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Evaluation of *in vitro* antifungal potential of several fungicides against *Alternaria alternata* (Fr.) Keissler, the causal agent of potato brown spot in Afghanistan

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Abstract

Potato brown spot caused by *Alternaria alternata* (Fr.) Keissler is one of the most destructive diseases of potato worldwide. The present study was conducted to identify the causal agent, and to test the efficacy of several fungicides with different modes of actions against *A. alternata*. Five different chemical fungicides including; Copper oxychloride (Sufer Copper Oxychloride® 50% WP), Carbendazim (Carbendazim Aria® 60% WP), Penconazole (Penconazole® 20% WE) , Mancozeb (Qadri Mancozeb® 80% WP) and Flutriafol 6.94% + Tebuconazole 20.8% (Topgaurd® 30% SC) were tested at four different concentrations of; 20, 100, 300 and 500 mg\ l, using the poisoned food technique. Among the tested fungicides, the highest *in vitro* inhibition (%) of mycelial growth of the pathogen was recorded on using Flutriafol 6.94% + Tebuconazole 20.8% causing complete inhibition (100 %) at 100, 300 and 500 mg\ l, followed by Penconazole causing 100 % inhibition at 300 and 500 mg\ l; moreover, it caused strong inhibition of 90.17 % at 100 mg\ l. Both fungicides proved to be the most effective expressing strong inhibition potency of 93.75 % even at the lowest concentration of 20 mg\ l after 5 days of fungal incubation, although the inhibitory efficacy decreased slightly over time after 10 days of incubation. Mancozeb was the third effective fungicide causing strong mycelial inhibition of (41.91, 75.24 and 84.21 %) at 100, 300 and 500 mg\ l, respectively. The least mycelial growth inhibition was observed with Copper oxychloride (8.77 %) followed by Carbendazim (21.05 %) at maximum concentration of 500 mg\ l. For the best of our knowledge, this is the first report of *A. alternata* as the fungal causal agent of potato brown spot in Afghanistan.

Keywords: *Alternaria alternata*, Fungicides, Poisoned food technique, Mycelial growth inhibition

1. Introduction

Potato (*Solanum tuberosum* L.) is one of the most valuable food crops in terms of quantities produced and consumption worldwide (Murmu *et al*., 2017). After the important staple cereal crops such as; wheat

(*Triticum aestivum* L.), rice (*Oryza sativa* L.) and maize (*Zea mays* L.), potato has the highest production worldwide (Tsedalye, 2014), and is ranked the 6th in terms of world most produced food commodity, as reported by Cook, (2015). In Afghanistan, it is also one of the most important staple vegetables both in terms of production and consumption, where annual production reaches more than 600,000 metric tons (MAIL. 2020) (Unpublished data).

Early studies conducted by Tsedalye, (2014); Ahmed, (2017) revealed that among hundred diseases of potato, foliar diseases (i.e. early blight and/or brown spot) are the most important and destructive diseases worldwide, particularly in areas with favorable weather conditions, where it causes reduction in quantity and quality of this crop. These foliar diseases are also reported from different potato regions in Afghanistan, as proposed by Popal, (2006); CABI. (2020).

Early blight and brown spot, which were considered as the same disease by Leiminger and Hausladen, (2012); Kapsa, (2009); Paurson *et al*., (2015), can damage potato foliage and tubers. Early blight can cause yield losses of 5- 50 % (Tsedalye, 2014), and in some continents it reaches up to 73 % (Kapsa, 2009). On the other hand, recent study of Ahmed, (2017) highlighted that crop losses due to brown spot are around 20 %, but in the case of severe infection or when combined with early blight, it can cause 70-80 % losses to potato production.

Two species of the genus *Alternaria*; *A. solani* and *A. alternata* are the causal agents of early blight and brown spots diseases, respectively (Nottensteiner *et al*., 2019). Both *Alternaria* spp. differ in some morphological features including; mycelium color and mycelium growth rate on the growth media, spore structures and temperature requirements. In the course of disease development, the morphological symptoms of both diseases are difficult to distinguish, thus both were considered as the causal agents of early blight (Kapsa, 2009; Zheng *et al*., 2015; Paurson *et al*., 2015). *A. alternata* has several pathotypes which cause number of diseases in other plants such as; stem canker of tomato (Grogan *et al*., 1975), *Alternaria* black spot of strawberry (Tsuge *et al*., 2011), leaf spot and fruit rot of chilli (Ginoya and Gohel, 2015), leaf spot of bean, black point of wheat (Cromey and Mulholland, 1988), stem rot of mango (Li *et al*., 2018), and *Alternaria* fruit rot of persimmon (Prusky *et al*., 1981).

