

Efficacy of Fungicides and Bioagents Against Leaf Spot Diseases of Safed Musali

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ABSTRACT

In an experimental finding it was observed that all chemicals including systemic, non systemic and combination products were significantly superior in reducing the spore germination of *Colletotrichum dematium* and *Alternaria alternata* within 24 hours. In vitro evaluation of different chemicals and bioagents revealed that after 7th days, the fungicide propiconazole achieved complete inhibition followed by 0.1% tridemorph (97.23%) and thiram 97.29% against *Colletotrichum dematium*. Maximum fungal growth was recorded in control i.e. 78.00 mm. In case of *Alternaria alternata* 0.1% propiconazole recorded maximum inhibition i.e. 77.88 per cent followed by carbendazim + 0.2% mancozeb i.e. 74.89 per cent. The minimum efficiency was observed in 0.3% copper oxychloride followed by 0.2% thiophanate M with 71.66 and 65.22 mm as against 74.33 mm radial mycelial growth in control. Among the bioagents, *T. viride* recorded maximum inhibition i.e. (48.41% and 57.82%) followed with *T. harzianum* (41.80%, 42.66%) and minimum inhibitory effect was observed in *P. fluorescens* (11.65%, 16.58%) respectively.

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INTRODUCTION

India is considered as the land of medicinal plants that are cultivated or grow as wild under diversified geographical environmental conditions. Medicinal plants are rich in secondary metabolites and are potential source of drug molecules including alkaloids, glycosides, coumarins, flavonoids and steroids etc. In spite of being natural source of bioactive molecules and toxins, even medicinal plants do succumb to diseases caused by plant pathogens. Among the medicinal and

aromatic plants safed musli is mainly prone to pathogen like *Colletotrichum dematium* and *Alternaria alternata* causing leaf spot diseases that cause severe losses in tuber yield. Looking towards the severity of disease and its resulting losses the present study was undertaken under laboratory condition to assess certain disease management practices through chemicals as well as bioagents.

MATERIAL AND METHODS

Spore germination method

In plates moist cotton swab and three layer of blotting paper were placed and used as moist

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chamber. Two cavity slides were kept on a pair of glass in each chamber. The whole set was sterilized with the help of denatured spirit. A drop of double strength concentration of fungicides and suspension of pathogen was placed in cavity slide. The plates were incubated at room temperature ($28^{\circ}\text{C} \pm 2^{\circ}\text{C}$). In control only water suspension was used. The emergence of germ tube was considered as positive germination. The inhibition of spore germination was calculated and analyzed statistically.

Poisoned food technique

The principle involved in this technique was to make the nutrient medium toxic with a fungitoxicant and allow the test fungi to grow on medium and study the mycelial inhibition in laboratory. Hundred ml of liquefied potato dextrose agar medium was taken in 250 ml flask, plugged with cotton and sterilized. Requisite quantities of fungicides were added as per desired concentration. Melted toxic PDA, 20 ml/plate was poured in sterilized petriplates and allowed to solidify. These petriplates were then inoculated by test organisms separately. Six mm disc of 10 days old test fungal culture were cut with sterilized cork borer and transferred aseptically in the centre of petriplates containing the poisoned medium. The surface of inoculum disc was kept in inverted position with agar surface in plates. The control plates were also grown under same condition on PDA without fungicides. All these operations were carried out under aseptic condition in sterilized isolation chamber. Three plates were inoculated for each fungus for every fungicides. Plates were incubated at room temperature at $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

Evaluation of *Trichoderma* spp. and *Pseudomonas fluorescens* against pathogen by dual culture methods

The culture of *Trichoderma harzianum*, *T. viride* and *Pseudomonas fluorescens* obtained from Department of Plant Pathology, Dr. PDKV, Akola were grown separately on PDA and Kings B media in plates. Cultures were used for evaluation of antagonistic effect against *Alternaria alternata* and *Colletotrichum dematium*. A well sterilized melted

PDA was poured in sterilized Petriplates and after solidification 6 mm diameter circular disc from *Trichoderma* culture with sterilized cork borer was placed in each, 4 disc of antagonists were inoculated at four peripheral points and at the centre 6 mm disc of test pathogen was inoculated separately. *Pseudomonas fluorescens* was streaked with the help of bacterial needle on the nutrient agar medium and in the centre 6 mm disc of test pathogen was placed and incubated at room temperature ($27^{\circ}\text{C} \pm 2^{\circ}\text{C}$). For each isolates three replications were maintained. In control only 6 mm disc was placed per plate at centre. Antagonistic effect of *T. viride*, *T. harzianum* and *Pseudomonas fluorescens* against *Alternaria alternata* and *Colletotrichum dematium*, was assessed by dual culture method.

