Journal of Natural Science Review

Vol. 3, No. 1, 2025
https://kujnsr.com
e-ISSN: 3006-7804

Evaluation of the Effect of Different Fungicides on Potato Brown Leaf Spot *Alternaria alternata* in Greenhouse

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ABSTRACT

One of the successful strategies to increase the yield of potatoes is the management of diseases and pests during the whole growing period. Potato brown spot caused by *Alternaria alternata* (Fr) Keissler is one of the most destructive diseases of potatoes worldwide. The present study compared two different fungicide programs under greenhouse conditions during 2022. Firstly, the resistance of five local potato varieties (Lora, Kofre-hemaloni, Kofre-Gibson, Evalovetion, and Morabell) was evaluated under greenhouse conditions to the pathogen to find the most susceptible variety for further experiment. The degree of infection by the pathogen was calculated. The study demonstrated that the Lora variety had the most tolerance, and the Morabell variety was sensitive to the pathogen. Then, the effects of three common chemical fungicides (Mancozeb®80%, Penconazole®20%, and Topguard®30% (Flutriafol (6.94% + tebuconazole (20.8%) were tested against the pathogen using the most sensitive potato variety (Morabell) in CRD (Completely Randomized Design) under greenhouse condition. The means of infection was calculated over ten days. Specific doses of the fungicides had a significant effect in reducing disease. The severity of the disease decreased with the increased use of all the applied fungicides. The study revealed that Mancozeb and Penconazole with 1g per liter doses were the most effective treatment in controlling brown leaf spots in potatoes.

ARTICLE INFO

Article history:

Received: June 10, 2024
Revised: March 02, 2025
Accepted: March 16, 2025

Keywords:

Alternaria alternata; Fungicide; Mancozeb; Penconazole;Potato, Topguard

To cite this article: Bahlolzada, H., Jamily, A. S., Yousufzai, A., Saleem, S., & Nabizada, F. (2025). Evaluation of the Effect of Different Fungicides on Potato Brown Leaf Spot Alternaria alternata in Greenhouse. *Journal of Natural Science Review*, *3*(1), 76–86. https://doi.org/10.62810/jnsr.v3i1.64

Link to this article: https://kujnsr.com/JNSR/article/view/64

INTRODUCTION

Because of their high yield potential and food value, potatoes (Solanum tuberosum L) are an important agricultural product (Koch et al., 2020). The food value of potatoes in Afghanistan is significant (FAO, 2022) both in terms of production and consumption, where annual production reaches more than 600,000 metric tons (MAIL. 2020) unpublished data. Afghanistan's annual agriculture sector report shows that the highest quantity of potatoes in Afghanistan is produced in Bamyan Province. Potato production is about 266,560 metric tons in Bamyan province, and the area under potato cultivation is 16,660 ha (Anonymous, 2021).

Alternaria spp. It causes several diseases in potato leaves and stems. To prevent or delay the development of fungicide resistance, alternate or combine fungicides with different modes of action (Odilbekov et al., 2019). Potato brown leaf spot disease is caused by *A. solani* and *A. alternata* (Jones et al., 1993). Diseases caused by *A. solani* usually appear as leaf spots and blight, which causes wilting, stem rot, and tuber or fruit rot. The leaf spot is usually dark, brown to black (Jones et al., 1993). Early susceptibilities of *A. solani* to difenoconazole and metronidazole (dimethylation inhibitors) using the mycelium growth assay showed similar intrinsic activity against the pathogen with mean EC50 (effective concentration at which fungal growth is inhibited by 50%) with values. It showed 0.09 µg/ml (Fonseka et al., 2016). Research conducted in the Hamedan province of Iran shows that, from 300 isolates, most of the species were identified as *A. alternata* (Fr.) Kessler in various plants, including potatoes (Bagherabadi et al., 2015). The leaf spots develop over time, and usually, their symptoms spread in the form of concentric circles, similar to those of a shooting target. Early blight destroys 38-78% of potatoes and tomato yield annually in the United States of America, Palestine, England, and India (Jones et al., 1993). Brown leaf spot (*A. alternata*) is a significant problem in potato, tomato, and eggplant production (Jindo et al., 2021; Agrios, 2005). Potato is susceptible to early blight, which is mainly caused by *Alternaria solani* Ellis and Martin (Hooker, 1981). This disease caused a decrease in yield and has been reported in Africa, Asia, Australia, Europe, North America, Central America, and South America (Miller and Pollard, 1976; CABI, 2020). Various species of *Alternaria* are the cause of potato early blight disease. Various studies revealed that *Alternaria* significantly reduces crop yields in most countries (Mirkarimi et al., 2013).