Several chemicals, derivatives of both systemic and non-systemic fungicides and various plant extracts have been studied *in vitro* and *in vivo* on both potato and tomato early blight, to find proper fungicides and\or plant extracts with proper rate and proper application time (Ghazanfar *et al*., 2016; Murmu *et al*., 2017; Nashwa and Abo-Elyousr, 2012). The majority of these studies focused on *A. solani*, however few research have been reported on *A. alternata* as the causal agent of brown spot/early blight of potato (Kapsa, 2009).

The effectiveness of fungicides in limiting development of both species of *Alternaria*; *A. solani* and *A. alternata* is also reported to be different (Kapsa, 2009). Moreover, inappropriate use of chemicals will lead to serious human health hazards, therefore; concentration of fungicides to a minimum rate, proper application time and intervals are recommended to minimize the adverse impacts on the agro-ecosystem, environment and danger to animals and human consumers.

The objectives of this study were: to identify the causal agent of brown spots of potato in Afghanistan, and to evaluate the in vitro efficacy of different chemical fungicides against the pathogen; to find the minimum effective concentration.

2. Material and methods

This study was carried out at Plant protection laboratory, Faculty of agriculture, Kabul University, Afghanistan, during 2019 to evaluate the in vitro effects of common synthetic fungicides on fungal growth using the poisoned food technique, according to Kulmitra *et al*., (2017).

2.1. Isolation and identification of the fungal pathogen

Fungal isolates were obtained from naturally infected potato leaves collected from research farm of the Faculty of Agriculture, showing similar early blight symptoms including; bull eye pattern in which centric rings with brown to black spots appeared on the infected leaves, although the size of these lesions was relatively smaller than a typical early blight symptom. The infected potato leaves were cut into small pieces along with healthy margins of about 1-1.5 cm. Surface sterilization of these cutting leaves were carried out using 0.1% $NaHOCl_3$ for approximately 3 min., washed three times with sterile dist., water, dried up between sterilized filter paper tissue, and then placed aseptically on the surface of potato dextrose agar (PDA) plates (250 g\ l potato, 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 single spore technique (Hansen, 1926). Identification of the pathogenic fungus was conducted through microscopic investigation according to Zheng *et al*., (2015).

2.2. Detection of the *in vitro* antifungal potential of the fungicides

Five fungicides mainly; Copper oxychloride (Super Copper Oxychloride®, 50% WP), Carbendazim (Carbendazim Aria®, 60% WP), Penconazole (Penconazole®, 20% WE), Mancozeb (Qadri Mancozeb®, 80% WP) and Flutriafol 6.94% + Tebuconazole 20.8% (Topgaurd®, 30% SC), were obtained from a local market. These fungicides were tested for their *in vitro* antifungal potential against the pathogen using the poisoned food technique at 4 different concentrations of; 20, 100, 300 and 500 mg\ l, according to Kulmitra *et al*., (2017). The fungicides were dissolved in sterilized H_2O to make stock solution of 10000 mg\ l, and then were filter sterilized. An appropriate volume of the stock solutions was added to the PDA medium prior to solidification. Before pouring the medium, streptomycin sulphate (1×10^6 IU), and penicillin (1×10^6 IU) antibiotics at 1 ml\ l were used to avoid bacterial contamination, according to Hajano *et al*., (2012). About 18-20 ml of poisoned media were poured into 90 mm Petri plates and allowed to solidify. After solidification, 5 mm diameter disc was cut under aseptic conditions from the actively growing margin of 10 d old culture of the pathogenic fungus using a cork borer, and then placed at the center of each of the seeded PDA plates. Suitable control was maintained by placing the fungal disc at the center of each non-treated medium. All plates were incubated at 26± 1 °C for 10 d, and the colony diameter was measured (in mm) using a calibrated ruler at 24 h intervals till 10 d of incubation, according to Kulmitra *et al*., (2017). Three replicate plates were used for each concentration of the respective fungicide, and the assay was repeated twice.

2.3. Determination of the percentage (%) of inhibition in radial growth of the pathogen

The percentage (%) of growth inhibition (PGI) of the fungus over control was calculated by using the formula given by Ginoya and Gohel, (2015):

$$PGI = \frac{100\ (DC-DT)}{DC}$$

Where; PGI: Percentage (%) of growth inhibition, DC: Average diameter of mycelial growth in control plates (mm), DT: Average diameter of mycelial growth in treated plates (mm)

The concentrations of all fungicides were adjusted in accordance with the commercial formulation present on the label of each fungicide.

