untreated control. Following the

third spray, the lowest PDI (34.75%) was achieved by fungicide chlorothalonil 78.12% WP (T1), followed by mancozeb 75% WP (T2) (35.33%) and tebuconazole 50% + trifloxystrobin 25% WG (36.33%) (T4). In contrast to the untreated control (T7), these treatments reduced the disease by 46.11, 45.21 and 43.66%, respectively. Propiconazole 25% EC (T3), fluxapyroxad 167 g L^{-1} + pyraclostrobin 333 g L^{-1} SC (T5) and azoxystrobin 11% + tebuconazole 18.3% SC (T6) recorded PDI values of 37.91, 39.00 and 40.08%, which, in comparison to the untreated control, represented disease reductions of 41.21, 39.52 and 37.84%, respectively.

The fungicidal treatments had AUDPC values ranging from 562.43 to 627.48, while the untreated control had 1038.64 (Table 3). The lowest AUDPC (562.43) and relative AUDPC (54.15%) were recorded by chlorothalonil 78.12% WP (T1), followed by mancozeb 75% WP (T2) (577.73 and 55.62%) and tebuconazole 50% + trifloxystrobin 25% WG (T4) (593.60 and 57.15%), demonstrating their superior ability to inhibit the development of brown leaf spot disease throughout the cropping period. In contrast, following the third spray, the untreated control (T7) showed the greatest AUDPC (1038.64) and the highest disease severity (64.48%).

Effect of fungicides on yield attributes

Fungicidal treatments significantly influenced potato tuber yield and yield attributes (Table 4) (Fig. 6). Among different fungicides, the highest number of tubers per plant (16.62), maximum tuber size (50.91 mm), tuber weight per plant (204.15 g), tuber weight per plot (6.11 kg/6 m^2 of plot) and tuber yield (28.06 t ha^{-1}) were recorded with chlorothalonil 78.12% WP (T1). Additionally, this treatment had the highest yield response ratio (1.83), largest yield increase over control (82.56%), maximum yield gain over the untreated control (12.69 t ha^{-1}) and highest relative yield index (1.18). Mancozeb 75% WP (T2) produced 14.40 tubers $plant^{-1}$, 48.49 mm tuber size, 200.83 g tuber weight per plant, 5.96 kg tuber weight per plot and 26.90 t ha^{-1} tuber yield and become the next most successful treatment. Tebuconazole 50% + trifloxystrobin 25% WG (T4) recorded a tuber yield of 26.09 t ha^{-1}, demonstrating good performance then followed by fungicide azoxystrobin 11% + tebuconazole 18.3% SC (T6) (24.74 t ha^{-1}), fluxapyroxad 167 g L^{-1} + pyraclostrobin 333 g L^{-1} SC (23.04 t ha^{-1}) (T5) and propiconazole 25% EC (T3) (22.72 t ha^{-1}). The untreated control (T7) exhibited the lowest observations for all yield parameters, with just 7.20 tubers per plant, a tuber size of 27.30 mm, a tuber weight of 165.66 g per plant, and tuber yield of 15.37 t ha^{-1}.

Table 3 Field efficacy of fungicides for the management of brown leaf spot of potato under natural epiphytotic conditions

Fungicides	Treatment	Percent Disease Index (PDI)				Disease reduction over control (%)	AUDPC	Relative AUDPC (%)
		After 1st spray	After 2nd spray	After 3rd spray	Mean			
Chlorothalonil 78.12% WP	T1	19.66	26.00	34.75	26.80	46.11	562.43	54.15
Mancozeb 75% WP	T2	18.33	27.33	35.33	27.00	45.21	577.73	55.62
Propiconazole 25% EC	T3	21.33	28.00	37.91	29.08	41.21	611.51	58.88
Tebuconazole 50% + Trifloxystrobin 25 WG	T4	17.66	28.33	36.33	27.44	43.66	593.60	57.15
Fluxapyroxad 167 g L^{-1} + Pyraclostrobin 333 g L^{-1} SC	T5	22.33	27.33	39.00	29.55	39.52	627.48	60.41
Azoxystrobin 11% + Tebuconazole 18.3% SC	T6	17.66	28.33	40.08	28.69	37.84	614.23	59.14
Control (untreated)	T7	30.00	49.00	64.48	47.83	-	1038.64	100.00
C.D. (P=0.05)		4.04	5.33	5.32	-	-	-	-
SE(m)		1.29	1.77	1.70	-	-	-	-
C.V.		10.67	10.19	7.24	-	-	-	-

(a). Disease reduction over control (%) was calculated based on percent disease index recorded after the third spray, (b). Area under disease progress curve (AUDPC) was calculated according to Campbell and Madden (1990) using the trapezoidal integration method and (c). Relative AUDPC (%) was calculated by expressing treatment AUDPC values as a percentage of the AUDPC value of the untreated control.

