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In vitro evaluation of fungicides and bio-agents against *Drechslera tetramera, Bipolaris sacchari, Nigrospora sphaerica* and *Alternaria alternata* causal agent of leaf spot of medicinal and aromatic grasses

Someshree Mane, RW Ingle, Priti Shinde and Ashwini Kumar

Abstract
India is a global leader in essential oil steam distilled from aromatic crops. Most of the species of *Cymbopogon*. e. *C. winter ianus Jowitt, C. flexuosus (Nees) Wats.* and *C. martinii* (Roxb.) *Wats.* have been cultivated for a long time for their essential oils. Few fungal pathogens like *Drechslera tetramera, Bipolaris sacchari, Nigrospora sphaerica* and *Alternaria alternata* are known to attack these grasses causing leaf spot which results into substantial yield losses. The present investigation was carried out to test the efficacy of fungicides and bio-agents In Vitro. Among fungicides tested, the highest (100%) mycelial inhibition of *Drechslera tetramera, Bipolaris sacchari, Nigrospora sphaerica* and *Alternaria alternata* were recorded in Propiconazole (0.1%) and Propiconazole + Difenoconazole (0.1%). The results of dual culture technique revealed that maximum growth inhibition of *Drechslera tetramera* (61.60%), *Bipolaris sacchari* (62.00%), *Nigrospora sphaerica* (67.88%) and *Alternaria alternata* (55.00%) was observed with *Trichoderma viride* followed by *Pseudomonas fluorescens* and *Bacillus subtilis*.

Keywords: citronella, lemongrass, palmrosa, leaf spot, fungicides, biological control agents

Introduction
India has a wealth of 2500 aromatic plants out of the 20,000 species occurring in the world. Organically grown medicinal and aromatic crop products are not only readily accepted in the global markets, but also command higher prices than those cultivated using chemical inputs. Globalizatron increased the demand for Indian medicinal and aromatrc plants. India is currently exporting Rs.1256 crores of crude drugs from medicinal plants and Rs.260 crores worth of essential oils annually to a number of countries including USA, Europe and Japan. India has an excellent potential to emerge as a global leader both in medicinal and aromatic plants sector (Rajeswara Rao and Rajput, 2005) [1].

Aromatic grasses produce essential oils, perfumes and flavours are in use with our civilization since several thousand years. Due to vast area and varied agro-climatic conditions, it can be commercially cultivated in different parts of India successfully. Essential oils and aroma chemicals are indispensible in various human activities. Some of the important aromatic grasses like Lemongrass, Citronella, Palmrosa etc. have great demand in our country.

Disease is one of the major constraint in economic crop production as they inflict heavy losses. Like other plants, aromatic grasses are also attacked by many diseases during their growth. Few fungal and bacterial pathogens are known to attack these grasses resulting into substantial yield losses. Due to foliar diseases the major oil constituents were depleted in diseased leaf as compared to the healthy one. The recovery of oil was decreased along with the quantity of citronellal, citronellol, citronellyl acetate, geranyl acetate and limonene in the diseased leaf oil. Among the fungal diseases, leaf spot caused by *Bipolaris sacchari, Drechslera tetramera, Alternaria alternata,* and *Nigrospora sphaerica*. So, it is necessary to control these fungi before causing damage by using suitable control measures i.e. chemicals and bio agents.

Materials and Methods

In present investigation, efforts were made to evaluate some fungicides and bio agents against the test pathogens by poison food and dual culture method. Required quantity of individual fungicide was added separately into sterilized molten PDA so as to get the desired concentration of the fungicides. Later, 20 ml of the poisoned media was poured into sterilized Petri plates. Mycelium discs of five mm diameter from seven days old culture of the fungus were cut out by sterile cork borer and one such disc was placed at the centre of each plate. PDA without any fungicide served as control. Three replications were maintained for each concentration. Plates were incubated at room temperature for seven days and radial growth was measured when fungus attained maximum growth in control plates. The efficacy of the fungicides was expressed as per cent inhibition of mycelial growth over control which was calculated by using the formula of Vincent (1927).