2.4. Statistical analysis

Analysis and interpretation of the experimental data was carried out by conducting completely randomized design (CRD) and Factorial CRD for laboratory studies. ANOVA was generated using Statistix 9.0 and Statistical Analysis Software (SAS), respectively (SAS Institute. 2002).

3. Results

3.1. Fungal isolation and identification

The pathogenic fungus was isolated from potato diseased leaves with bull eye pattern, in which centric rings with brown to black spots appeared in the infected leaves. Since early blight and brown spot diseases are associated with two species of *Alternari* spp. (i.e. *A. alternata* and *A. solani*) and both pathogens produce similar symptoms; the isolated fungus was further subjected to microscopic study and conidial characterization.

Colony appearance of the isolate is dark grey to black brown and densely turfy on PDA plates, as clear in Fig. (1a). On PDA plates, the isolate produced conidial chains of 8-12 spores. Conidia are pyriform to ellipsoid, 20.8-40.5× 7.6-12.0 µm, with 3-8 transversal and 0-3 longitudinal septa (Fig. 1b). According to Zheng *et al*., (2015), *A. solani* produces conidia with one beak long-ovoid with 9-11 transverse septa and 1-2 longitudinal septa, and were 2-3 times longer (102.7-115.0 × 15.0-25.3 µm) than conidia produced by *A. alternata*. Hence, the current fungal isolate is identified as *A. alternata*.

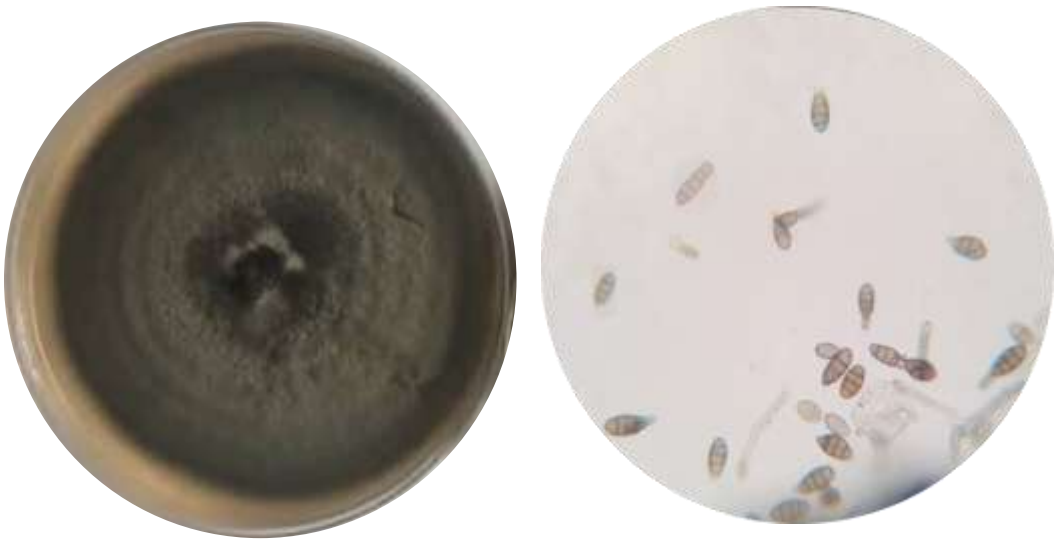

Fig. 1 (a): Colony appearance of *A. alternata* on PDA plate; (b): Microscopic observation of *A. alternata* conidia

3.2. *In-vitro* evaluation of the inhibitory activity of the synthetic fungicides on *A. alternata* mycelial growth

Complete randomized design (CRD) factorial data analysis showed that all tested fungicides significantly (F= 507.73, df= "5, 61", P≤ 0.01) inhibited the mycelial growth of the pathogen compared to the control. The data presented in Table (1) indicated that at 20 mg\ l and after 5 d of fungal growth, Flutriafol 6.94% + Tebuconazole 20.8% and Penconazole are the most effective, and they strongly reduced the average linear mycelial growth of *A. alternata* to 1.00 mm, with growth inhibition of 93.75 %, followed by Mancozeb (12.00 mm) with growth inhibition of 25.00 %, as clear in Table (1), Fig. (2). Carbendazim has a minimum effect (15.67 mm) with least growth inhibition percentage of 2.08 %, while Copper oxychloride does not express any growth inhibition (0 %). The fungal growth inhibition decreased over time.