Chemical fungicides are the most meaningful management strategies for controlling early blight pathogens (Madadi et al., 2021; Rodriguez et al., 2022). Several systemic and non-systemic fungicides are involved in the control of potato brown leaf spot (Rodriguez et al., 2022). Alidadi et al. (2020) reported that in a field experiment, Mancozeb 75% WP fungicide at a dosage of 0.25% and Hexaconazole EC 0.05% were applied to control late blight in potatoes, in which Mancozeb 75% WP demonstrated greater efficacy in controlling late blight (Patel et al., 2019). According to Sharifnabi (2021), Mancozeb was an effective fungicide controlling potato brown leaf spot disease. This study aims to determine the minimum effective dose of certain fungicides against brown leaf spot pathogens. It investigates the maximum efficacy of fungicides at the lowest doses on potato brown leaf spot. Fungicides such as Topguard (Flutrifol 6.94% + Tebuconazole 20.8%), Penconazole, and Mancozeb were evaluated in greenhouse conditions against the potato brown leaf spot pathogen, and their efficacy was evaluated. The main objective of this study is to conduct a pathogenicity test of the pathogen through the method of spore application on sensitive potato variety to verify its successful infection process in *vivo* and to evaluate the efficacy of common fungicides in the control of potato brown spot pathogen.

MATERIALS AND METHODS

Environmental conditions for growth

The present study was conducted under the greenhouse conditions at the agriculture research farm of Kabul University. In the present study, five local potato varieties (Lora, Evalovetion, Kofrehemaloni, Kofregepsonum, and Morabell) were planted in 4-liter pots filled with agricultural soil. After 45 days of growth, the plants were inoculated with the fungal pathogen *A. alternata* to find the most susceptible variety. The pots were arranged in CRD, and each treatment was replicated using trice. The plants were irrigated regularly every four days until the soil in the pots was saturated. Next, the chemical fungicides (Mancozeb®80%, Penconazole®20%, and Topguard®30% (Flutriafol (6.94% +tebuconazole (20.8%) were used in amounts of 0.2, 0.5, and 1g per liter doses to eliminate brown leaf spots (Table,1). A total of 36 pots were prepared with the Morabell variety. There were 10 treatments; each replicated trice in a CRD design.

Table 1. Characteristics of different fungicides used against potato blight under greenhouse conditions

Common name	Mode of Action	Trade name	Chemical name
Penconozole	Systemic	Penconazole® 20% WE	1-[2-(2,4 Dichlorophenyl) pentyl]-1H-1,2,4-triazole
TopGuard (flutriafol (6.94%)+ tebuconazole (20.8%)	Systemic	TOPGAURD® 30% SC	1H-1,2,4-Triazole-1-ethanol, α-(2-fluorophenyl)-α-(4-fluorophenyl) (RS)-1-(4-Chlorophenyl)- 4,4-dimethyl-3-(1H, 1,2,4-triazole-1-ylmethyl) pentane- 3-ol
Mancozeb	Contact	Qadri Mancozeb® 80% WP	((2-((dithiocarboxy) amino) ethyl) carbamodithioato)) (2-)- kS,kS')manganese mixture with((2-((dithiocarboxy)amino) ethyl)carbamodithioato))(2-)- kS,kS')zinc

Isolation and identification of the fungal pathogen

Fungal isolates were obtained from naturally infected potato leaves collected from the agriculture research farm of Kabul University, showing similar early blight symptoms, including a bull eye pattern in which centric rings with brown to black spots appeared on the infected leaves. However, the size of these lesions was relatively smaller than a typical early blight symptom. The infected potato leaves were cut into small pieces with healthy margins of about 1-1.5 cm. Surface sterilization of these cutting leaves was carried out using 0.1% NaHOCl3 for approximately three min., washed three times with sterile distilled water, dried up between sterilized filter paper tissue, and then placed aseptically on the surface of potato dextrose agar (PDA) plates (250 g/l potatoes, 2 % dextrose, 1.5 % agar), supplemented with Hygromycin 1 ml/ l; to avoid bacterial contamination. These plates were incubated at 26 ±1°C for one week (Zheng et al., 2015). After incubation, fungal purification was carried out using a single-spore technique (Hansen, 1926).

