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Evaluation of Biocontrol Agents and Fungicides against Alternaria Leaf Spot of Spinach

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ABSTRACT

Background: Spinach (*Spinacia oleracea*) is a worldwide cultivated crop and is attacked by a complex of foliar diseases. Alternaria leaf spot has become quite a problem as it results in poor quality and low yield.

Methods: The experiment was conducted during 2021-22 with a total of four fungicides *viz* Carbendazim 12% + Mancozeb 63% WP, Hexaconazole 5% SC, Azoxystrobin 18.2% + Difenoconazole 12.5% W/V, Copper oxychloride 50% and three bio-control agents *viz*. *Trichoderma viride*, *Trichoderma asperellum*, *Trichoderma harzianum* to evaluate their effectiveness against Alternaria leaf spot of spinach. Fungicides were evaluated at four concentrations *viz* 25, 50, 75 and 100 ppm for their efficacy.

Result: All the tested chemicals and bioagents were effective to reduce the disease severity, but hexaconazole at 100 ppm was the most effective in controlling the radial growth (82.55% inhibition) followed by Azoxystrobin (79.97%). The study established the efficacy of fungicides and bio-agents against Alternaria leaf spot of spinach caused by *Alternaria alternata*. The levels of inhibition revealed the potential to manage pathogen and can be used for better yield and good quality product thereby minimizing risks of crop failures.

Key words: Alternaria, Bioagents, Fungicide, Severity.

INTRODUCTION

Spinach (*Spinacia oleracea*) is an edible flowering plant. Grown for its leaves it is a very important vegetable crop across the world (Wachira *et al.,* 2014). In 2019, 2580 tonnes of spinach was sold by India costing 4.34 m USD. Raw spinach contains 3.6% carbohydrates, 2.9% protein and about 91.4% water and is a rich source of iron, manganese, potassium, magnesium, calcium and dietary fibre (Roberts and Moreau, 2016). Spinach is an important crop for consumption and health purposes with over 26 million tons production on about 921000 ha area globally (FAO, 2018).

European countries cultivate 31,770 ha, with around 555,416 tons annual production. Spinach covers around 5100 ha with annual production of around 86,990 tons in Spain. About 89% of the spinach is consumed domestically (raw 15%, frozen 80% and canned 5%) whereas rest 11% is exported (MAPAMA, 2016). Spinach gives a significant positive effect on bioavailability of iron (Yu Wei Luo and Jing Li 2016). For domestic use spinach is grown mainly on a small scale and the surplus is sold in local markets (Anonymous, 2017). With just a little improvement most farmers grow spinach in traditional farming. This is the main reason why there is low production of spinach. Ivan *et al.* (2014) noticed that the yield in spinach varies according to locations, growing practices, cultivars, climates, disease and pest resistance. Just like any other vegetable, Spinach is infected by diseases and pests (Singh *et al.,* 2015). Mohammed *et al.* (2019) reported that spinach crop can be attacked by several fungal species that causes foliar diseases. It was also reported that due to Alternaria leaf spot disease, the mortality of plants is very high (Czajka *et al.,* 2015). Alternaria leaf spots are some of the diseases which cause huge losses. In Saudi Arabia *Alternaria spp*. caused huge economic losses where the losses were in the range of 20-80%. Both quality and quantity are affected by *Alternaria* species causing leaf spot and leaf blight (Marraiki *et al*., 2012). The occurrence and the amount of damage this disease causes tend to vary according to regions and the management options deployed.

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MATERIALS AND METHODS

Field experiments were conducted in *rabi* season of 2021-22 at Agriculture Research Farm, while *in vivo* experiments were performed in the laboratory of department of Plant Pathology, Lovely Professional University, Phagwara (Punjab).

In-vitro evaluation of fungicides against *A. alternata*

The efficacy of the fungicides was checked *in vitro* based on the mycelial growth inhibition of *A. alternata* using the poisoned food technique (Plate 1). All four fungicides *viz*.

Copper oxychloride 50% WP, Azoxystrobin 18.2% SC + Difenoconazole 11.4% SC, Hexaconazole 5% SC, Carbendazim 12% + mancozeb 63% WP) were tested against the pathogen at four different concentrations given 25, 50, 75 and 100 ppm. In Erlenmeyer flasks (250 ml), a stock solution of 100 ml was prepared by adding distilled water to each fungicide. Later working solution was made by adding the stock solution to a 250 ml flask containing potato dextrose agar medium (PDA). The chemicals were added only after sterilization was done in the autoclave. The potato dextrose agar having the required concentrations was poured into Petri plates that were sterilized before work in a hot air oven for about an hour. After the media solidified each plate was inoculated with the pathogen culture (min 7 days old) and a single Petri plate with no fungicide was also inoculated, it served as untreated control. Each treatment consisted of 3 replicates. Later the Petri plates were kept in an incubator at 26±1°C Inspection of Petri plates was done regularly for a week. The mycelial growth was measured when the control plate was fully covered (Noman *et al*., 2018).