Results after 10 d of fungal growth at 20 mg\ l showed that growth inhibition by Flutriafol 6.94% + Tebuconazole 20.8% (13.33 mm) and Penconazole (14.00 mm), are 63.25 %, 61.54 %; respectively, which exhibited relatively slight decrease in the growth inhibition compared to that observed at 5 d of incubation (Table 2, Fig. 3). This is followed by Mancozeb (31.00 mm) causing growth inhibition by 17.95%. On the other hand, Carbendazim (37.00 mm) and Coper oxychloride (38.00 mm) presented minimal inhibitory effects, expressing 2.56 % and 0.00 % growth inhibition, respectively. At 100, 300 and 500 mg\ l, Flutriafol 6.94 % + Tebuconazole 20.8 % are highly effective and caused 100 % inhibition of mycelial growth after both 5 d and 10 d of incubation. This is followed by Penconazole which caused 100 % inhibition of mycelial growth at 300 mg\l and 500 mg\ l, at both incubation periods. However, the same fungicide demonstrated lowest mycelial growth (3.67 mm) and highest growth inhibition (90.27 %), at 100 mg\ l after 10 d of fungal incubation. With respect to Mancozeb, the mycelial growth of *A. alternata* is significantly different within all four concentrations tested (20, 100, and 300 and 500 mg\ l) in both two incubation periods, although expressed highest growth inhibition at 500 mg\ l. Mycelial growth after 10 d is the lowest growth (6.00 mm) and highest inhibition (84.21 %) observed at 500 mg\ l, compared to the control (38.00 mm). This is followed by 300 mg\ l (9.33 mm) presenting strong growth inhibition (75.24%), while minimum growth inhibition (41.91 %) is observed at 100 mg\ l. Carbendazim fungicide treatment expressed the lowest growth diameter (30.67 mm) and highest inhibition (21.05 %) at 500 mg\ l, followed by 300 mg\ l (30.70 mm, 18.66 %) and 100 mg\ l (34.00 mm, 8.85 %), compared to the control (37.30 mm) after 10 d (Table 2, Fig. 3). There is also a significant difference between the two incubation periods, showing stronger growth inhibition at 5 d (34.56 % at 500 mg\ l), while the effect is remarkably decreased to 21.05 % at the same concentration after 10 d of incubation. The last synthetic fungicide; Copper oxychloride expressed the lowest inhibitory effects compared to all the other fungicides, and little differences are observed among all the tested concentrations, particularly after 10 d of incubation. Least mycelial growth (34.67 mm) is detected at 500 mg\ l with growth inhibition of 8.77 %, which is followed by 300 mg\ l (37.00 mm) with only 1.86 % inhibition of mycelial growth. Mycelial growth was not inhibited at 100 mg\l after 10 d; however, at the same concentration after 5 d of incubation, there is a significant mycelial growth inhibition by 16.18 %.

Comparative statistical analysis among the different fungicides in different concentration at two different observation times (5 d and 10 d) was carried out, and results are depicted in Fig. (2), Fig. (3). Observation after two different periods (5 d and 10 d) clearly indicated the decrease in fungicidal effects over time throughout all the fungicides at all the tested concentrations, except for Flutriafol 6.94% + Tebuconazole 20.8 % at 100, 300 and 500 mg\ l, and Penconazole at 300 and 500 mg\ l, which remained 100 % inhibitory effects after both incubation periods.

Table 1: Detection of *in vitro* efficacy of different synthetic fungicides against the mycelial growth of *A. alternata* after 5 d of incubation

	20 mg\l		100 mg\l		300 mg\l		500 mg\l	
Fungicides/ Concentrations	Mycelial Growth (mm)	Percent (%) Inhibition	Mycelial Growth (mm)	Percent (%) Inhibition	Mycelial Growth (mm)	Percent (%) Inhibition	Mycelial Growth (mm)	Percent (%) Inhibition
Carbendazim	15.6 ± 1.5^{a}	2.08	13.0 ± 1^{b}	22.00	11.6 ± 1.5^{c}	32.56	10.6 ± 0.6^{c}	34.56
Coper Oxychloride	16.0 ± 1^{a}	0.00	15.0 ± 1^{ab}	10.18	14.0 ± 1^{b}	19.08	14.0 ± 1^{b}	14.11
Mancozeb	12.0 ± 1^{b}	25.00	4.0 ± 1^{c}	76.05	0.0 ± 0^{d}	100.00	0.0 ± 0^{d}	100.00
Penconazole	1.00 ± 0^{c}	93.75	0.0 ± 0^{d}	100.00	0.0 ± 0^{d}	100.00	0.0 ± 0^{d}	100.00
Flutriafol 6.94% + Tebuconazole 20.8%	1.0 ± 0^{c}	93.75	0.0 ± 0^{d}	100.00	0.0 ± 0^{d}	100.00	0.0 ± 0^{d}	100.00
Control	16.0 ± 1^{a}	0.00	16.6 ± 0.6^{a}	0.00	17.3 ± 0.6^{a}	0.00	16.3 ± 1.5^{a}	0.00
C. D (P=0.05)	2.58		2.04		2.14		2.14	
S.E (d)	0.76		0.60		0.63		0.63	
S.E.	0.54		0.43		0.45		0.45	
C.V.	9.17		9.19		10.91		11.44	

