

Effect of bioagents and chemicals for the management of aerial blight and dry root rot of blackgram incited by *Rhizoctonia bataticola*

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ABSTRACT

Blackgram (*Vigna mungo* L.) is an important pulse crop grown throughout India. A new disease of blackgram *i.e.* aerial blight and dry root rot caused by *Rhizoctonia bataticola* is primarily a soil inhabitant. An attempt was made to manage the disease with biocontrol agents and fungicides. Among the biocontrol agents tested against *Rhizoctonia bataticola*, *Pseudomonas fluorescens* (DAPG⁺ve)-RP46 was found more effective as compared to other bio-control agents and inhibited maximum mycelial growth (68.95%) of *R. bataticola* followed by *T. harzianum* (Th-R) (61.85%) and *T. viride* (Tv-R) (61.11%) under *in vitro* condition. Fungicides like contact, systemic and combi products were also tested against the aforementioned pathogen. Among five contact fungicides captan, propineb and zineb recorded cent per cent inhibition (100%) of mycelial growth at all the concentrations (*i.e.*, 0.1, 0.2 and 0.3%) among seven systemic fungicides and combi fungicides tested, benomyl, carbendazim, hexaconazole, thiophanate methyl and tridemefon showed 100 per cent mycelia inhibition and also in carbendazim 12% + mancozeb 63%, cymoxanil 8%+ mancozeb 64%, captan 70% + hexaconazole 5 %, tricyclozole 18% + mancozeb 62% and mancozeb (64 %) + metalaxyl (4 %) showed cent per cent (100%) inhibition at all the concentrations (0.05, 0.10 and 0.2%), respectively. The maximum vigour index of 2652.83 was recorded in *Pseudomonas fluorescens* (+DAPG)- RP46 treated blackgram seeds followed by 2042.00 and 1997.80 vigour index in *Trichoderma harzianum*-II(R) and *T. viride*-II(R) and poor vigour index of 1258.00 was observed in untreated control.

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INTRODUCTION

Blackgram (*Vigna mungo* L.) is an important pulse

crop grown throughout India. Blackgram is a rich protein food containing about 25 per cent protein, which is almost three times more than that of cereals. Blackgram

supplies a major share of protein requirement of vegetarian population of the country. In India, it occupies an area of 3.5 million ha. With a production of 1.5 to 1.9 million tonnes and productivity of 500 kg/ha whereas in Karnataka, blackgram occupies in an area of 1.26 lakh ha with the production of 0.64 lakh tonnes and productivity of 508 kg per ha. Blackgram is grown in Bidar, Kalaburagi, Raichur, Yadgir, Vijayapur, Dharwad, Bellari and Belagavi districts of Northern Karnataka (Anonymous, 2012).

Blackgram is known to be affected by more than twenty diseases. Among them, anthracnose caused by *Colletotrichum lindemuthianum*, *Cercospora* leaf spot caused by *Cercospora canescens*, powdery mildew caused by *Erysiphe polygoni*, leaf crinkle disease caused by Leaf Crinkle Virus and a new disease of blackgram *i.e.* aerial blight and dry root rot caused by *Rhizoctonia bataticola* is more destructive and known to cause more than 60 per cent yield loss. Hence, in the present study an attempt was made to manage this soil borne disease with some bio agents and chemicals.

MATERIAL AND METHODS

Isolation and characterization of pathogen :

The stems/roots or leaves samples was collected used for isolation of fungal pathogen by following standard tissue isolation method. For this purpose, black gram leaves showing typical spots or stem canker symptoms were cut into small bits measuring about 2 mm and surface sterilized in 0.1 per cent mercuric chloride or sodium hypo chloride solution for one minute, such bits were transferred to Petri dishes containing sterile water successively for three times and then into the Petri dishes containing 20 ml of potato dextrose agar (PDA) medium and incubated at $28 \pm 2^{\circ}$ C for 10 days. The culture of *R. bataticola* was maintained at 5° C in the refrigerator and sub cultured periodically at an interval of 20 to 25 days during the course of the investigation.

