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Aparna Tekade
Dr. Panjabrao Deshmukh Krishi Vidypeeth, Akola (Maharashtra), India

Mina D Koche
Dr. Panjabrao Deshmukh Krishi Vidypeeth, Akola (Maharashtra), India

RB Kothikar
Dr. Panjabrao Deshmukh Krishi Vidypeeth, Akola (Maharashtra), India.

AN Surpam
Dr. Panjabrao Deshmukh Krishi Vidypeeth, Akola (Maharashtra), India

Correspondence
Aparna Tekade
Dr. Panjabrao Deshmukh Krishi Vidypeeth, Akola, Maharashtra, India

Efficacy of fungicides and bioagents against *Curvularia lunata* causing blight of coleus under laboratory conditions

Aparna Tekade, Mina D Koche, RB Kothikar and AN Surpam

Abstract
Experiment was conducted to assess the effect of different bioagents like *Trichoderma viride*, *T. harzianum* and *Pseudomonas fluorescence* and fungicides like Carbendazim (0.1%), Curzate M-8 (0.2%), Topsin M (0.2%), Chlorothalonil (0.2%), Metalaxyl (0.2%), Tridemorph (0.1%), Propiconazole (0.1%), Mancozeb (0.2%), Copper oxychloride (0.3%), Thiram (0.3%), Carbendazim + Mancozeb (0.2%), Tricyclazole + Mancozeb (0.2%) and Zineb + Hexaconazole (0.2%) against *Curvularia lu*nata. After 7th days the *T. viride* recorded maximum inhibition 60.81% followed with *T. harzianum* (50.71%) and minimum inhibitory effect was observed in *P. fluorescens* (33.41%). Among all the systemic non systemic and combination products, Complete inhibition was achieved in tridemorph, propiconazole, dithane, carbendazim + mancozeb, tricyclozole + mancozeb and zineb + hexaconazole and minimum inhibition (10.17%) was recorded in carbendazim 0.1 per cent while 65.33 mm radial mycelial growth was recorded in control.

Keywords: Fungicides, Bioagents, *Colletotrichum dematium*, Safed musali

1. Introduction
Human dependence on plant for curing of diseases is supported by facts of Ayurveda. Medicinal plants are rich in secondary metabolites and are potential source of drugs includes alkaloids, glycosides, coumarins, flavonoids and steroids etc. Among the medicinal and aromatic plants coleus is an important indigenous medicinal and aromatic plant and was mainly proned with the pathogen like *Curvularia lunata* leaf spot causing severe losses in yield. Initial symptoms of the disease are small brown water soaked flecks appears on the upper leaf surface with diameter ranging from 0.5 to 3 cm which later coalesced to form dark brown lesions with a well-defined border. Lesions often merged to form large necrotic areas, covering more than 90% of the leaf surface, which contributed to plant death. The disease significantly reduces the number of functional leaves. Late in the disease progression, stems and rhizomes were also affected thereby reducing oil yield and quality. Therefore, in view of the magnitude of damage caused by the fungus on this important plant, the present investigation was undertaken to screen out the most efficient fungicides and bioagents against *C. lunata* for management of the disease.

2. Material and Methods
2.1 Spore germination method
In plates moist cotton swab and three layer of blotting paper were placed and used as moist 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 °C ± 2 °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.

2.2 Poisoned food technique
The principle involved 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 °C ± 2 °C.

2.3 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 *Curvularia lunata*. 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 °C ± 2 °C). For each isolates three replications were maintained. In control only 6 mm disc was placed per plate at centre. Antanonistic effect of *T. viride, T. harzianum* and *Pseudomonas fluorescens* against *Curvularia lunata* 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.