Table 4 Effect of fungicides on yield related attributes of potato under field conditions in response to brown leaf spot disease

Fungicides	Treatment	Number of tubers per plant^{-1}	Tuber size (mm)	Tuber weight per plant^{-1} (g)	Tuber weight per plot^{-1} (kg)	Tuber yield (t ha^{-1})	Yield gain over control (t ha^{-1})	Yield response ratio	Increase in yield over control (%)	Relative yield index
Chlorothalonil 78.12% WP	T1	16.62	50.91	204.15	6.11	28.06	12.69	1.83	82.56	1.18
Mancozeb 75% WP	T2	14.40	48.49	200.83	5.96	26.90	11.53	1.75	75.01	1.13
Propiconazole 25% EC	T3	11.77	43.52	189.33	4.96	22.72	7.35	1.48	47.82	0.95
Tebuconazole 50% + Trifloxystrobin 25 WG	T4	12.90	46.71	197.66	5.86	26.09	10.72	1.70	67.74	1.09
Fluxapyroxad 167 g L^{-1} + Pyraclostrobin 333 g L^{-1} SC	T5	9.58	36.54	178.75	4.06	23.04	7.67	1.50	49.90	0.97
Azoxystrobin 11% + Tebuconazole 18.3% SC	T6	10.27	42.09	186.73	4.76	24.74	9.37	1.61	60.96	1.04
Control	T7	7.20	27.30	165.66	3.57	15.37	0.00	1.00	-	0.64
C.D. (P=0.05)		1.77	3.40	15.61	0.734	3.09	-	-	-	-
SE(m)		0.57	1.09	5.25	0.238	0.99	-	-	-	-
C.V.		8.39	4.48	7.35	8.16	7.20	-	-	-	-

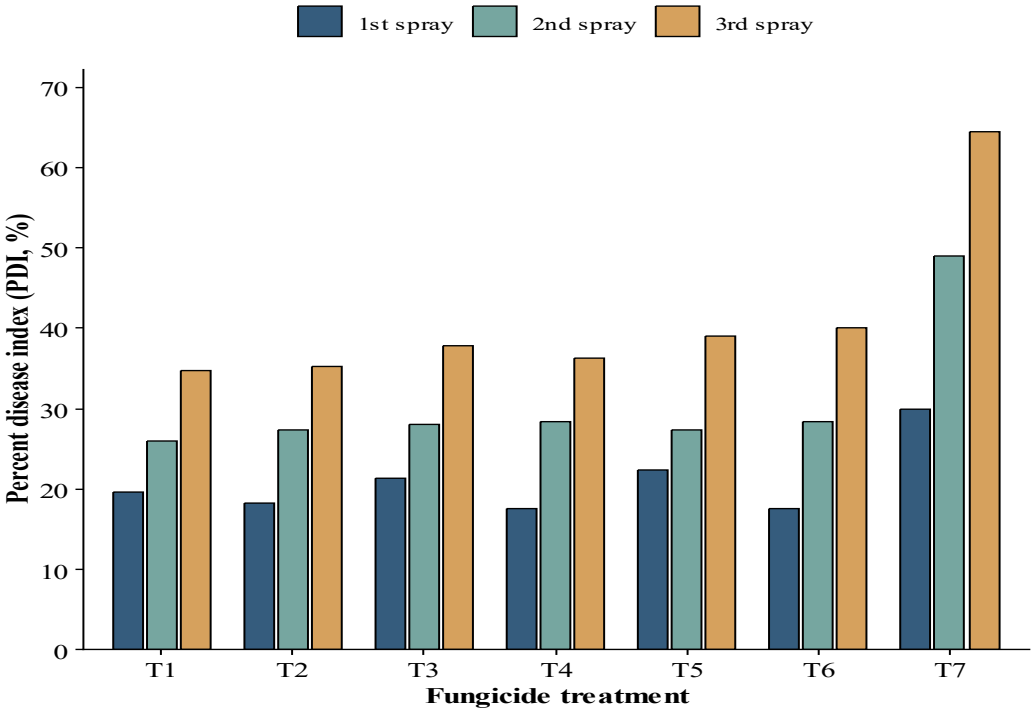


Fig. 5 Progression of potato brown leaf spot following successive fungicide sprays under field conditions