In vitro* evaluation of bio-agents against *A. alternata

By using the dual culture technique three bio-agents were tested against *Alternaria* alternata *viz*. *Trichoderma viride*, *T. harzianum* and *T. asperellum* with three replications each. PDA was poured into Petri plates and sterilized before the procedure later pathogen inoculum was carefully put into a corner of the Petri plate and on the other corner a species of *Trichoderma*. Later kept in an incubator at 25±1°C (Kurhade *et al.,* 2021).

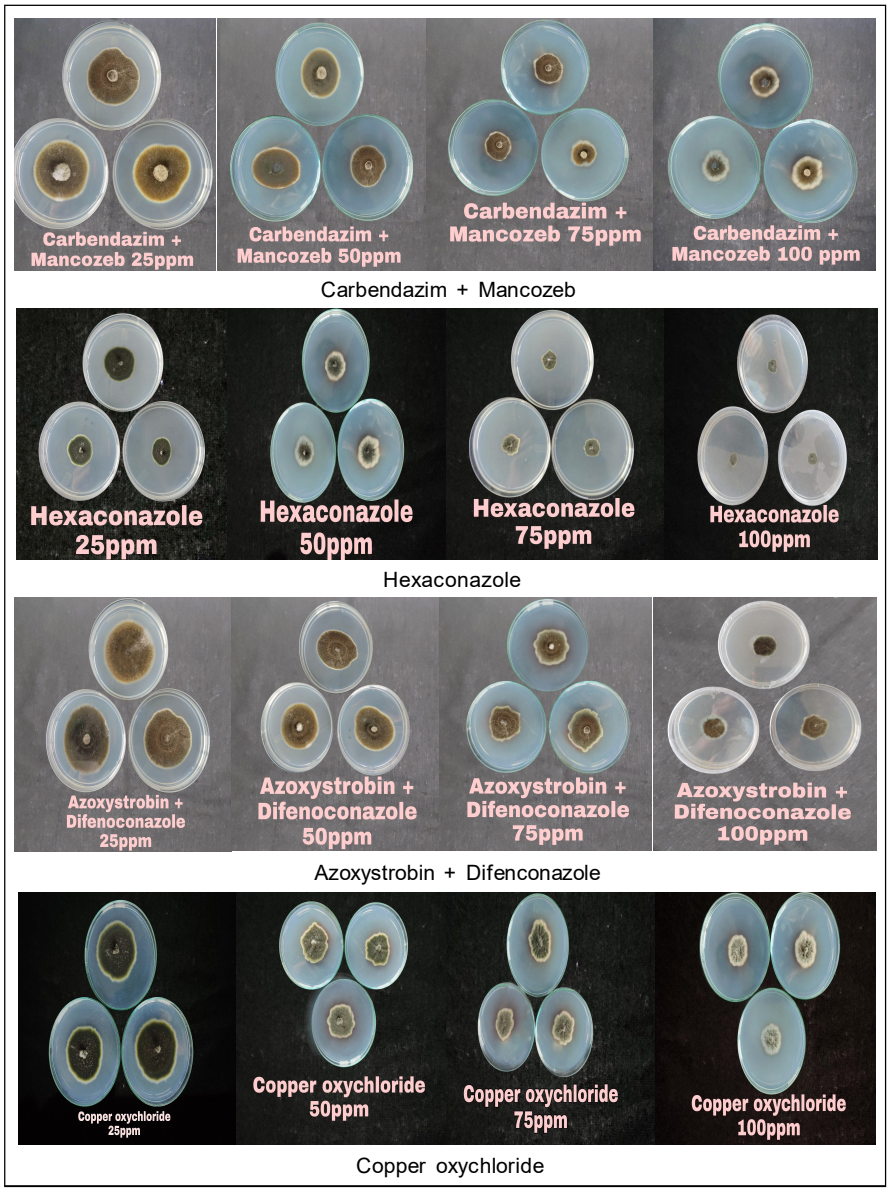


Plate 1: *In-vitro* evaluation of fungicides at different concentrations against *A. alternata.*