Evaluation of bioagents and fungicides against *Rhizoctonia bataticola* :

Six bioagents were evaluated for their efficacy through dual culture technique. The fungal bio-agent and the test pathogen were inoculated side by side on a single Petri plate containing solidified PDA medium. Whereas, the bacterial bioagents were streaked one day earlier to

the test pathogen. Three replications were maintained for each isolate with one control by maintaining only pathogen and bioagent. They were incubated for seven days. The diameter of the colony of both bioagent and the pathogen was measured in both directions and average was recorded and the per cent inhibition on growth of the test pathogen was calculated by using the formula given below by Naik *et al.* (2009).

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

where;

I = Per cent inhibition

C = Radial growth of the pathogen in control

T = Radial growth of pathogen in treatment

Poison food technique (Sharvelle, 1961) was followed to test the efficacy of the mentioned fungicides. The pathogen *R. bataticola* was grown on PDA medium in Petri-plates for ten days prior to setting up the experiment. Fungicide suspension was prepared in PDA by adding required quantity of fungicide to obtain the desired concentration on the basis of active ingredient present in the chemical. Twenty ml of poisoned medium was poured in each of the sterilized Petri plates. Mycelial disc of 0.5 cm was taken from the periphery of ten day old culture and placed in the centre and incubated at $28 \pm 2^{\circ}$ C till growth of the fungus touched the periphery in control plate. Suitable checks were also maintained without addition of any fungicide, three replications were maintained for each treatment. The diameter of the colony was measured in two directions and average was worked out. The per cent mycelial inhibition was calculated by using the formula given by Vincent (1927) and data were analyzed statistically by using Completely Randomized Design (CRD).

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

where,

I = Per cent inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

Effect of different bioagents and chemicals on blackgram seedlings under *in vitro* :

Effect of different bioagents and chemicals were assessed based on the seedling vigour index using the standard roll towel method (ISTA, 1993). Fifty blackgram seeds treated with different bioagents and

fungicides were kept over the presoaked germination paper. The seeds were held in position by placing another presoaked germination paper strip and gently pressed. The polythene sheet along with seeds were then rolled and incubated in growth chamber for 15 days. Three replications were maintained for each treatment. Untreated seeds served as control. Root length and shoot length (cm) of individual seedlings were measured and the germination percentage of seeds was recorded. The vigour index was calculated by using the formula as described by Abdul-Baki and Anderson (1973). Vigour index (VI) = Seedling length (Mean root length + mean shoot length) x germination per cent. Five seedlings were taken randomly from each treatment and their fresh weight was recorded. Later, the seedlings were kept in the hot air oven for 4 days at 60°C for complete desiccation and dry weight of the seedlings were recorded.

RESULTS AND DISCUSSION

Bacterial antagonists, *P. fluorescens* (+DAPG)-RP46, *P. fluorescens* and *Bacillus* sp. were screened against *R. bataticola* under *in vitro*. Among them *P. fluorescens* (+DAPG) recorded maximum (68.95%) inhibition of mycelial growth of *R. bataticola* followed by *P. fluorescens* (55.55%) and the least was in *Bacillus subtilis* (51.85 %) (Table 1). Anand *et al.* (2009) and Vinod Kumar *et al.* (2007) observed and identified a superior performance of *Pseudomonas fluorescens* isolate against a broad spectrum of pathogens. The mechanism involved in the inhibition of growth might be due to the agglutination potential of *P. fluorescens* and colonization of sclerotia of *Macrophomina phaseolina* as observed by Jana *et al.* (2000), Manjunatha *et al.* (2012); Vanithal and Ramjegathesh (2014).

Among the fungal bio-agents tested, *T. harzianum*

Sr. No.	Bio-agents	Per cent of inhibition *
1.	<i>Trichoderma harzianum</i> (Th-B)	59.56 (50.51)**
2.	<i>Trichoderma harzianum</i> (Th-R)	61.85 (51.85)
3.	<i>Trichoderma viride</i> (Tv-B)	59.10 (50.24)
4.	<i>Trichoderma viride</i> (Tv-B)	61.11 (51.42)
5.	<i>Pseudomonas fluorescens</i> (+ve DAPG)- RP46	68.95 (56.14)
6.	<i>Pseudomonas fluorescens</i>	55.55 (48.19)
7.	<i>Bacillus subtilis</i> (Bs-1)	51.85 (46.06)
8.	Control	0.00 (0.00)
	S.E.±	2.44
	C.D. (P=0.01)	7.06