3. Results and Discussion

3.1 Spore inhibition technique

Curvularia lunata causes leaf blight in coleus and the pathogen was tested against different fungicides *in vitro* by spore inhibition (Table 1). All test chemicals were effective in reducing the germination and significantly superior over control. The maximum germination was noted in control i.e. 48.39, 53.77 and 57.84 per cent at 4, 12 and 24 hours respectively. Propiconazole 0.1% and tricyclozole + mancozeb 0.2% were observed to be effective and recorded 100 per cent inhibition till 24 hours. Tridemorph 0.1%, dithane M-45 0.2% and carbendazim + mancozeb 0.2 per cent were efficient and recorded more than 90 per cent inhibition of spore germination. After 24 hour of incubation the efficiency was reduced to lesser extent that the toxicity was reduced with the increase in incubation time. The minimum spore inhibition was achieved due to chlorothalonil 0.2% and thiophanate M 0.2% with 30.06, 35.09 and 49.42 per cent inhibition respectively. Similar, more or less results were reported by Aparna Tekade *et.al*. (2015) [1] while studying on occurrence of diseases on medicinal plants of Vidarbha region of Maharashtra. Avasthi *et.al*. (2015) [2] studied on occurrence of leaf spot diseases on *Aloevera* and reported that, among the pathogens, *Curvularia* spp. are the major incitants for the leaf blight disease and can be inhibited with the use of fungicides under laboratory conditions.

3.2 Poisoned food technique

Table 2 revealed that, after 3rd day of incubation Ridomil MZ 0.2%, tridemorph 0.1%, propiconazole 0.1%, dithane M-45 2%, carbendazim + mancozeb 0.2%, tricyclozole + mancozeb 0.2% and zineb + hexaconazole 0.2% were observed to be highly efficient in reducing the growth of *Curvularia lunata* to the extent of 100%, Curzate M-8 0.2% exhibited 91.78 per cent inhibition while, 28.89 per cent inhibition was recorded in carbendazim 0.1 per cent. Similar efficiency was recorded after 7th days of incubation. Mycelial growth was minimum i.e. 7.44 mm in ridomil and in thiram 9.16 mm with i.e. 88.61 and 85.97 per cent inhibition respectively while, 65.33 mm radial mycelial growth was recorded in control. Similar results were reported by Rakshpal Singh *et.al.* (2011) [4].

3.3 Bioagent

Different bioagents were assessed against *Curvularia lunata* by dual culture method. Minimum radial mycelial growth i.e. 18.25 mm with higher inhibition i.e. 60.60 per cent was achieved in *Trichoderma viride* followed with *T. harzianum* 51.09 per cent. The minimum inhibitory effect was observed in *Pseudomonas fluorescens* i.e. 32.13 per cent (Table 3). After 7 day *Trichoderma viride* recorded maximum inhibition i.e. 60.81 per cent, while 50.71 per cent inhibition was achieved in *T. harzianum* and 33.41 per cent in *Pseudomonas fluorescens*. Chijamo Kithan and Daiho (2014) [3] tested bioagents against *Curvularia lunata* causing leaf blight in *Etlingera linguiformis* a medicinal herb and found that, among the bioagents, treatment containing rhizome + foliar spray of *T. harzianum* and rhizome treatment + foliar spray with *T. viride* were found most effective.

Table 1: Efficacy of different fungicides against *Curvularia lunata* (spore inhibition) causing leaf blight of coleus (*in vitro*)