Bio agents were evaluated for their efficacy through dual culture technique. Twenty ml of sterilized and cooled potato dextrose agar medium was poured into sterilized Petri plates. Fungal antagonists were evaluated by inoculating a pathogen at one side of the Petri plate and the antagonist at exactly opposite side of the same plate by leaving about 4 cm gaps. For this, actively growing cultures were used. In case of bacterial antagonist evaluation, one mycelial disc of pathogen were inoculated in the centre of the petri plate and bacterial antagonist was streaked two times at the periphery of the same plate. After required period of incubation the radial growth of the pathogens was measured. Per cent inhibition over control was worked out according to the equation given by Vincent. The different antagonistic organisms used against *Bipolaris sacchari, Drechslera tetramera, Alternaria alternata,* and *Nigrospora sphaerica*. includes, *Trichodermavride, Pseudomonas fluorescens* and *Bacillus subtilis.*

Where, I = Per cent inhibition C = Growth in control T = Growth in treatment

Table 1: Fungicides used in the *In Vitro* study

Tr. No	Trade name	Common name	Active ingredients	Formulation	Conc.%
T1	Nativo	Tebuconazole + Trifloxystrobin	50%+25%	WG	0.1
T2	Sprint	Carbendazim + Mancozeb	25%+50%	WS	0.2
T3	Reader	Propiconazole + Difenoconazole	13.9%+13.9%	EC	0.1
T4	Tilt	Propiconazole	25%	EC	0.1
T5	Amistar	Azoxystrobin	23%	SC	0.2
T6	Contaf	Hexaconazole	5%	WP	0.1
T7	Bavistin	Carbendazim	50%	WP	0.1

Results and Discussion

Seven fungicides were tested at different concentrations in the laboratory for their efficacy against *Drechslera tetramera, Bipolaris sacchari, Nigrospora sphaerica* and *Alternaria alternata* causal agent of leaf spot of aromatic grasses as described in 'Material and Methods'. The results are presented here under. Poisoned food method was employed to test the efficacy of various fungicides against *Drechslera tetramera, Bipolaris sacchari, Nigrospora sphaerica* and *Alternaria alternata*. Propiconazole (0.1%) and Propiconazole+ Difenoconazole (0.1%) was found most effective for arresting 100% mycelial growth of Drechslera tetramera, Bipolaris sacchari, Nigrospora sphaerica and Alternaria alternata. Hexaconazole (0.3%) and Carbendazim + Mancozeb (0.2%) showed 100% mycelial inhibition *of Drechslera tetramera, Bipolaris sacchari* and *Nigrospora sphaerica*. Tebuconazole + Trifloxystrobin (0.1%) showed 100% mycelial inhibition of *Bipolaris sacchari, Nigrospora sphaerica* and *Alternaria alternata*. Whereas Carbendazim (0.1%) was found most effective for arresting 100% mycelial growth of *Alternaria alternata* and *Nigrospora sphaerica*. Tebuconazole + Trifloxystrobin (0.1%) showed 81.96% mycelial inhibition of *Drechslera tetramera*. Hexaconazole (0.3%) showed 78.24% mycelial inhibition of *Alternaria alternata*. Carbendazim (0.1%) showed 23.78% and 35.11% mycelial inhibition of *Drechslera tetramera* and *Bipolaris sacchari* respectively. Azoxystrobin (0.2%) showed 65.10%, 80.85%, 53.18% and 39.54% mycelial inhibition of *Drechslera tetramera, Nigrospora sphaerica, Alternaria alternata* and *Bipolaris sacchari* respectively.

In dual culture technique, maximum growth inhibition of *Drechslera tetramera* (61.60%), *Bipolaris sacchari* (62.00%), *Nigrospora sphaerica* (67.88%) and *Alternaria alternata* (55.00%) was observed with *Trichoderma viride* followed by *Pseudomonas fluorescens* and *Bacillus subtilis*.

In present investigation, all the fungicides tested showed better inhibition of the mycelial growth and sporulation of *Drechslera tetramera, Nigrospora sphaerica, Alternaria alternata* and *Bipolaris sacchari*. Propiconazole (0.1%) and Propiconazole+ Difenoconazole (0.1%) was found effective against all fungi *Drechslera tetramera, Nigrospora sphaerica, Alternaria alternata* and *Bipolaris sacchari*. Hexaconazole (0.3%) and Carbendazim + Mancozeb (0.2%) was found second effective fungicides of *Drechslera tetramera, Bipolaris sacchari* and *Nigrospora sphaerica.* Also Carbendazim (0.1%) was recorded effective against *Nigrospora sphaerica* and *Alternaria alternata.* This variation in fungus sensitivity is to be expected with the more specific benzimidazole and dithiocarbamate compounds and is well documented (Kiran *et al.* 2011, Mahendra *et al*. 2012) [5, 6], Triazoleare known to inhibit the sterol biosynthesis pathaway (Nine and Thapliyal). Simimilar observations were reported by Jakatimath *et al*. (2017) [7] and Yamashita *et al.* (2010) [8].