Inhibition of different fungus was calculated by deducting growth of isolates grown in association with *T. viride*, *T. harzianum* and *Pseudomonas fluorescens* from that of individually grown fungal colony.

RESULTS AND DISCUSSION

Spore inhibition

Colletotrichum dematium and *Alternaria alternata* infects different medicinal crop causing leaf spot disease. Data presented in Table 1. revealed that, all the chemicals including systemic, non systemic and combination products were significantly superior in reducing the spore germination. After 24 hours minimum inhibition was observed in copper oxychloride 0.3%, ridomil MZ 0.2% and chlorothalonil 0.2% i.e. 5.34, 4.37 and 8.20 per cent respectively. Highest inhibitory activities were achieved in thiram (97.27%) propiconazole 0.1% (95.29%) and zineb + hexaconazole 0.2% (94.54 %) against *Colletotrichum dematium*. In case of *Alternaria alternata* all fungicides were found to be efficient in reducing the spore germination and recorded significant differences. Maximum inhibition was observed in propiconazole 0.1% (84.56%) followed by Thiram 0.3%, carbendazim + mancozeb 0.2% and Tridemorph 0.1% with inhibition of 81.52, 84.00 and 74.04 per cent respectively after 24 hours

Table 1. *In vitro* Efficacy of different fungicides against *Colletotrichum dematium* and *Alternaria alternata* (spore inhibition) causing leaf blight of safed musli

Treatment	Fungicides	Conc. (%)	<i>Colletotrichum dematium</i> After 24 hour		<i>Alternaria alternata</i> After 24 hour	
			Mean spore germination	% Inhibition	Mean spore germination	% Inhibition
T ₁	Carbendazim	0.1	15.90 (23.50)	78.32	45.65 (42.48)	33.27
T ₂	Curzate M-8	0.2	56.33 (48.62)	23.21	21.37 (27.56)	68.76
T ₃	Thiophanate M	0.2	15.63 (23.26)	78.69	53.42 (46.95)	21.92
T ₄	Chlorothalonil	0.2	67.34 (55.12)	8.20	43.55 (41.27)	36.34
T ₅	Ridomil MZ	0.2	70.15 (56.85)	4.37	45.98 (42.65)	32.79
T ₆	Tridemorph	0.1	5.34 (13.31)	92.72	17.76 (24.95)	74.04
T ₇	Propiconazole	0.1	3.45 (10.63)	95.29	10.56 (19.00)	84.56
T ₈	Dithane M-45	0.2	52.77 (46.61)	28.06	19.24 (25.99)	71.81
T ₉	Copper oxychloride	0.3	69.44 (56.42)	5.34	64.30 (53.38)	6.02
T ₁₀	Thiram	0.3	2.00 (8.13)	97.27	12.64 (20.79)	81.52
T ₁₁	Carbendazim + Mancozeb	0.2	10.95 (19.28)	85.07	13.68 (21.72)	80.00
T ₁₂	Tricyclozole + Mancozeb	0.2	39.54 (38.94)	46.10	27.09 (31.31)	60.40
T ₁₃	Zineb + Hexaconazole	0.2	4.00 (11.54)	94.54	28.36 (32.20)	58.55
T ₁₄	Control		73.36 (58.89)	-	68.42 (55.80)	-
	'F' test		Sig.		Sig.	
	SE (m) ±		1.19		0.42	
	CD (P = 0.01)		4.67		1.63	

of incubation.

Poisoned food technique

Efficacy of different fungicide was assessed by poisoned food technique against *Colletotrichum dematium* and *Alternaria alternata* (Table 2).

Significant differences were observed among the chemical after 7th DAI. Complete inhibition was achieved in propiconazole followed by tridemorph

0.1% (97.23%) and thiram 97.29 per cent. Maximum fungal growth was recorded in control i.e. 78.00 mm in *C. dematium*. The fungi toxicity of different fungicides was evaluated by Sharma and Gupta (1986). Kardale (1991) observed the complete inhibition of *C. gloesporioides* by carbendazim and thiram. In this study it was observed the efficient toxicity of mancozeb, hexaconazole and propiconazole correlated the

Table 2. *In vitro* Efficacy of fungicides against *Colletotrichum dematium* and *Alternaria alternata* causing blight of safed musli.