Where; C.D. (Critical difference), S.E (d) (Standard deviation), S.E. (Standard error), C.V. (Coefficient of variation). Different superscript letters indicate significant differences among treatments, according to the least significant difference (P =0.05)

Table 2: Detection of *in vitro* efficacy of different synthetic fungicides against the mycelial growth of *A. alternata* after 10 d of incubation

	20 ppm		100 ppm		300 ppm		500 ppm	
Fungicides/ Concentrations	Mycelial Growth (mm)	Percent (%) Inhibition	Mycelial Growth (mm)	Percent (%) Inhibition	Mycelial Growth (mm)	Percent (%) Inhibition	Mycelial Growth (mm)	Percent (%) Inhibition
Carbendazim	37.0 ± 1^{a}	2.56	34.0 ± 1.7^{b}	8.85	30.7 ± 1.2^{b}	18.66	30.0 ± 1^{c}	21.05
Coper Oxychloride	38.0 ± 1^{a}	0.00	37.3 ± 0.6^{a}	0.00	37 ± 1^{a}	1.86	34.7 ± 1.5^{b}	8.77
Mancozeb	31.0 ± 1^{b}	17.95	21.7 ± 1.5^{c}	41.91	9.3 ± 1.2^{c}	75.24	6.0 ± 1^{d}	84.21
Penconazole	14.0 ± 1^{c}	61.54	3.7 ± 0.6^{d}	90.17	0.0 ± 0^{d}	100.00	0.0 ± 0^{e}	100.00
Flutriafol 6.94% + Tebuconazole 20.8%	13.3 ± 1.5^{c}	63.25	0.0 ± 0^{e}	100.00	0.0 ± 0^{d}	100.00	0.0 ± 0^{e}	100.00
Control	38.0 ± 0^{a}		37.3 ± 1.5^{a}	0.00	37.7 ± 1.5^{a}	0.00	38.0 ± 1^{a}	0.00
C. D (P=0.05)	2.81		3.23		2.74		2.58	
S.E (d)	0.59		0.96		0.81		0.76	
S.E.	0.83		0.68		0.57		0.54	
C.V.	3.60		5.28		5.23		5.21	

Where; C.D. (Critical difference), S.E (d) (Standard deviation), S.E. (Standard error), C.V. (Coefficient of variation). Different superscript letters indicate significant differences among treatments, according to the least significant difference (P =0.05)