In-vivo* evaluation of fungicides and bioagents against *A. alternata

Trials were conducted with eight treatments in three replications using randomized block design (RBD). The variety 'Harit Sobha' was sown with a plot size of 6 m × 2.5 m with the row-to-row distance of 12 inches and plant to plant 5 cm. The sowing of the crop was done on 10 December 2021 at the research farm of Lovely Professional University. A spore suspension of 10^6 conidia/ml of 14-day-old culture of *A. alternata* was sprayed on 2 week old spinach plants to confirm pathogenicity. Symptoms of the disease were visible in the field after 45 days of sowing. With the advancement of the disease, the necrotic spots get turned into blight (Aslam *et al*., 2019). Treatments were given at a 15 days interval. The severity of the disease was measured one week after each spray using a 0-4 scale (Biswas and Ghosh, 2018) where 0, no disease; 1, 1-10%; 2, 11-15%; 3, 16-50%; 4, 51-100% plant showing leaf spot. Four fungicides *viz*. Hexaconazole 55% WP, Carbendazim 12% + Mancozeb 63% WP, Azoxystrobin 18.4% SC + Difenoconazole 11.4% and Copper oxychloride 50%WP were selected to check their efficacy against *Alternaria spp.* causing Alternaria leaf spot in spinach. Three biological control agents *viz*. *Trichoderma viride*, *T. harzianum* and *T. asperellum* at various concentrations. A total of three sprays were administered in the gap of fifteen days. Data was recorded and later converted into Percent disease index.

Statistical analysis

The experiments were designed as RBD in the field and CRD under laboratory condition with 3 replicates of each treatment. To evaluate the treatment effect, ANOVA was used to compare the means between treatments and OPSTAT software was applied for data analysis.

RESULTS AND DISCUSSION

During the survey of Alternaria leaf spot, different symptoms were observed, these symptoms included small irregular dark spots on the upper portion of the leaf. Loss of vigour, yellowing and chlorosis was also seen on leaves, in some cases leaves drying was there and in severe infection whole plant died. To test the efficacy of fungicides and bio-agent, studies were carried out. The most effective method for the control of this disease is mainly fungicide, however wide use of chemicals may result in contamination of the environment (Bourguet *et al.,* 2016). Moreover, these plant pathogens have been found to develop resistance against the fungicides (Humaira, 2015). Therefore, chemicals should only be used only when required. The results are discussed below.

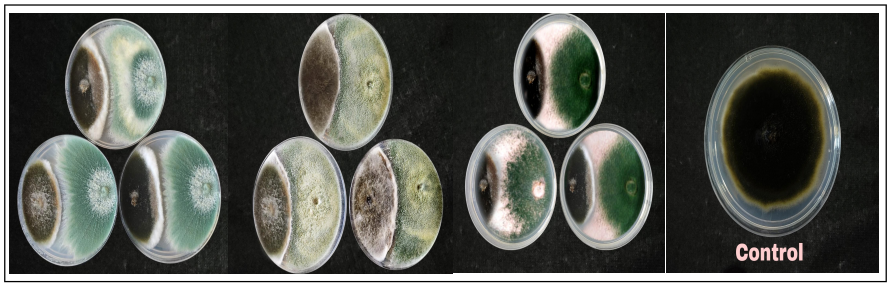
In vitro* evaluation of fungicides and biocontrol agents against *A. alternata

The leaves brought to the lab had irregular brown spots on the surface and dark brown spots were visible on the lower surface of the leaves. The spots coalesce together and the leaves drop off.

The efficacy of 4 fungicides and 3 bio-agents was tested *in vitro* as well as *in vivo*. *In vitro* tests involved 4 concentrations viz. 25, 50, 75, 100 with 3 replications against *Alternaria alternata* on PDA by Poison Food Technique (Plate 1). The results showed that among the fungicides, hexaconazole (82.55) at 100 ppm significantly obtained higher growth inhibition percentage followed by azoxystrobin + difenoconazole (79.97%) and Carbendazim + Mancozeb (76.86%) whereas Copper Oxychloride (49.17%) was found least effective (Plate 1) (Table 1). Among biocontrol agents, *Trichoderma viride* (50.96%) was more effective and *T. harzianum* (47.26%) was at par with *T. asperellum* (43.71%) (Plate 2) (Table 2). According to Kimani, 2014; Kumar, 2015, the biocontrol agents are selective in their target, available easily and do not show any residual effect. Kumar and Rathi (2018) tested five fungicides against *Alternaria* of Indian mustard *viz.* Azoxystrobin, Mancozeb, Difenoconazole, Hexaconazole and Propiconazole out of which Hexaconazole was significantly effective in controlling severity followed by Mancozeb. Ginoya and Gohel (2015) found out the *in vitro* efficiency of synthetic fungicides out of which, Hexaconazole, tebuconazole, difenconazole (11.40%) + azoxystrobin (18.20%) had completely inhibited the growth of *Alternaria alternata*. Balai *et al*. (2020) reported Mancozeb as the most effective fungicide against alternaria blight of Pigeon pea pathogen (75.26%).