Treatment	Fungicides	Conc. (%)	<i>Colletotrichum dematium</i>		<i>Alternaria alternata</i>	
			MCD after 7 days (mm)	Per cent growth inhibition	MCD after 7 days (mm)	Per cent growth inhibition
T ₁	Carbendazim	0.1	14.16	81.84	56.33	24.21
T ₂	Curzate M-8	0.2	60.83	22.01	28.00	62.33
T ₃	Thiophanate M	0.2	15.44	80.20	65.22	12.25
T ₄	Chlorothalonil	0.2	67.11	13.96	51.00	31.38
T ₅	Ridomil MZ	0.2	75.66	3.00	64.33	13.45
T ₆	Tridemorph	0.1	2.16	97.23	24.66	66.88
T ₇	Propiconazole	0.1	0.0	100.0	16.44	77.88
T ₈	Dithane M-45	0.2	53.77	31.06	25.33	65.92
T ₉	Copper oxychloride	0.3	73.25	6.08	71.66	3.59
T ₁₀	Thiram	0.3	2.11	97.29	22.00	70.40
T ₁₁	Carbendazim + Mancozeb	0.2	10.00	86.21	18.66	74.89
T ₁₂	Tricyclozole + Mancozeb	0.2	9.25	88.14	33.33	55.15
T ₁₃	Zineb + Hexaconazole	0.2	6.33	91.88	35.33	52.46
T ₁₄	Control		78.00		74.33	
	'F' test		Sig.		Sig.	
	SE(m) ±		4.58		3.85	
	CD (P = 0.01)		17.87		12.87	

present findings [2,5,8,9]. The findings of Gawande [4], Dod and Deshmukh [3], Singh *et al.* [10] and Kaur *et al.* [7] also corroborates the results.

In case of *Alternaria alternata* After 7th DAI, all test chemicals were found to be efficient in reducing the growth however, propiconazole 0.1% recorded maximum inhibition i.e. 77.88% followed by carbendazim + mancozeb 0.2% i.e. 74.89%. The minimum efficiency was observed in copper

oxychloride 0.3% followed by thiophanate M 0.2% with 71.66 and 65.22 mm as against 74.33 mm radial mycelial growth in control. Singh and Rai [10] evaluated the efficiency of different fungicides including Indofil M-45, chlorothalonil, carbendazim, thiram, tridemorph, copper oxychloride and ridomil and stated that Indofil M-45 and chlorothalonil inhibited the growth to the extent of 100%. But the present results revealed that propiconazole 0.1%,

Table 3. Efficacy of bioagent against *Colletotrichum dematium* and *Alternaria alternata* causing leaf blight of safed musli (*in vitro*)

Bioagents	<i>Colletotrichum dematium</i>		<i>Alternaria alternata</i>	
	MRD 7 th day (mm)	Per cent growth inhibition	MRD 7 th day (mm)	Per cent growth inhibition
<i>T. viride</i>	16.25	48.41	14.83	57.82
<i>T. harzianum</i>	18.33	41.80	20.16	42.66
<i>Pseudomonas fluorescens</i>	27.83	11.65	29.33	16.58
Control	31.50		35.16	
'F' test	Sig.		Sig.	
SE(m) ±	0.97		1.52	
CD (P = 0.01)	4.59		7.21	

tridemorph 0.1%, carbendazim + mancozeb 0.2% were highly efficient and at par with each other. The minimum efficiency was achieved due to ridomil 0.2%, chlorothalonil 0.2% and copper oxychloride 0.3%, might be due to variation in strains of *A. alternata* tested from different geographical areas. Akbari and Parkhia [1] proved the efficiency of systemic tridemorph, propiconazole, hexaconazole while thiram and mancozeb among non systemics recorded cent per cent inhibition of *A. alternata*. The similar trend was observed in the present studies. Copper oxychloride was least efficient in reducing the growth. Systemic fungicides have shown their superiority over non systemic in restricting the growth of *A. alternata*. The effectiveness may be attributed to their greater ability to inhibit the metabolic activities of pathogen.

Efficiency Bioagents

The fungal and bacterial bioagents were tested against *Colletotrichum dematium* and *Alternaria alternata* (Table 3). After 7th days the *T. viride* recorded maximum inhibition i.e. 48.41 per cent and in *T. harzianum* 41.80 per cent. The minimum inhibitory effect was observed in *P. fluorescens* i.e. 11.65 per cent against *Colletotrichum dematium*. In case of *Alternaria alternata* after 7th day *T. viride* recorded maximum inhibition i.e. 57.82 per cent followed with *T. harzianum* 42.66 per cent. The minimum inhibition was observed in *P. fluorescens* i.e. 16.58 per cent while 35.16 mm radial mycelial growth was noted in control.

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