Culture of the pathogen and its purification

To purify the Alternaria alternata, the mycelium was first obtained from the Plant Protection Department, Agriculture Faculty, Kabul University laboratory. The purification was repeated twice, and the morphology of the culture colony was studied to minimize the risk of identification. To identify morphologically, the mycelium was incubated at 24°C in PDA media, forever put for spore germination, under white fluorescent light with photoperiod for 8 hours at the light and 16 hours in darkness for 7 days. Morphological identification was made using the identification key of Ghosta et al. (2003). The Macroscopic and microscopic characteristics of *Alternaria alternata* were also identified (Ghosta et al., 2003).

Macroscopic characteristics

After 7 days, the mycelium of *A. alternata* on the PCA (potato carrot agar) culture medium exhibited a dark gray-brown color. The mycelium grew both above and on the surface of the culture medium. The aerial filaments of the mycelium did not alter the color of the culture medium. The conidiophores were single and sometimes branched at the ends, with conidia forming clusters on the conidiophores. The mycelium and spores covered the culture surface uniformly, without forming concentric circles, due to the difference in sporulation between darkness and light (Fig. 1).

Conidiophores from yellow up to light brown are mostly simple, short, 61-91 micrometers long, and have knee-like bends at the end. Short Secondary conidiophores chain conidia were branched and often single-celled (Fig. 1). It was similar to the studies conducted by other researchers (Ghosta et al., 2003; Madadi et al., 2021). The isolate was consistent with the previous sample identified by experts from the Plant Protection Department of the Faculty of Agriculture, Kabul University (Madadi et al., 2021).

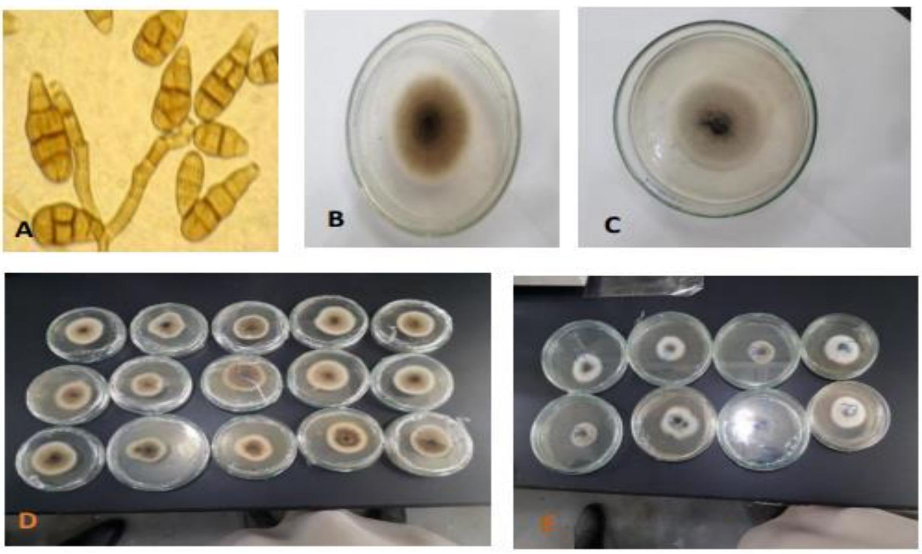

Figure 1. (A) Conidia and conidiophores of A. alternata. (B, C, d, and E) mycelium color in PDA culture after 7 days

Pathogenicity test

The 45-day-old potato plants were prepared in 36 pots under greenhouse conditions arranged in CRD design. There were 10 treatments (the three fungicides, each with three dosses and a water control), each replicated trice. However, before sporulation, the potato leaves were thoroughly washed, and 300 ml spore solution of *A. alternata* amount of 10^7 spore/ml was sprayed on the potato leaves. The potato plants were kept under the growth chamber in humidity of 90-95% and temperature between 23-30 °C for 48 h. Primary symptoms of leaf spot appeared after two days in potatoes; its severity t was recorded daily for ten days according to the grading methods of Rodríguez (2007), as detailed in Table.2

Table 2. Grading method of potato blight under greenhouse conditions

Description of symptoms	Rating of severity
Spots on lower leaves	10
Spots on most of the lower leaves	20
Spots on all lower and some of the middle leaves	30
Developed blight lesions in lower leaves	40
Blight lesions in lower leaves spread to some middle leaves.	50
Blight lesions developed in all inferior and most of the middle leaves.	60
Blight lesions developed in all lower and middle leaves.	70
Blight lesions developed in all lower and middle leaves and spread to upper leaves.	80
Total blight (death of the plant)	100