Tt. No.	Fungicides	Conc. (%)	4 hour		12 hour		24 hour	
			Mean spore germination	Per cent inhibition	Mean spore germination	Per cent inhibition	Mean spore germination	Per cent inhibition
T_1	Carbendazim	0.1	28.55 (32.27)	41.00	38.23 (38.17)	28.90	40.45 (39.47)	30.06
T_2	Curzate M-8	0.2	6.75 (15.00)	86.05	10.25 (18.63)	80.93	14.95 (22.71)	74.15
T_3	Thiophanate M	0.2	16.29 (23.81)	66.33	23.44 (28.93)	56.40	29.25 (32.71)	49.42
T_4	Chlorothalonil	0.2	25.48 (30.33)	47.34	31.67 (34.27)	41.10	37.54 (37.76)	35.09
T_5	Ridomil MZ	0.2	3.00 (9.98)	93.80	5.30 (13.31)	90.14	7.45 (15.79)	87.11
T_6	Tridemorph	0.1	1.45 (6.80)	97.00	1.98 (7.90)	96.31	2.00 (8.13)	96.54
T_7	Propiconazole	0.1	0	100.0	0	100.0	0	100.0
T_8	Dithane M-45	0.2	2.00 (8.13)	95.86	2.00 (8.13)	96.28	3.75 (11.09)	93.51
T_9	Copper oxychloride	0.3	6.78 (15.12)	85.98	8.95 (17.36)	83.35	10.30 (18.72)	82.19
T_{10}	Thiram	0.3	5.96 (14.06)	87.68	8.65 (17.04)	83.91	9.54 (17.95)	83.50
T_{11}	Carbendazim +	0.2	4.00 (11.54)	91.73	5.98 (14.06)	88.87	6.99 (15.23)	87.91

	Mancozeb							
T_{12}	Tricyclozole + Mancozeb	0.2	0	100.0	0	100.0	0	100.0
T_{13}	Zineb + Hexaconazole	0.2	3.47 (10.78)	92.82	5.98 (14.06)	88.87	7.33 (15.68)	87.32
T_{14}	Control		48.39 (44.08)	-	53.77 (47.18)	-	57.84 (49.49)	-
	'F' test		Sig.		Sig.		Sig.	
	SE(m) ±		0.48		0.50		0.54	
	CD (P = 0.01)		1.88		1.96		2.10	

Table 2: Efficacy of fungicides against *Curvularia lunata* (*in vitro*) causing leaf blight of coleus

Tt. No.	Fungicides	Conc. (%)	Mean colony diameter after 3rd day (mm)	Per cent growth inhibition	Mean colony diameter after 7 days (mm)	Per cent growth inhibition
T_1	Carbendazim	0.1	40.33	28.89	58.32	10.71
T_2	Curzate M-8	0.2	4.66	91.78	15.25	76.65
T_3	Thiophanate M	0.2	17.25	69.58	30.16	53.83
T_4	Chlorothalonil	0.2	25.83	54.46	38.00	41.83
T_5	Ridomil MZ	0.2	0.0	100.0	7.44	88.61
T_6	Tridemorph	0.1	0.0	100.0	0.0	100.0
T_7	Propiconazole	0.1	0.0	100.0	0.0	100.0
T_8	Dithane M-45	0.2	0.0	100.0	0.0	100.0
T_9	Copper oxychloride	0.3	7.16	87.37	10.82	83.43
T_{10}	Thiram	0.3	4.11	92.75	9.16	85.97
T_{11}	Carbendazim + Mancozeb	0.2	0.0	100.0	0.0	100.0
T_{12}	Tricyclozole + Mancozeb	0.2	0.0	100.0	0.0	100.0
T_{13}	Zineb + Hexaconazole	0.2	0.0	100.0	0.0	100.0
T_{14}	Control	-	56.72	-	65.33	-
	'F' test		Sig.		Sig.	
	SE(m) ±		0.93		1.23	
	CD (P = 0.01)		3.62		4.80	

Table 3: Efficacy of bioagent against *Curvularia lunata* causing blight of coleus (*in vitro*)

Bioagents	Mean radial diameter 3rd day (mm)	Per cent growth inhibition	Mean radial diameter 7th day (mm)	Per cent growth inhibition
Trichoderma viride	18.25	60.60	21.33	60.81
Trichoderma harzianum	22.66	51.09	26.83	50.71
Pseudomonas fluorescens	31.44	32.13	36.25	33.41
Control	46.33	-	54.44	-
'F' test	Sig.	-	Sig.	-
SE(m) ±	1.61	-	2.63	-
CD (P = 0.01)	7.65	-	12.51	-

4. References

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