*Mean average of three replication

**Figures in parentheses are arcsine values

Sr. No.	Fungicides	% mycelia inhibition at different concentration*			
		0.10%	0.20%	0.30%	Mean
1.	Captan	100 (90.00)**	100 (90.00)	100 (90.00)	100 (90.00)
2.	Copper hydroxide	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
3.	Propineb	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
4.	Sulphur	0.00 (0.00)	0.00 (0.00)	11.66 (19.96)	3.88 (11.36)
5.	Zineb	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
6.	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	Mean	50.00 (45.00)	(50.00) (45.00)	51.94 (46.11)	50.64 (45.36)

	S.E.±	C.D. (P=0.01)
Fungicides (F)	0.12	0.46
Concentration (C)	0.08	0.32
F x C	0.20	0.79

*Mean average of four replication

**Figures in parentheses are arc sine values

Table 3 : *In vitro* evaluation of systemic fungicides on the mycelial growth of *Rhizoctonia bataticola*

Sr. No.	Fungicides	% mycelia inhibition at different concentration*			Mean
		0.05%	0.10%	0.20%	
1.	Benomyl	100 (90.00)**	100 (90.00)	100 (90.00)	100 (90.00)
2.	Carbendazim	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
3.	Difenconazole	98.79 (83.68)	99.07 (84.47)	99.49 (85.90)	99.11 (84.58)
4.	Hexaconazole	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
5.	Thiophanate methyl	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
6.	Tridemefon	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
7.	Carboxin	15.60 (23.26)	52.58 (46.48)	60.25 (50.91)	42.81 (40.86)
8.	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	Mean	76.79 (61.19)	81.45 (64.48)	82.46 (65.24)	80.24 (63.60)
		S.E.±		C.D. (P=0.01)	
Fungicides (F)		0.04		0.15	
Concentration (C)		0.02		0.09	
F x C		0.07		0.26	

*Mean average of three replication **Figures in parentheses are arcsine values

Table 4 : *In vitro* evaluation of combi fungicides on the mycelial growth of *Rhizoctonia bataticola*

Sr. No.	Fungicides	% mycelia inhibition at different concentration*			Mean
		0.05%	0.10%	0.20%	
1.	Hexaconazole 4% + zineb 68%	50.00 (45.00)**	99.53 (86.06)	100.00 (90.00)	83.17 (65.77)
2.	Carbendazim 12% + mancozeb 63%	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
3.	Cymoxanil 8%+ mancozeb 64%	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
4.	Captan70% + hexaconazole 5 %	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
5.	Tricyclozole 18% + mancozeb 62%	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
6.	Mancozeb (64 %) + metalaxyl (4 %)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
7.	Control	0.00 (0.00)	0.00 (0.00)	(0.00) (0.00)	0.00 (0.00)
	Mean	78.57 (62.42)	85.64 (67.73)	85.71 (67.78)	83.31 (65.88)
		S.E.±		C.D. (P=0.01)	
Fungicides (F)		0.01		0.04	
Concentration (C)		0.0072		0.02	
F X C		0.01		0.07	

* Mean average of three replication

**Figures in parentheses are arcsine value

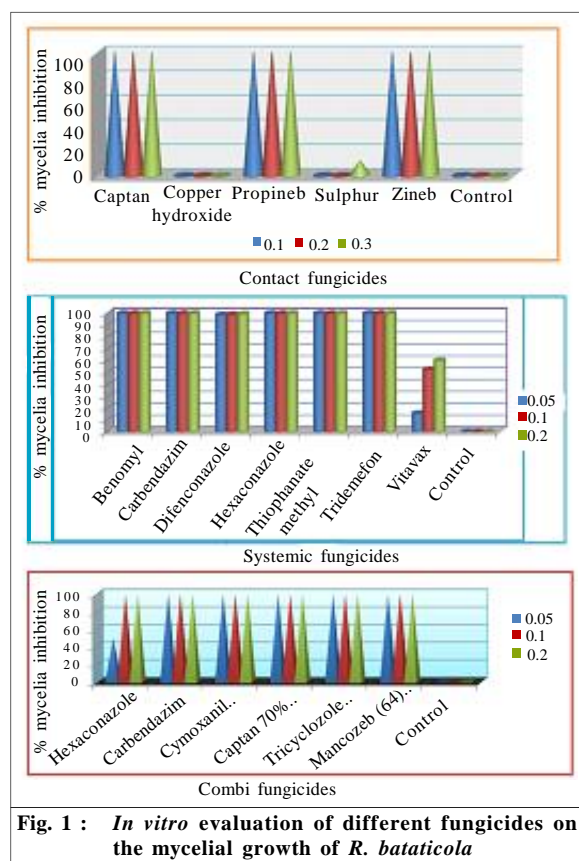
Table 5 : Effect of different bioagents and chemicals on blackgram seedlings under *in vitro*