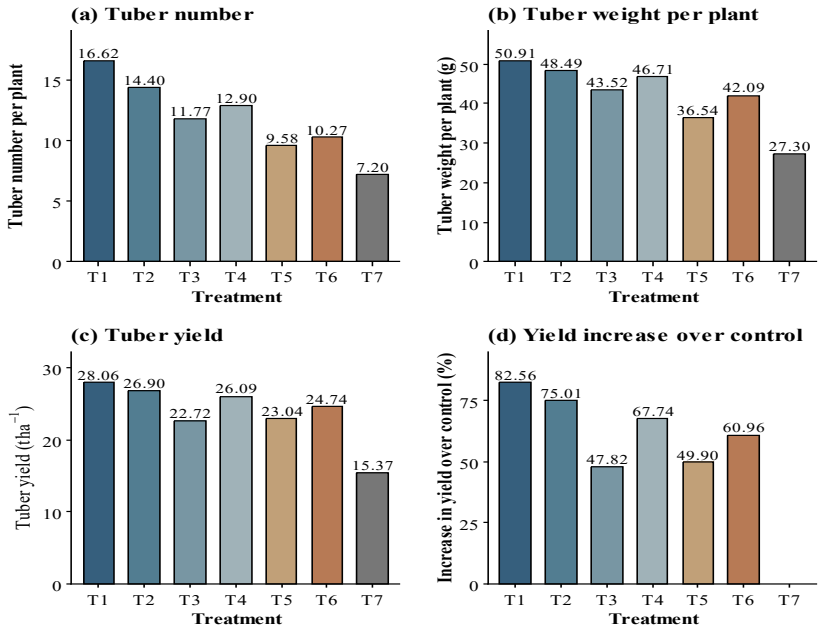


Fig. 6 Effect of fungicide treatments on yield related attributes of potato: **a** tuber number per plant, **b** tuber weight per plant, **c** tuber yield and **d** increase in yield over the untreated control.

Economics

The economic analysis (Table 5) showed significant variation amongst fungicidal treatments. Mancozeb 75% WP exhibited the highest benefit:cost (BC) ratio (2.54) and incremental cost-benefit ratio (ICBR) (20.50) because to its relatively lower application cost, despite chlorothalonil 78.12% WP producing the highest gross return (₹2,80,600 ha^{-1}) and net return (₹1,67,424 ha^{-1}). Tebuconazole 50% + trifloxystrobin 25% WG exhibited a B:C ratio of 2.21 with a net return of ₹1,42,900 ha^{-1}, while azoxystrobin 11% + tebuconazole 18.3% SC exhibited a B:C ratio of 2.14. Despite effectively controlling disease, fungicide fluxapyroxad 167 g L^{-1} + pyraclostrobin 333 g L^{-1} SC had the lowest economic return among the fungicidal treatments because of its high application cost, yielding an ICBR of 1.20 and a B:C ratio of 1.40. The untreated control had the lowest B:C ratio of 1.53, net return of ₹53,700 ha^{-1} and gross return of ₹1,53,700 ha^{-1}.

Table 5 Economics

Fungicides	Cost of fungicides (₹ ha^{-1})	Cost of cultivation (₹ ha^{-1})	Total cost of cultivation (₹ ha^{-1})	Tuber yield (t/ha)	Gross return (₹ ha^{-1})	Additional return over control (₹ ha^{-1})	Net return (₹ ha^{-1})	B:C ratio	ICBR
Chlorothalonil 78.12% WP	13,176	1,00,000	1,13,176	28.06	2,80,600.00	1,26,900	1,67,424.00	2.47	9.63
Mancozeb 75% WP	5,625	1,00,000	1,05,625	26.90	2,69,000.00	1,15,300	1,63,375.00	2.54	20.50
Propiconazole 25% EC	16,080	1,00,000	1,16,080	22.72	2,27,200.00	73,500	1,11,120.00	1.95	4.57
Tebuconazole 50% + Trifloxystrobin 25 WG	18,000	1,00,000	1,18,000	26.09	2,60,900.00	1,07,200	1,42,900.00	2.21	5.96
Fluxapyroxad 167 g L^{-1} + Pyraclostrobin 333 g L^{-1} SC	63,675	1,00,000	1,63,675	23.04	2,30,400.00	76,700	66,725.00	1.40	1.20
Azoxystrobin 11% + Tebuconazole 18.3% SC	15,600	1,00,000	1,15,600	24.74	2,47,400.00	93,700	1,31,800.00	2.14	6.01
Control (untreated)	0.00	1,00,000	1,00,000	15.37	1,53,700.00	-	53,700.00	1.53	-

(a). Gross return was calculated considering the prevailing market price of potato tubers at ₹10,000 t^{-1}. (b). ICBR was calculated as the ratio of additional return over control to the cost of fungicides.

