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Integrated Management of Foliar Blight of Medicinal Crop (Turmeric) Caused by *Alternaria alternata*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The infected samples were collected from three districts viz; Kathua, Samba and Jammu for the study of the disease. The causal fungus of the disease was isolated and identified as *Alternaria alternata* on the basis of morphological characteristics. The cultivars PH-1 and local cultivar of turmeric were sown with three replications in the year 2014 and 2015 respectively, each to study the disease development and its management. During *in vitro* studies, the five groups of fungicide (Mancozeb, Carbendazim, Copper Oxychloride, Hexaconazole and Propiconazole) were evaluated against *Altenaria alternata* causing disease, best results were obtained with hexaconazole. The antagonistic activity of two fungal and two bacterial biocontrol agents were studied by dual culture thus *Trichoderma viride* and *Pseudomonas flurescens* portrayed better results. Under compatibility of fungal bioagents and bacterial bioagents best compatibility was observed between copper oxychloride and *Trichoderma viride* and hexaconazole and *Pseudomonas flurescens.* Under the

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field conditions the best results among all fifteen treatments, with minimum disease intensity was observed in terms of integration i.e (hexaconazole + *Trichoderma viride*) (4.2%) in PH-1 cultivar and (6.7%) in local cultivar.

Keywords: Foliar blight of turmeric; alternaria alternate; chemical management; biological management; soil amendment; integrated management.

1. INTRODUCTION

Turmeric (*Curcuma longa* L.) (Haridra or Haldi) is a rhizomatous herbaceous perennial medicinal plant of the ginger family i.e., Zingiberaceae. It is native to southern Asia, requiring temperatures between 20 and 30°C (68 and 86°F) with a considerable amount of annual rainfall to thrive. Turmeric is one of the most important spice crop cultivated in India. It has versatile uses like flavoring, dye making, drug preparation, cosmetics and medicine (Dixit, et al. [1]. Turmeric is officially entered in the ayurvedic pharmacopoeia of India, pharmacopoeia of the People's Republic of China and in Japanese standards of herbal medicines which is also called as "Hidden Lilly" or "turmeric of commerce". The most important chemical components of turmeric are a group of compounds which include Curcumin (Diferuloylmethane), Demethoxycurcumin, and Bisdemethoxycurcumin. The best-studied compound is Curcumin, which constitutes 3.14 per cent (on an average) of powdered turmeric. Turmeric is the ancient and sacred spice crop of India. India is a leading producer and exporter of turmeric in the world and accounts for 80 per cent of the world's production. This herbal plant is highly prone to several fungal diseases Naidu, [2]. The leaf blight caused by *Alternaria alternata* (Fr.) Keissler, is an important foliar disease of turmeric, damaging the crop to a greater extent by reducing the size and weight of the rhizome. Mallikarjun [3] reported that leaf blight was a serious disease and caused considerable damage to the plant in almost all turmeric growing areas of Northern Karnataka. The leaf blight disease of turmeric caused by *Alternaria alternata* was first reported from Madhya Pradesh Choudhury, [4]. Mamatha and Hedge [5,6] found that leaf blight caused by *A. alternata* was an important foliar disease of turmeric commonly found in Karnatka and it damaged the crop up to a great extent destroying the active photosynthetic area leading to the reduction in size and weight of rhizome. Rukhsana et al. [7] reported that leaf spot was incited by *A. alternata* that could cause economic losses in the cultivation. The available literature regarding the foliar blight of turmeric caused by *Alternaria alternata* is few so, considering the economic importance of the crop and the foliar blight disease, the present investigation was taken up in the Division of Plant Pathology, FOA, SKUAST-J, Chatha to develop eco-friendly and economically feasible integrated disease management approach for the Jammu condition through bio-agents, Soil amendment and need based fungicide application for the foliar blight of turmeric against two cultivars i.e., PH-1 and local.