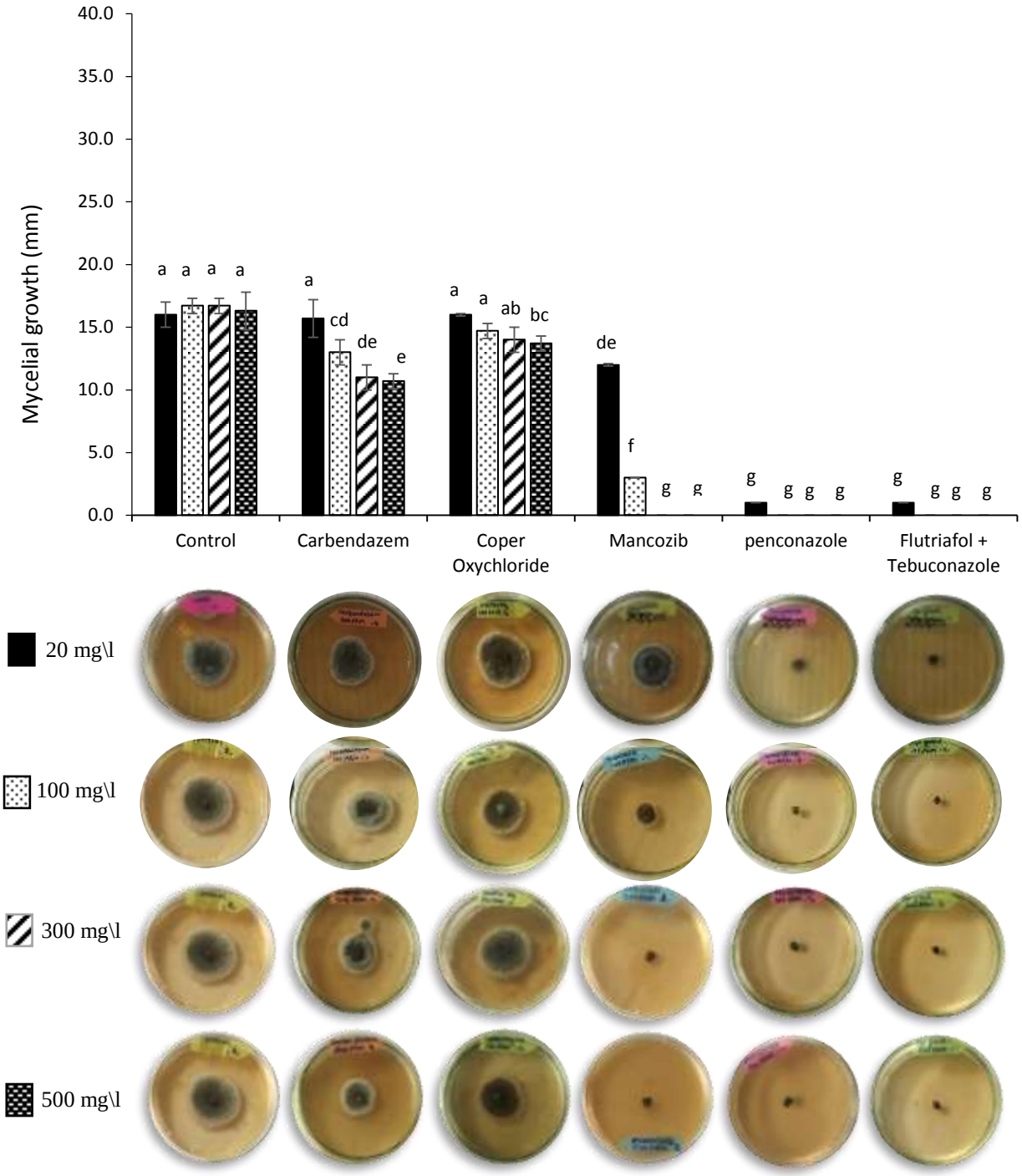

Fig. 2: *In vitro* effects of different fungicides on mycelial growth of *A. alternata* after 5 d of incubation. Different letters indicate significant differences among treatments, according to the least significant difference (P =0.05). Results are averages of 3 replicates for each fungicide concentration