In present investigation, the bio-control agents viz., *Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis* were tested In Vitro against *Drechslera tetramera, Nigrospora sphaerica, Alternaria alternata* and *Bipolaris sacchari* Maximum mycelial inhibition of Drechslera tetramera, Nigrospora sphaerica, *Alternaria alternata* and Bipolaris sacchari were recorded against *Trichoderma viride*. The results are in accords with Pandey and Hussain (2010) [3] who observed that both the spacies of Trichoderma i.e *T. viride* and *T. harzianum* was most effective for inhibition of mycelial growth of *Drechslera tetramera* in *Capsicum frutescens*. Similar results were recorded by Pandey (2010) [2] recorded that *Trichoderma viride* inhibits 66.67% of mycelial growth of *Alternaria alternata,* a common and destructive pathogen of *Capsicum frutescens*.

Table 2: Efficacy of fungicides against *Drechslera tetramera* by poisoned food method

Tr.no	Fungicides	Conc. (%)	3rd day		5th day		7th day	
			MCD (mm)	PGI (%)	MCD (mm)	PGI (%)	MCD (mm)	PGI (%)
T1	Tebuconazole(50%) + Trifloxystrobin(25%)WG	0.1	0.00	100	12.00	76.69	12.00	81.96
T2	Carbendazim (25%) + Mancozeb (50%) WS	0.2	0.00	100	0.00	100	0.00	100
T3	Propiconazole (13.9%) + Difenoconazole (13.9%) EC	0.1	0.00	100	0.00	100	0.00	100
T4	Propiconazole25%EC	0.1	0.00	100	0.00	100	0.00	100
T5	Azoxystrobin 23%SC	0.2	0.00	100	21.19	58.85	23.21	65.10
T6	Hexaconazole 5% WP	0.1	0.00	100	0.00	100	0.00	100
T7	Carbendazim 50% WP	0.1	0.00	100	32.05	37.76	50.70	23.78
T8	Control	-	35.33	-	51.50	-	66.52	-
	'F test	-	-	-	Sig.	-	Sig.	-
	SE(m)±	-	-	-	0.51	-	0.46	-
	CD(P=0.01)	-	-	-	2.11	-	1.92	-

MCD=Mean Colony Diameter (mm), PGI=Per cent Growth Inhibition (%)

Table 3: Efficacy of fungicides against *Nigrospora sphaerica* by poisoned food method

Tr. no	Fungicides	Conc. (%)	3rd day		5th day		7th day	
			MCD (mm)	PGI (%)	MCD (mm)	PGI (%)	MCD (mm)	PGI (%)
T1	Tebuconazole(50%) + Trifloxystrobin(25%)WG	0.1	0.00	100	0.00	100	0.00	100
T2	Carbendazim (25%) + Mancozeb (50%) WS	0.2	0.00	100	0.00	100	0.00	100
T3	Propiconazole (13.9%) + Difenoconazole (13.9%) EC	0.1	0.00	100	0.00	100	0.00	100
T4	Propiconazole25%EC	0.1	0.00	100	0.00	100	0.00	100
T5	Azoxystrobin 23%SC	0.2	0.00	100	13.00	84.32	17.23	80.85
T6	Hexaconazole 5% WP	0.1	0.00	100	0.00	100	0.00	100
T7	Carbendazim 50% WP	0.1	0.00	100	0.00	100	0.00	100
T8	Control	-	51.58	-	82.91	-	90.00	-
	'F test	-	-	-	Sig.	-	Sig.	-
	SE(m)±	-	-	-	0.52	-	0.48	-
	CD(P=0.01)	-	-	-	2.17	-	2.00	-

MCD=Mean Colony Diameter (mm), PGI=Per cent Growth Inhibition (%)

Table 4: Efficacy of fungicides against *Alternaria alternata* by poisoned food method