2. MATERIALS AND METHODS

2.1 Isolation of Pathogen and Purification

The diseased samples collected from different locations, were washed under fresh tap water and dried in the folds of blotting paper and then small bits (2-3mm) were cut with the help of flame sterilized blade from the junction of diseased and healthy portion in the ratio of 1:3. These bits were surface sterilized by dipping in 0.1 per cent mercuric chloride solution for 20 seconds. These bits were given three consecutive wash to remove the mercuric chloride from the surface and dried by placing the bits on sterilized blotter paper. These bits were then transferred on sterilized PDA poured in Petri-plates under aseptic conditions of Laminar flow bench and incubated in a BOD chamber at 25±2°C. The plates were regularly observed for fungal growth. The growing fungal colonies were transferred to PDA slants by hyphal tip culture technique. After proper growth of culture in the test tubes, pure cultures were maintained in a refrigerator at 4±1°C. Stock cultures were maintained by continuous sub-culturing at an interval of 45 days in winter and 30 days in summer for further investigations.

2.2 Management

2.2.1 *In vitro* evaluation of fungicides against the pathogen and the bio-control agents

In order to find out the efficacy of the different fungicides against the pathogen (*Alternaria*

alternata) and compatibility of fungicides with potential fungal bio-control agents were tested under *in vitro* conditions by using poisoned food technique Dhingra and Sinclair, [8] and was evaluated using seeded plate technique for bacterial bio-control Skinner, [9]. Evaluation of five fungicides i.e., mancobzeb, carbendazim, copper oxychloride, hexaconazole and propiconazole was done at recommended dose (2000 ppm for Mancozeb, 1000 ppm for Carbendazim, 3000 ppm for Copper Oxychloride, 100 ppm each for Hexaconazole and Propiconazole and half of the recommended dose. In poisoned food techniques the desired amount of chemicals were dissolved in 20 ml media per plate and 5 mm bit of fully grown culture of pathogen was placed in the centre of the plate and growth of the culture was recorded. In case of seeded plate method the bacterial bioagents were added in the media at the rate of 2 per cent by adding suspension cells having count 1x 10^6 cells per ml of the solution after cooling of the media. Then the bit of blotter paper (5 mm Diameter) were soaked in the desired concentration of fungicides for 30 minutes and dried in the shade were placed in the centre of the plate. Then the plates were incubated for four days at 25 + 2°C and observations regarding inhibition zone was recorded. Experiment was conducted in CRD and was replicated five times. Antagonistic activity of two fungal and two bacterial bio-agents i.e., *Trichoderma harzianum, Trichoderma viride, Pseudomonas flurescens* and *Bacillus subtilis,* were evaluated following the dual culture technique Dennis and Webster, [10]. Percentage reduction in radial growth over control was taken on 4th day and 8th day and calculated by using the formula previously described by Vincent [11] and readings regarding diameter of the inhibition zone were taken on 4th day and 8th day.

The growth inhibition of pathogen (*Alternaria alternata*) and potential fungal bio-control agents over control for each treatment was calculated as per Vincent [11].

$$I = \frac{C-T}{C} X\ 100$$

Where,

I = Per cent reduction in growth of test pathogen
C = Radial growth (mm) in control
T = Radial growth (mm) in treatment

2.2.2 *In vivo* evaluation of soil amendments, biocontrol agents and fungicides against the pathogen

Soil amendments with Farm Yard Manure, mustard oil cake, and application bio-control agents viz; *Trichoderma viride*, *Pseudomonas fluorescens* and chemicals viz. mancozeb and hexaconazole alone and in different combinations were evaluated under field conditions against the cultivar PH-1 and Local in two consecutive years 2014 and 2015 for each variety. The soil application was done by the drenching of one liter per plot of spore or cell suspension at the rate of 1 x 10^6 spores / cells per ml. The foliar spray of biological agents was done with same suspension. In case of integration with chemicals in the recommended dose was used as first spray and bio control agents were used as second and third spray after 15 days interval. The plots were first sprayed with the spore suspension of the pathogen and after appearance of the symptoms the management practice was started under field condition. The experiment was conducted in Randomized Block Design design with three replications in the research farm of SKUAST- J chatha.