Statistical analysis

Data were subjected to statistical analysis using the Statistical Analysis System (SAS9.4.). Means were analyzed using least significant differences (LSD) at a significance level of ($p < 0.05$). Also, the statistical analysis system (SPSS9.1) was used to determine the effect of three types of chemicals with different doses on potato leaf spot disease; in this test, Mean rank was analyzed by non-parametric method, using the Kruskal Wallis test at the level of $p_{<}$ 0.01.

RESULTS

Resistance of varieties

In the present study, the Laura variety, with an average disease intensity of 5.33%, was recognized as the most resistant. Evalovetion variety with 30% disease severity was recognized as the second resistant variety, Kofre-gebsonum and Kofre-hemaloni varieties with 50% disease severity were calculated as sensitive varieties, and the Morabell variety with 56% disease severity was recognized as the most sensitive variety ($p < 0.05$) (Fig. 2, and Table 3).

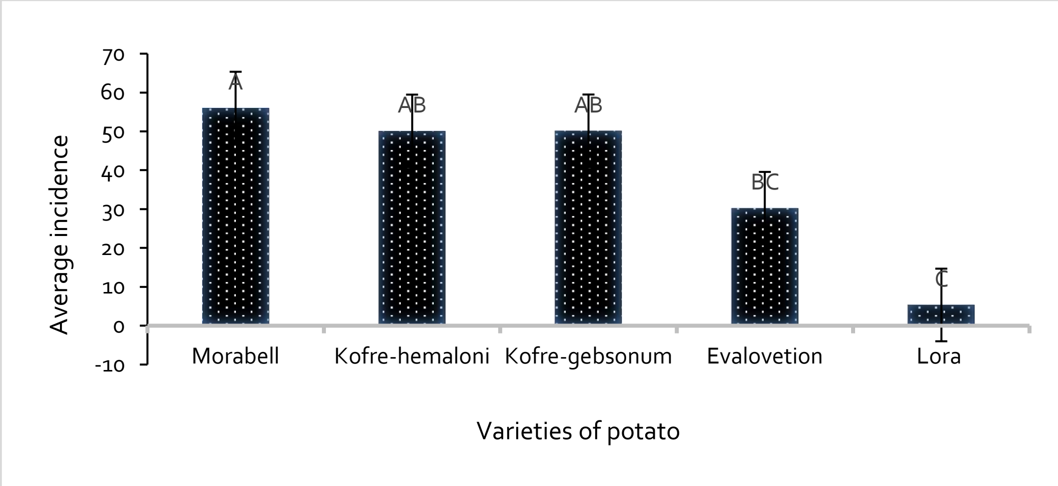

Figure 2. The infection rate of A. alternata on different potato varieties under greenhouse conditions

Figure3. Potato brown leaf spot Symptoms developed on the leaves of the Morabell variety after 10 days' inoculation with A. alternata

Table 3. Comparison of the average incidence of the disease on the leaves of inoculated varieties with the A. alternata

Varieties of potatoes	Average incidence
Morabell	56 ± 0^{a}
Kofre-hemaloni	$50 \pm 0a^{b}$
Kofre-gebsonum	50 ± 0^{ab}
Evalovetion	$30+0b^{c}$
Lora	$5+33^{c}$

This means that the same letters in the same column are not statically different at p <0.05 LSD test.

Efficacy of fungicides

The study revealed that, on the tenth day of fungicide application, the treatment containing 1g per liter of Mancozeb®80% had a greater inhibitory effect than all other treatments, including control and other chemicals of the same dose (Tables 4 and 5). Penconazole®20% with a concentration of 1 g per liter was found on par with Mancozeb ®80% in inhibitory effect on potato brown leaf spot.