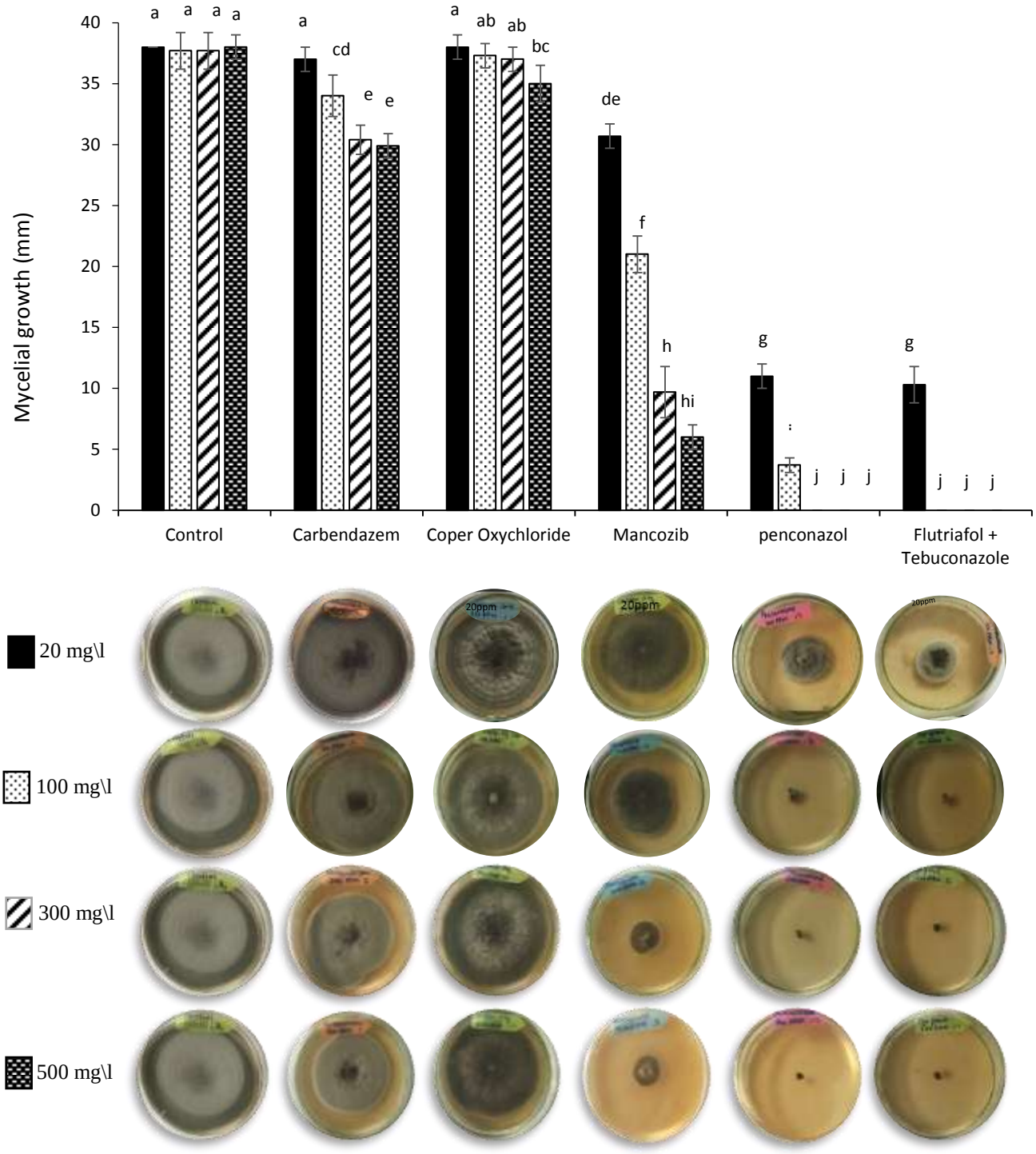

Fig. 3: *In vitro* effects of different fungicides on mycelial growth of *A. alternata* after 10 d of incubation. Different letters indicate significant differences among treatments, according to the least significant difference (P =0.05). Results are averages of 3 replicates for each fungicide concentration

4. Discussion

In the current study, efficacy of two systemic, one combination product and two contact fungicides were evaluated at 4 different concentrations, using the poisoned food technique.

Obtained data revealed that Penconozole a member of systemic fungicides from Trizole family was highly effective and caused mycelial growth inhibition of *A. alternata* at 300 and 500 mg\ l, and caused significant reduction (96.01 %) in linear growth of *A. alternata* at lowest concentration of 20 mg\ l after 5 d of incubation was observed, although this inhibition was slightly reduced over time. To the best our knowledge, this is the first report indicating mycelial growth inhibition of *A. alternata* at low concentration of 20 mg\ l by a synthetic fungicide.

In accordance with the current results, a previous study conducted by Gynoya and Gohel, (2015) reported that systemic fungicides including; tebuconazole, hexaconazole and difenoconazole from the same trizole families have given excellent results of inhibition (%) of *A. alternata* (causal agent of Chilli leaf spot) at 500 mg\ l. Earlier study of Singh and Singh, (2006) screened several fungicides against *A. alternata* and recorded that hexaconazole gave excellent inhibition (%) of mycelial growth, at a low concentration of 50 mg\ l. Gohel and Solanky, (2012) have reported that difenconazole, propiconazole and hexaconazole caused inhibition (%) of mycelial growth at minimum of 200 mg\ l. Akbari and Parakhia, (2007) highlighted that difenconazole and hexaconazole of the Triazole family were completely inhibitory to *A. alternata* the fungal causal agent of sesame blight at lowest concentration of 50 mg\ l. All these data were in line with our study and indicate that systemic fungicides from the Triazole family have excellent effects of growth inhibition on *A. alternata*. Furthermore, this study confirmed that inhibition of mycelial growth could be achieved at minimum fungicide concentration of 20 mg\ l.

In addition to *A. alternata*, similar effects of Triazole systemic fungicides were observed on growth inhibition of *A. solani*, the causal agent of tomato early blight, potato early blight, rice blast and other fungal diseases (Gohel and Solanky, 2012; Murmu *et al*., 2017; Kulmitra *et al*., 2017; Singh *et al*., 2018).