Discussion

Based on colony morphology, conidial traits and ITS sequence analysis, the pathogen isolated from symptomatic potato leaves was determined to be *A. alternata*. The morphological characteristics observed in the present study, including obclavate to pyriform conidia with

transverse septa and grey to dark olivaceous colonies were consistent with the descriptions illustrated by Simmons (2007). The pathogen's identity was further confirmed by ITS sequence analysis, which also concurred with the phylogenetic classification of *Alternaria* spp., published by Woudenberg et al. (2015). Lingwal et al. (2022) have previously reported similar morphological and molecular traits of *A. alternata* linked to potato leaf spot. In the genus *Alternaria*, where morphological overlap between taxa is frequent, the combination of morphological and molecular techniques has been regarded as a trustworthy method for species identification (Woudenberg et al. 2015).

The mycelial growth of *A. alternata* was considerably reduced by all fungicides tested *in vitro*, however their effectiveness varied depending on the active ingredient and dose. Complete suppression by fungicide fluxapyroxad + pyraclostrobin at all tested dosages demonstrated the pathogen's great sensitivity to fungicides based on succinate dehydrogenase inhibitor (SDHI) and quinone outside inhibitor (QoI), as they interfere with fungal respiration and ATP synthesis by blocking various locations in the mitochondrial electron transport chain. Avenot and Michailides (2010) have emphasized the effectiveness of fungicides including succinate dehydrogenase inhibitor (SDHI) against *Alternaria* spp., which consistent with the complete inhibition demonstrated by fluxapyroxad + pyraclostrobin in present investigation. Similarly, at relatively low doses, propiconazole 25% EC, tebuconazole 50% + trifloxystrobin 25% WG and azoxystrobin 11% + tebuconazole 18.3% SC showed complete inhibition. While propiconazole's activity is linked to the inhibition of ergosterol biosynthesis, the complementing mechanisms of action of triazole and strobilurin fungicides may account for the high efficacy of the combination fungicides. Pasche et al. (2004) published similar results, showing that *Alternaria* spp. were highly sensitive to QoI fungicides. Similarly, Park et al. (2024), demonstrated the efficacy of several fungicides against *Alternaria* spp., infecting potato. However, they also noted resistance to the QoI fungicide kresoxim-methyl, underscoring the necessity of ongoing sensitivity monitoring. Additionally, the considerably greater effectiveness of mancozeb and chlorothalonil among the contact fungicides in this study underscores the ongoing significance of multisite fungicides in integrated disease control and fungicide resistance management.

When compared to the untreated control, all fungicidal treatments considerably decreased the severity of the disease under field settings. The superior performance of chlorothalonil in present

study might be due to its multisite mode of action and long lasting protective activity on foliage, which successfully limit pathogen infection and disease progression. Leiminger and Hausladen (2012) showed similar decreases in disease severity in potatoes after fungicide application. Fungicide rotation and the combination of multisite and site-specific fungicides are crucial for lowering selection pressure and delay in the development of fungicide resistance has been widely emphasized (Corkley et al. 2022).

Potato brown leaf spot was effectively suppressed in fungicide treated plots, which resulted in a better tuber production. It appears that effective disease control maintained the functional green leaf area and extended canopy photosynthetic activity during tuber bulking because chlorothalonil, which had the lowest disease severity and AUDPC, also provided the highest tuber production. In a similar vein, the increased yield attained with mancozeb highlights even more how important efficient disease control is to optimizing potato productivity. Similarly, improvements in disease suppression and tuber yield by fungicide application have been reported in case of *A. solani* infecting potato crop (Sharma et al. 2022).

The generation, partitioning and translocation of photoassimilates to developing tubers were probably improved by maintaining a positive source-sink relationship, which improved tuber growth and overall productivity (Li et al. 2016). Additionally, Kapsa (2008) emphasized the need of integrated disease management in reducing losses brought on by main potato pathogens. Economic analysis demonstrated that mancozeb demonstrated improved economic efficiency due to its higher benefit:cost ratio and lower application cost, whereas chlorothalonil maximized returns. According to these results, both fungicides are practical and affordable solutions for controlling *A. alternata* induced potato brown leaf spot in *tarai* ecosystem of Uttarakhand, India.

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Contribution

Conceptualization: A Sharma, S Sharma, R Tewari; Methodology: A. Sharma, S. Sharma; Formal analysis and investigation: A. Sharma; Writing-original draft preparation: A. Sharma; Writing-review and editing: S. Sharma, R. Tewari; Resources: S. Sharma, R. Tewari; Supervision: S. Sharma, R. Tewari. All authors commented on previous versions of the manuscript and approved the final manuscript.

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Declarations

The authors state that there are no recognized conflicts of interest.

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