In vivo* evaluation of fungicides and bioagents against *A. alternata

Progress of disease during 2021 is represented in Table 3. The data revealed that PDI was minimum in hexaconazole



A B C D

Plate 2: (A) *T. viride* (B) *T. asperellum* (C) *T. harzianum*, (D) Control plate.

Table 1: *In vitro* growth inhibition of *A. alternata* at different concentrations of fungicides.

Treatments	Percent growth inhibition			
	Fungicide conc. (ppm)			
	25	50	75	100
Carbendazim 12% + Mancozeb 63% WP	61.72	66.33	71.73	76.86
Hexaconazole 5% SC	65.16	69.5	76.43	82.55
Azoxystrobin 18.2% + Difenoconazole 11.4% SC	63.48	67.14	73.48	79.97
Copper oxychloride 50% WP	30.26	35.92	42.43	49.17
CD	1.74	1.49	1.51	1.77
SE(m)	0.21	0.42	0.14	0.21
SE(d)	0.29	0.59	0.20	0.31
CV	0.81	1.46	0.44	0.60

Table 2: *In vitro* evaluation of bioagents against *A. alternata.*

Treatments	Colony diameter (mm)			Total	Mean colony diameter	Inhibition %
Trichoderma viride	14.12	15.23	14.78	44.13	14.71	50.96
Trichoderma harzianum	15.33	15.68	16.45	47.46	15.82	47.26
Trichoderma asperellum	16.54	17.14	16.98	50.66	16.88	43.71
Control	90	90	90			
C.D.						0.699
SE(m)						0.198
SE(d)						0.280
C.V.						0.999

Table 3: *In vivo* evaluation of fungicides and bioagents against *A. alternata* (disease severity).

Treatment	45 DAS	60 DAS	75 DAS	Mean
Carbendazim + Mancozeb	43.18	39.13	34.97	39.09
Hexaconazole	36.86	31.03	26.23	31.37
Azoxystrobin + Difenoconazole	40.67	34.05	31.25	35.32
Copper oxychloride	54.14	49.43	46.17	49.91
Trichoderma viride	62.44	61.18	58.51	60.71
Trichoderma harzianum	64.27	62.48	59.13	61.97
Trichoderma asperellum	66.07	63.98	60.37	63.47
Control	74.45	76.14	75.33	75.30
C.D.				3.16
SE(m)				1.03
SE(d)				1.46
CV				3.32

(31.37%) treated plots followed by Azoxystrobin + Difenoconazole (35.32%) whereas *Trichoderma viride* showed PDI of 60.71%. The minimum PDI was observed in the check plot (75.30%) (Table 3). Aqeel and Ashraf (2016) found 48.5% PDI in *Trichoderma harzianum* treated plot followed by *Trichoderma viride* (48.14%) against *Alternaria brassicae.* Herle and Kamanna (2014) observed decrease in PDI in chemical treated plots. Zineb 68% + hexaconazole 4% was found to be most effective (21.50% PDI) followed by tebuconazole 25% EC (22% PDI) and captan 70%+ hexaconazole 5% (24.66% PDI) against early blight of Potato.

CONCLUSION

Spinach (*Spinacia oleracea*), primarily grown for leaves, is a very important leafy vegetable. Spinach is rich in iron and vitamins. During the survey of Alternaria leaf spots different symptoms were observed, these symptoms included small irregular dark spots on the upper portion of the leaf. Loss of vigour, yellowing and chlorosis was also seen on leaves, in some cases leaves drying was there and in severe infection whole plant died. To test the efficacy of fungicides and bio-agents studies were carried out.

Hexaconazole among other fungicides showed prominent growth inhibition against *A. alternata*. And in bio-

agents *T. viride* performed better in bio-agents. The levels of inhibition observed under *in-vitro* and *in-vivo* revealed the potential to manage pathogens and can be used for better yield and good quality products thereby minimizing risks of crop failures.

Hexaconazole and Azoxystrobin + Difenoconazole can be recommended for use in the proper management of this disease however excessive use of fungicides can cause environmental pollution so the use of bio-agents should be prioritized. The use of chemicals should be done only if the other means fail to control the disease.

Conflict of interest: None.

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