3. RESULTS

3.1 *In Vitro* Evaluation of Fungicides against *Alternaria alternata* Causing Foliar Blight of Turmeric

The results of the experiments (Table 1.) reveal that all the fungicides significantly inhibited the growth of the pathogen over control. In case of half dose hexaconazole showed maximum inhibition over control (87.62%) with colony diameter of 5.32 mm on 4th day and (88.41%) per cent inhibition with colony diameter 10.43 mm on 8th day which was statistically at par with propiconazole, which caused 84.88 per cent inhibition with colony diameter of 6.50 mm on 4th day and 86.25 per cent inhibition and colony diameter of 12.37 mm on 8th day. Among older fungicides mancozeb showed inhibition (75.58%) with colony growth of 10.50 mm on 4th day and 74.63 per cent inhibition with 22.83 mm colony growth on 8th day. Carbendazim showed the least effectiveness against the pathogen with 48.09 per cent inhibition and 22.32 mm colony diameter on 4th day and 53.0 per cent inhibition and 42.30 mm colony diameter.

Table 1. *In vitro* evaluation of fungicides against *Alternaria alternata* causing foliar blight of Turmeric

Treatment	Concentration (ppm)	4th day		8th day		Concentration (ppm)	4th day		8th day	
		Colony Diameter (mm)	Inhibition (%)	Colony Diameter mm	Inhibition (%)		Colony Diameter (mm)	Inhibition (%)	Colony Diameter (mm)	Inhibition (%)
Mancozeb	1000	10.50	75.58	22.83	74.63	2000	7.33	82.95	14.53	83.85
Carbendazim	500	22.32	48.09	42.30	53.0	1000	17.70	59.06	37.13	58.74
Copper Oxychloride	1500	16.40	61.86	35.63	60.41	3000	13.83	67.67	28.40	68.44
Hexaconazole	50	5.32	87.62	10.43	88.41	100	5.13	88.37	10.40	88.44
Propiconazole	50	6.50	84.88	12.37	86.25	100	5.20	87.90	10.53	88.32
Control		43.00		90.00			43.00		90.00	
SEm ±		0.72		0.53			037		0.40	
CD (P=0.05)		1.60		1.19			0.82		0.89	

In case of recommended dose same trend of result was observed. The hexaconazole showed the maximum inhibion of 88.37 over control with the colony diameter of 5.13 mm on 4th day and (88.44%) inhibition with 10.40 mm colony growth which was statistically at par with the Propiconazole showing the inhibition of (87.90%) with 5.20 mm colony growth on 4th day and (88.32%) with 10.53 mm colony diameter on 8th day. Among older fungicides mancozeb showed maximum efficacy and carbendazim showed least effectiveness against the pathogen.

3.2 *In vitro* Evaluation of Bio Control Agents against *Alternaria alternata* Causing Foliar Blight of Turmeric

The efficacy of two fungal bioagents i.e. *Trichoderma harzianum* and *Trichoderma viride* and two bacterial bioagents i.e. *Pseudomonas fluorescens* and *Bacillus subtilis* was evaluated under the laboratory conditions and the data observed is present in the Table 2. The data indicates that all the bio-agents significantly inhibited the mycelial growth of *A. alternata*. *T. viride* showed maximum inhibition (58.85%) of the pathogen over control with the minimum growth of pathogen (14.40 mm) followed by *T. harzianum* with (54.0%) inhibition with 16.10 mm colony diameter of the pathogen on 4th day. Among bacterial bio agents *P. flourescens* showed 33.57 per cent inhibition with 23.25 mm colony diameter on 4th day. *B. subtilis* showed least effectiveness.

The same trend was observed after 8th day. *T. viride* was observed as the best treatment with maximum inhibition of (63.86%) followed by *T. harzianum* with (54.65%) inhibition and among bacterial bio agents *P. fluorescens* inhibited 39.76 per cent of the growth of pathogen. *B. subtilis* was observed as least effective bio-control agents.