In the present study, carbendazim a member of the systemic fungicides from Benzimidazoles family was not very effective in inhibiting the mycelial growth and caused only 21.05 % inhibition at maximum 500 mg\l, while the effects were decreasing in lower concentrations recording 0.00 % inhibition at 20 mg\ l. This result is in line with the study carried out by Gohel and Solanky, (2012), which reported carbendazim as the least effective (37.15 %) even at high concentration of 1000 mg\ l. In previous works, Kumar *et al*., (2013); Ghazanfar *et al*., (2016) reported that several fungicides including carbendazim (Bavistin) and copper oxychloride (Blitox-50) strongly inhibited the fungal mycelial growth at 0.1 %, or at higher concentrations. These results further confirmed our investigation that carbendazim could only inhibit the mycelial growth of *A. alternata* at high concentration.

Several studies conducted by Ghazanfar *et al*., (2009); Murmu *et al*., (2017); Kumar *et al*., (2018) were carried out to understand the effects of combination product on the growth of *A. alternata*, *A. solani* and other important plant pathogenic fungi. Our investigation indicated that Flutriafol 6.94% + Tebuconazole 20.8 % (Topgaurd® 30 % SC) have given superior effects on growth inhibition of *A. alternata*, compared to all the tested fungicides. It presented high % of mycelial growth inhibition even at lowest concentration of 20 mg\ l after 5 d of incubation, with strong inhibitory potential at the same concentration after 10 d of incubation. Moreover, Kumar *et al*., (2018) also investigated the efficacy of several combination products in controlling *A. solani*, where among all the tested fungicides; Fenamidone 10% + Mancozeb 50 % gave excellent results both

under *in vitro* and *in vivo* conditions. Similarly, Murmu *et al*., (2017) reported that along with Triazole systemic fungicides, propineb 61% + iprovalicarb 5.25% also caused high % inhibition of *A. solani* mycelial growth. In a previous study, Singh and Chowdhary, (2008) revealed that among six fungicides, combination of systemic and non-systemic fungicides (carbendazim 12 % + mancozeb 67 %) had highest inhibition of 83.93 %, 94.27 % and 97.64 % at 500, 750 and 1000 mg\ l concentrations, respectively on *Alternaria* leaf spots of chilli. These studies demonstrated the effectiveness of fungicides combination products on *Alternaria* diseases.

Mancozeb, which is a Dithiocarbamate non-systemic fungicide with a protective action, caused 100 % mycelial growth inhibition at 500 mg\ l after 5 d of incubation, although the effects was slightly decreasing (84.21 %) after 10 d. In agreement with the current results, an early study of Akbari and Parakhia, (2007) reported that non-systemic fungicides including mancozeb gave 100 % inhibition of *A. alternata* (causal agent of sesame blight) at minimum concentration of 500 mg\ l. Furthermore, Gohel and Solanky, (2012) highlighted that mancozeb at 2000 mg\ l caused 100 % inhibition, whereas at 1500 mg\ l; it strongly inhibited the mycelial growth by 90.43 %.

Copper oxychloride which is a protective wettable fungicide having double effects of systematic and contact fungicide with preventive action was proved to be non-effective on mycelial growth inhibition even at high concentration of 500 mg\ l. In contrast with the present study, Gohel and Solanky, (2012) have reported that copper oxychloride caused 100 % inhibition of mycelial growth and conidial germination at minimum 1000 mg\ l, while Kumar *et al*., (2013) demonstrated that copper oxychloride at concentration of 0.2 %; could cause 81.4 % mycelial growth inhibition. Our investigation confirmed that copper oxychloride, at concentration of 500 mg\ l or lower; could not strongly inhibit mycelial growth of *A. alternata*. Copper oxychloride is much more effective in inhibiting conidial germination rather than inhibiting mycelial growth, since copper kills the spores by combining with the sulphahydral groups of certain enzymes. Spores actively accumulate copper and thus germination of these spores is inhibited, even at lower concentrations.

Conclusion

This is the first report of *A. alternata* as the causal agent of potato brown spot in Afghanistan. Our study suggested that systemic fungicides (penconazole) or combined compounds of two systemic fungicides viz., Tuboconzole + Flutriafol from the Triazole family have superior inhibitory effects on the target pathogen even in a minimum concentration of 20 mg\ l, which has not been reported so far. Our future prospectus is to carry out greenhouse and\or field experiments, to find out the lowest effective concentration of the mentioned fungicides under in vivo conditions, and to avoid unnecessary excessive use of the chemical fungicides.

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Conflict of interest

The authors declare that there is no conflict of interests.

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Ethical approval

Non-applicable.

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