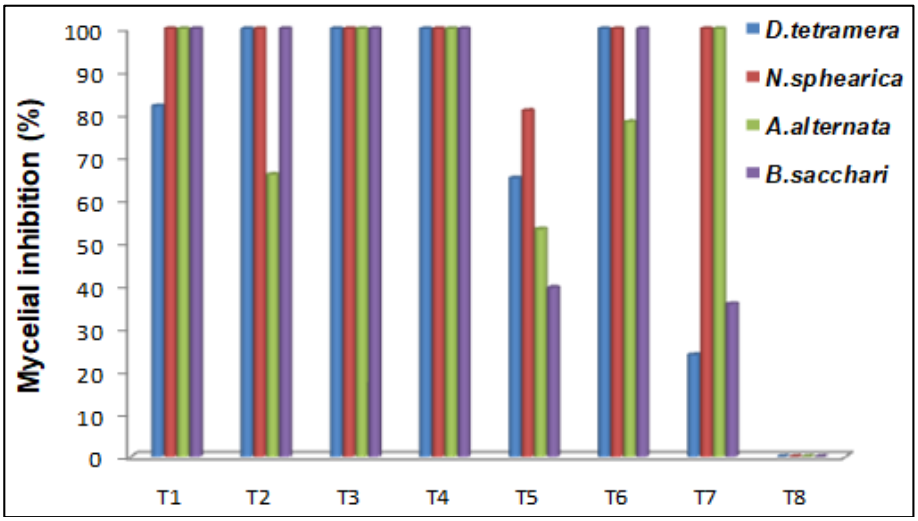
Tr. no	Fungicides	Conc. (%)	3rd day		5th day		7th day	
			MCD (mm)	PGI (%)	MCD (mm)	PGI (%)	MCD (mm)	PGI (%)
T1	Tebuconazole (50%) + Trifloxystrobin(25%)WG	0.1	0.00	100	0.00	100	0.00	100
T2	Carbendazim (25%) + Mancozeb (50%) WS	0.2	14.46	59.73	21.27	59.09	23.57	65.95
T3	Propiconazole (13.9%) + Difenoconazole (13.9%) EC	0.1	0.00	100	0.00	100	0.00	100
T4	Propiconazole25%EC	0.1	0.00	100	0.00	100	0.00	100
T5	Azoxystrobin 23%SC	0.2	16.66	64.16	26.16	49.69	32.41	53.18
T6	Hexaconazole 5% WP	0.1	0.00	100	11.83	77.25	15.06	78.24
T7	Carbendazim 50% WP	0.1	0.00	100	0.00	100	0.00	100
T8	Control	-	35.91	-	52.00	-	69.23	-
	'F test	-	Sig	-	Sig.	-	Sig.	-
	SE(m)±	-	0.22	-	0.48	-	0.54	-
	CD(P=0.01)	-	0.92	-	2.01	-	2.24	-

MCD=Mean Colony Diameter (mm), PGI=Per cent Growth Inhibition (%)

Table 5: Efficacy of fungicides against *Bipolaris sacchari* by poisoned food method

Tr. No	Fungicides	Conc. (%)	3rd day		5th day		7th day	
			MCD (mm)	PGI (%)	MCD (mm)	PGI (%)	MCD (mm)	PGI (%)
T1	Tebuconazole(50%) + Trifloxystrobin(25%)WG	0.1	0.00	100	0.00	100	0.00	100
T2	Carbendazim (25%) + Mancozeb (50%) WS	0.2	0.00	100	0.00	100	0.00	100
T3	Propiconazole (13.9%) + Difenoconazole (13.9%) EC	0.1	0.00	100	0.00	100	0.00	100
T4	Propiconazole25%EC	0.1	0.00	100	0.00	100	0.00	100
T5	Azoxystrobin 23%SC	0.2	0.00	100	18.16	37.37	21.16	39.54
T6	Hexaconazole 5% WP	0.1	0.00	100	0.00	100	0.00	100
T7	Carbendazim 50% WP	0.1	0.00	100	20.50	29.31	22.50	35.71
T8	Control	-	21.00	-	29.00	-	35.00	-
	'F test	-	-	-	Sig.	-	Sig.	-
	SE(m)±	-	-	-	0.19	-	0.22	-
	CD(P=0.01)	-	-	-	0.79	-	0.92	-

MCD=Mean Colony Diameter (mm), PGI=Per cent Growth Inhibition (%)



T1- Tebuconazole + Trifloxystrobin T2- Carbendazim + Mancozeb T3- Propiconazole + Difenoconazole T4- Propiconazole T5- Azmstrobin T6- Hexaconazole T7- Carbendazim T8- Control

Fig 1: Efficacy of fungicides against *Dresclera tetramera*, *Nigrospora sphaerica*, *Aftemata afternata* and *Bipolaris sacchari*

Table 6: Efficacy of bio-agents against *Drechslera tetramera* by dual culture method

Tr. no	Bio-agents	3rd day		5th day		7th day	
		MCD (mm)	PGI (%)	MCD (mm)	PGI (%)	MCD (mm)	PGI (%)
T1	*Trichoderma viride*	23.18	31.21	26.10	45.07	26.88	61.60
T2	*Pseudomonas fluorescens*	23.60	29.97	39.06	17.80	51.76	26.05
T3	*Bacillus subtilis*	20.00	40.65	40.00	15.82	55.92	20.11
	Control	33.70	-	47.52	-	70.00	-
	'F test	Sig	-	Sig.	-	Sig.	-
	SE(m)±	0.55	-	1.00	-	1.02	-
	CD(P=0.01)	1.62	-	2.97	-	3.03	-