Treatments	Germination (%)	Seedling length (cm)			Dry weight (g)	Seedling vigour index (SVI)
		Shoot	Root	Total		
<i>Trichoderma harzianum</i> (Th-B)	84.00 (66.42)*	9.17	13.00	22.17	0.18	1864.33
<i>Trichoderma harzianum</i> (Th-R)	86.00 (68.03)	10.17	13.58	23.75	0.19	2042.00
<i>Trichoderma viride</i> (Tv-B)	84.00 (66.42)	10.00	13.67	23.67	0.20	1984.67
<i>Trichoderma viride</i> (Tv-B)	84.67 (66.95)	10.67	12.90	23.57	0.21	1997.80
<i>Pseudomonas fluorescens</i> (⁺ ve DAPG)- RP46	91.33 (72.88)	13.33	15.67	29.00	0.30	2652.83
Carbendazim	80.00 (63.43)	9.00	10.33	19.33	0.15	1540.00
Thiophanate methyl	81.33 (64.40)	9.33	10.67	20.00	0.16	1622.00
Control	74.00 (59.34)	8.33	8.67	17.00	0.12	1258.00
S.E.±	1.07	0.65	0.64	1.13	0.02	94.58
C.D. (P=0.01)	3.10	1.88	1.86	3.27	0.05	274.00

*Figures in the parenthesis are arcsine transformed values

(TH-R) was found more effective as compared to other biocontrol agents, maximum mycelial inhibition of (61.85%) *R. bataticola* followed by *T. viride* (TV-R) (61.11%) were recorded (Table 1). These findings are similar to that of other researchers (Shekhar and Kumar, 2010; Nagamma *et al.*, 2012; Kumari *et al.*, 2012 and Manjunatha *et al.*, 2013).

In vitro evaluation of fungicides provides useful preliminary information regarding its efficacy against a pathogen with in a shortest period of time and therefore serves as guide for further field testing. In the present study among contact fungicides, captan, propineb and zineb recorded maximum inhibition of (100%) mycelial growth at all the concentrations (0.10%, 0.20% and 0.30%) (Table 2 and Fig. 1). Among the systemic and combi fungicides, benomyl, carbendazim, hexaconazole, thiophanate methyl, tridemefon and carbendazim 12% + mancozeb 63%, cymoxanil 8% + mancozeb 64%, captan 70% + hexaconazole 5 %, tricyclozole 18% + mancozeb 62% and mancozeb 64 % + metalaxyl 4 % showed 100 per cent inhibition at all the concentration 0.05, 0.10 and 0.20% tested (Table 3 and 4 and Fig. 1).



Complete inhibition of carbendazim, thiophanate methyl, carbendazim+ mancozeb and tricyclozole + mancozeb was observed against *R. bataticola*. These findings are supported by other researchers (Konde *et al.*, 2008; Prabhu *et al.*, 2012 and Nagamma *et al.*, 2012).

Effect of different bioagents and chemicals on blackgram seedlings :

In roll towel method, different bioagents showed more than 80 per cent seed germination and produced higher shoot and root length as well as dry weight of black gram seedlings with enhanced vigour index after 15 days of incubation. The maximum vigour index of 2652.83 was recorded in *Pseudomonas fluorescens* (+ DAPG)- RP-46 treated seeds followed by 2042.00 and 1997.80 vigour index in *Trichoderma harzianum*-II(R) and *T. viride*-II(R) treated seeds, respectively. Least germination (74%) with less shoot and root length as well as less dry weight of seedlings and poor vigour index of 1258.00 was observed in untreated control. The observations made in the present study corroborate with the results by other researchers (Ramamoorthy *et al.*, 2002 and Venuturla Bharathi *et al.*, 2014). Maximum inhibition of mycelial growth of *Fusarium solani* with enhanced germination and seedling vigour was recorded in tomato and hot pepper, respectively by seed treatment with *P. fluorescens* and *B. subtilis*.

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