Table 2. *In vitro* evaluation of bio control agents against *Alternaria alternata* causing foliar blight of turmeric

Treatment	4th day		8thday	
	Mycelial growth of *A. alternata*(mm)	Inhibition (%)	Mycelial growth of *A.alternata*(mm)	Inhibition (%)
T. harzianum	16.10	54.0	26.23	54.65
T. viride	14.40	58.85	20.38	63.86
P. fluorescens	23.25	33.57	33.68	39.76
Bacillus subtilis	25.50	27.14	36.33	35.34
Control	35.00		42.48	
SEm ±	**0.28**		**0.24**	
CD (P=0.05)	**0.62**		**0.74**	

3.3 *In vitro* Evaluation of Fungicides against Trichoderma Viride

The compatibility of fungal bio-agents (*T. harzianum* and *T. viride*) with the fungicides were evaluated at two different concentrations i.e. half of the recommended dose and recommended dose of selected fungicides and the result is presented in Table 3. The data shows that all the fungicides inhibited the growth of *T. viride* at all the concentrations tested. However, copper oxychloride showed maximum radial growth of *T. viride* which was observed as 16.83 mm on 4th day with the inhibition of (54.59%) at half dose of fungicide whereas, 30.70 mm radial growth with (44.18%) inhibition was observed on 8th day this was followed by propiconazole with 5.00 mm radial growth and (86.48%) inhibition on 4th day and 6.75 mm radial growth with (87.72%) inhibition on 8th day.

Other fungicides inhibited more than (90%) at half dose. The same trend was observed in case of full dose of fungicides. Copper oxychloride showed minimum inhibition of *T. viride* i.e., (62.97%) with 13.70 mm colony diameter on 4th day and (49.09%) inhibition with 28.00 mm radial growth on 8th day of observation. Rest of the fungicides like hexaconazole and propiconazole showed total inhibition of the bio-control agent at recommended dose.

3.4 *In virto* Evaluation of Fungicides against Bacterial Bio-control Agents i.e., *Pseudomonas fluorescens* and *Bacillus subtilis*

The compatibility of bacterial bio-agents with five fungicides at two concentrations i.e half of the recommended dose and recommended dose was evaluated using seeded plate technique and results are present in the Table 4. In case of *Pseudomonas fluorescens* hexaconazole was found highly effective chemical showing no inhibition zone i.e., 0.00 mm at half dose and 2.50 mm inhibition zone at full dose followed by carbendazim showing 3.00 mm inhibition zone at half dose and 6.00 mm inhibition zone at full dose. The copper oxychloride was observed as least effective chemical giving maximum inhibition zone.

The results of the experiment shows that in case of *Bacillus subtilis* propiconazole showed no inhibition zone i.e., 0.00 mm at half dose but 8.00 mm inhibition zone was observed at full dose followed by carbendazim with the inhibition zone of 7.00 mm at half dose and 10.33 mm at full dose. Copper oxychloride was observed as the least effective chemical showing maximum inhibition zone.

3.5 *In vivo* Evaluation of Soil Amendments, Biocontrol Agents and Fungicides against Foliar Blight of Turmeric

The efficacy of different treatments viz; soil amendments, biocontrol agents and fungicides alone and in possible combinations were evaluated under field conditions in case of cultivars PH-1 and Local cultivar in two different years 2014 and 2015.

The data show that among all the management practices i.e soil amendments Mustard oil cake and FYM, biocontrol agents *Trichoderma viride* and *Pseudomonas flurescens,* fungicides mancozeb and hexaconazole, alone and in various combinations significantly inhibited the foliar blight disease under field conditions. It was observed that in case of cultivar PH-1 the minimum disease intensity observed was in the combination of hexaconazole + *T. viride i.e*

(4.2%), which was statistically at par with hexaconazole + *P. fluorescens*(4.8%), hexaconazole + FYM (5.1%), hexaconazole + mustard oil cake (5.2%) and hexaconazole alone (5.3%) followed by mancozeb + FYM (9.2%) which was statistically at par with mancozeb + *T. viride* (10.1%), mancozeb + mustard oil cake (10.4%) and mancozeb + *P. fluorescens*(10.6%) and the least effective was mustard oil cake.

In case of Local cultivar the minimum disease intensity was observed in combination of hexaconazole + *T. viride i.e* (6.7%) which was statistically at par with hexaconazole + *P. fluorescens*(6.7%) , hexaconazole + FYM (7.2%), hexaconazole + mustard oil cake (7.4%) and hexaconazole alone (7.7%) followed by mancozeb + FYM (11.6%) which was statistically at par with mancozeb + *T. viride* (12.3%), mancozeb + mustard oil cake (12.7%) and mancozeb+ *P. fluorescens* (12.8%) and the least effective was mustard oil cake.