MCD=Mean Colony Diameter (mm), PGI=Per cent Growth Inhibition (%

Table 7: Efficacy of bio-agents against *Nigrospora sphaerica* by dual culture method

Tr. no	Bio-agents	3rd day		5th day		7th day	
		MCD (mm)	PGI (%)	MCD (mm)	PGI (%)	MCD (mm)	PGI (%)
T1	*Trichoderma viride*	12.97	74.83	27.34	67.04	28.90	67.88
T2	*Pseudomonas fluorescens*	28.32	45.06	56.80	31.52	77.30	14,11
T3	*Bacillus subtilis*	25.56	50.40	64.32	22.45	82.50	8.33
	Control	51.55	-	82.95	-	90.00	-
	'F test	Sig	-	Sig.	-	Sig.	-
	SE(m)±	0.59	-	0.65	-	0.53	-
	CD(P=0.01)	1.75	-	1.91	-	1.56	-

MCD=Mean Colony Diameter (mm), PGI=Per cent Growth Inhibition (%)

Table 8: Efficacy of bio-agents against *Alternaria alternata* by dual culture method

Tr. no	Bio-agents	3rd day		5th day		7th day	
		MCD (mm)	PGI (%)	MCD (mm)	PGI (%)	MCD (mm)	PGI (%)
T1	*Trichoderma viride*	13.86	43.15	26.66	33.35	27.00	55.00
T2	*Pseudomonas fluorescens*	14.60	40.11	33.44	16.40	45.75	23.75
T3	*Bacillus subtilis*	22.90	6.07	34.65	13.37	41.30	31.16
	Control	24.38	-	40.00	-	60.00	-
	'F test	Sig	-	Sig.	-	Sig.	-
	SE(m)±	0.47	-	0.75	-	0.97	-
	CD(P=0.01)	1.40	-	2.22	-	2.86	-

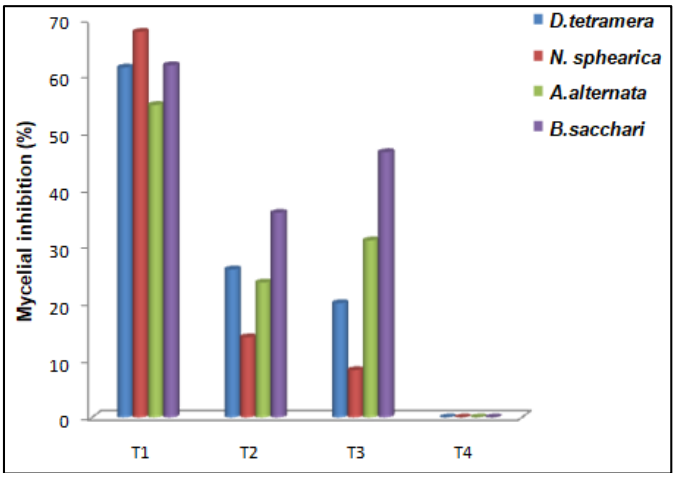
MCD=Mean Colony Diameter (mm), PGI=Per cent Growth Inhibition (%)

Table 9: Efficacy of bio-agents against *Bipolaris sacchari* by dual culture method

Tr. no	Bio-agents	3rd day		5th day		7th day	
		MCD (mm)	PGI (%)	MCD (mm)	PGI (%)	MCD (mm)	PGI (%)
T1	*Trichoderma viride*	5.04	78.38	15.20	47.96	15.20	62.00
T2	*Pseudomonas fluorescens*	5.08	75.80	10.40	64.39	25.59	35.02
T3	*Bacillus subtilis*	10.34	50.76	19.28	33.39	21.32	46.70
	Control	21.00	-	29.21	-	40.00	-
	'F test	Sig	-	Sig.	-	Sig.	-

	SE(m)±	0.37	-	0.33	-	0.60	-
	CD(P=0.01)	1.09	-	0.99	-	1.78	-

MCD=Mean Colony Diameter (mm), PGI=Per cent Growth Inhibition (%)



T1- *Trichoderma viride* T2- *Pseudomonas fluorescens*
T3- *Bacillus subtilis* T4- Control

Fig 2: Efficacy of different bioagents against *Dresclera tetramera, Nigrospora sphaerica, Anemone alternate* and *Bipolaris sacchari*

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