Table 4. Comparison of the effect of different doses of fungicides on the severity of the potato brown leaf spot disease during 10 days after application

Fungicides	Dose (g/lit)	Disease severity % (days after application)				
		2	4	6	8	10
mancozeb®80%	0	7.78ab	9.18a	12.52a	20.56a	24.89a
	0.2	6.89ebcd	8.81ab	10.96ab	17.85cb	18.11b
	0.5	6.22efd	6.08d	8.11ced	9.44e	11.11cd
	1	5.85f	5.92d	6.89e	8.11e	9.19de
penconazole®20%	0	7.44abc	8.63ab	12.85a	19.18ab	25.36a
	0.2	8.07a	8.44ab	9.96cb	15.78cd	17b
	0.5	6.89ebcd	6.63cd	8.67ced	9.22e	10.44cde
	1	5.93ef	6.33d	8.21ced	8.67e	8.66e
topguard®30%(flutriafol(6.94%+tebuconazole(20.8%)	0	7.18abcd	9.48a	12.14a	18.48ab	25.44a
	0.2	7.4abc	8.74ab	9.55cbd	14.85d	16.41b
	0.5	7.26abc	7.81cb	9.11cbd	10.44e	12.44c
	1	6.55efcd	6.18d	7.78ed	9.78e	11.29cd

This means that the same letters in the same column are not statically different at p <0.05 LSD test.

Table 5. Analysis of the inhibitory effect of three types of chemicals with different doses on A. alternata

Different doses of fungicides	N	Mean Rank
Mo	15	124.40
Po	15	119.97
To	15	122.53
M1	15	110.47
P1	15	110.07
T1	15	108.00
M2	15	64.90
P2	15	72.00

T2	15	89.97
M3	15	42.70
P3	15	52.63
T3	15	68.37
Total	180	
Chi-Square	51.632	
Df	11	
Asymp. Sig.	.000	

at $p < 0.01$ Kruskal Wallis Test Fungicide type are Grouping Variable. M= Mancozeb, P=Penconazole, T=Topguard, 0= 0g, 1=0.2g, 2=0.5g, 3=1g per liter

Figure 4. A: Morabell Potato Variety inoculated with A. alternata suspension as a control, B: Morabell potato variety, treated with 1g Mancozeb®80%fungicide after inoculated with A. alternata suspension for ten days

DISCUSSION

In the pathogenicity test, almost all the tested potato verities were infected with the pathogen in some degrees, among which the Morabell variety showed high disease incidence and was thus selected for further evaluation of the fungicide efficacy on potato brown leaf spot pathogen. In this study, the Lora variety has shown more tolerance than other verities, and the other three verities showed similar sensitivities; these results are in agreement with the results obtained by Mirkarimi et al. (2013), who conducted the same research on seven potato varieties in 2013 and stated that all the varieties have showed a different level of resistance against potato early blight diseases.

All fungicide treatments of lower dose (0.2 gm), medium dose (0.5 gm), and higher dose (1 gm) reduced the occurrence of potato brown leaf spot pathogen, respectively. The results were similar to the results expected by Iqbal et al. (2019) and the results obtained by Chourasiya et al. (2013) in both studies, the fungicide Mancozeb with a high dose (1 gm/L) had maximum effects in reduction of the severity of the disease. Also, Gondal et al. (2012) reported that Mancozeb application at a dose of 1 gm/L significantly reduced the potato brown spot pathogen incidence. Although previously, the use of different fungicides on spore germination and mycelium growth of the pathogen *A. alternate* in PDA culture media was reported by Madadi et al. (2021).

The second best fungicide for controlling potato brown spot disease under greenhouse conditions was found to be Penconazole at a dose of 1 gm/L; the results following the results of Chourasiya et al. (2013) in which the application of Penconazole successfully reduced the pathogen severity.

CONCLUSION

Evaluation of the resistance of five potato varieties against the fungus that causes brown leaf spot A. alternata. It was done in 2021. 45-days-old potato plants were arranged in the CRD Factorial design and inoculated with a seven-days-old spore solution of A. alternata. The observations showed that all the treatments affected potato to brown leaf spot and there is a significant difference between the treatments. In this research, Lora variety was evaluated as tolerant compared to other varieties. The effects of three common fungicides in the Afghan market with three different doses were investigated. The results show that the most effective fungicide was Mancozeb, which was used with the highest dose in this research (1g/L), followed by the fungicide Penconazole with a dose 1 g/L. Our study suggested Mancozeb and Penconazole that have superior inhibitory effects on the target pathogen even in a minimum concentration of 1g/L. Our future prospectus is to carry out field experiments, to find out the lowest effective concentration of the mentioned fungicides under in vivo condition, and to avoid from environmental pollution

Acknowledgments

We gratefully acknowledge the support from the Agriculture Faculty of Kabul University.

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