4. DISCUSSION

The fungicide Hexaconazole was highly effective in minimizing the colony growth under *in vitro* condition (88.44 per cent), which was statistically at par with Tebuconazole (88.32 per cent). Under laboratory condition *T. viride* was found highly effective in the colony growth suppression (63.86 per cent). Among fungicides only Copper) oxychloride allowed the growth of the bioagent T. viride, which was observed as 28.00 mm radial

Table 3. *In vitro* evaluation of fungicides against Trichoderma viride

Treatment	Concentration (ppm)	4th day		8th day		Concentration (ppm)	4th day		8th day	
		Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)		Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)
Mancozeb	1000	2.20	94.05	4.50	91.81	2000	1.40	96.21	3.20	94.18
Carbendazim	500	0.70	98.10	0.00	100.00	1000	0.00	100.00	0.00	100.00
Copper Oxychloride	1500	16.83	54.59	30.70	44.18	3000	13.70	62.97	28.00	49.09
Hexaconazole	50	0.50	98.64	3.25	94.09	100	0.00	100.00	0.00	100.00
Propiconazol	50	5.00	86.48	6.75	87.72	100	0.00	100.00	0.00	100.00
Control		37.00		55.00			37.00		55.00	
SEm ±		**0.38**		**1.50**			**0.22**		**0.48**	
CD (P=0.05)		**0.84**		**3.34**			**0.50**		**1.06**	

Table. 4. *In vitro* evaluation of fungicides against *Pseudomonas fluorescens* and *Bacillus subtilis*

Fungicide	Dose (ppm)	Inhibition Zone (mm)	
		Pseudomonas fluorescens	***Bacillus subtilis***
Mancozeb	1000	05.00	15.00
Carbendazim	500	03.00	07.00
Copper Oxychloride	1500	13.33	22.33
Hexaconazole	50	00.00	08.75
Propiconazole	50	07.75	00.00
Mancozeb	2000	09.66	18.00
Carbendazim	1000	06.00	10.33
Copper Oxychloride	3000	15.00	26.33
Hexaconazole	100	02.50	11.50
Propiconazole	100	10.75	08.00
Control		**0.00**	**0.00**

Table 5. *In vivo* evaluation of soil amendments, bio-control agents and fungicides against *Alternaria alternata* causing foliar blight of turmeric

Treatment	Per cent Disease Intensity (%)		Inhibition (%)	
	PH 1 (2014)	Local (2015)	PH 1 (2014)	Local (2015
Mustard oil Cake	28.3	30.6	10.05	9.20
FYM	27.2	29.8	13.54	11.57
Trichoderma viride	22.6	25.3	28.25	24.93
Pseudomonas fluorescens	24.4	26.3	22.54	21.96
Mancozeb	11.2	13.7	64.44	59.35
Hexaconazole	5.3	7.7	83.28	77.25
Mancozeb + Mustard oil Cake	10.4	12.7	66.98	62.41
Mancozeb + FYM	9.2	11.6	70.69	65.58
Mancozeb + *Trichoderma viride*	10.1	12.3	67.83	63.50
Mancozeb + *Pseudomonas fluorescens*	10.6	12.8	66.35	62.02
Hexaconazole + Mustard oil Cake	5.2	7.4	83.49	77.94
Hexaconazole + FYM	5.1	7.2	83.81	78.64
Hexaconazole + *Trichoderma viride*	4.2	6.7	86.67	80.12
Hexaconazole + *Pseudomonas fluorescens*	4.8	6.9	84.76	79.53
Control	31.5	33.7		
SEm ±	**0.60**	**0.49**		
CD (p=0.05)	**1.72**	**1.42**		

growth at 3000 ppm concentration. In case of field studies minimum disease was observed in case of Hexaconazole + *T. viride* in both the cultivars PH-1 (4.20 per cent) and local Cultivar (6.70 per cent), which was statistically at par with Hexaconazole + *Pseudomonas fluorescens* **in** both the cultivars PH-1 (4.80 per cent) and local Cultivar (6.90 per cent).

5. CONCLUSION

Combination of fungicide and bioconrol agent is beneficial in effective management of the disease along with the reduction of toxicity in the produce and reduction of production cost.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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