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Effect of bio-fungicides on quality parameters of brinjal seeds infected with *Phomopsis vexans*

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Abstract

Brinjal is an important vegetable crop in India and its production is mainly constrained up to 30 per cent due to the seedling and fruit rot caused by fungus, *Phomopsis vexans*. Although carbendazim is effectively used to control the *P. vexans*, it is detrimental to human and environment with long term usage. Hence, the present study was conducted to identify the bio-fungicide which can be used as an alternative to carbendazim. The results revealed that the seed germination was significantly superior with *Trichoderma viride*, *T. viride* + *Pseudomonas fluorescens* and *T. viride* + *T. asperellum* as compared to the carbendazim in blotter method while it was at par in pot culture experiment. The *T. viride* resulted in significantly higher seedling length (cm), seedling dry weight (mg), SVI-I and SVI-II as compared to the carbendazim in pot culture experiments. The disease incidence was significantly low and the disease control was significantly high in *T. viride* and its combinations with *P. fluorescens* or *T. asperellum* as compared to carbendazim in blotter method while, at par under pot culture conditions. Therefore, *T. viride* (5 g kg^{-1} seed) can be effectively used as an alternative to carbendazim to improve the seedling vigour and control of *P. vexans* of brinjal.

Key words: Brinjal, bio-fungicides, carbendazim, *Phomopsis vexans*, seed quality, *Trichoderma viride*

Introduction

Brinjal (*Solanum melongena* L.) is a common vegetable crop grown in India but its production is constrained by seedling and fruit rot caused by *Phomopsis vexans*. This leads in decreased marketable value and yield losses up to 50 per cent (Das, 1998; Beura *et al*., 2008). *P. vexans* remain as mycelia in seed, as spores on seed coat and as both in soil (Chaudhary and Hasija, 1979) and can be controlled by the seed treatment with chemical fungicide namely carbendazim (Beura *et al*., 2008, Singh *et al*., 2012; Phansawan *et al*., 2015; Rohini *et al*., 2016b; Sanjeev *et al*., 2017). However, use of synthetic chemicals for a longer period results in the build-up of toxic chemicals potentially hazardous to mankind and environment in addition to pathogen resistance (Rajavel, 2000; Avinash and Hosmani, 2012). Alternatively, the bio-fungicides are natural, low cost, safer, eco-friendly and improve the seed quality parameters like, seed germination, vigour and fruit yields. In this regard, use of bio-fungicide like *Trichoderma viridae* (Das *et al*., 2014) and beneficial bacteria (Rohini *et al*., 2016a) has shown to inhibit the growth of *P. vexans*.

T. viride, T. asperellum, T. asperellum and *Pseudomonas fluorescens* have been reported effective as antifunal bioagents (Islam *et al*., 2011; Singh *et al*., 2012). Few studies have compared the effect of chemical fungicides with the bio-agents. Therefore, study of bio-fungicides in comparison with chemical fungicides could be effective to suggest the eco-friendly control measure in the changing climate scenario. Hence, the present study was conducted with an objective of evaluating different bio-fungicides on quality parameters of brinjal seeds infected with *P. vexans* and to identify the bio-fungicide which can be used as an alternative to the carbendazim in brinjal cultivation.

Materials and methods

Two experiments (laboratory and pot culture) were conducted to study the effect of bio-fungicides on seed quality parameters of *P. vexans* infected seeds of brinjal. These experiments were conducted at the Department of Seed Science and Technology, CCS HAU, Hisar during October-November, 2016. The seeds used for these experiments were harvested during February-March, 2016 (high yielding popular variety, H-8). The seed germination (79 %) was above Indian Minimum Seed Certification Standards.

In laboratory (blotter method), the infected brinjal seeds were treated with *T. viride, T. asperellum* and *P. fluorescens* powder formulations @ 5 g kg^{-1} seed and their combinations. *T. asperellum* was obtained from Bio-control Laboratory, Government of Haryana and the rest from local market. Twenty five treated seeds were placed equidistantly in petri plates lined with two layers of blotter paper (Whatman No.1) and 16 petri plates per replication was maintained for planting of 400 seeds. These petri plates were placed in BOD incubator maintaining temperature at 25±1°C and three replications were maintained for each of the treatment. Distilled water was added whenever the blotter paper appeared nearly to dry.

The control treatments were untreated infected seed, healthy seed and carbendazim treated seed (2 g kg^{-1} seed). For pot culture experiment, pots (27.5 cm diameter and 30 cm height) were filled with 4 kg of the oven sterilized soil. The seeds were treated as detailed in laboratory experiment, and twenty five seeds were placed at a depth of 1-2 cm in each of the pot with 16 pots per replication. The pots were watered daily up to 14 days. The weeds were uprooted whenever seen.

Final count was made on 14th day and only normal seedlings were

considered for germination testing. Ten normal seedlings per replication were selected at random at the time of final count for observations on shoot length, root length and total seedling length. After taking the shoot and root length, the same seedlings were kept for drying in oven at 70±1 °C until they attained a constant dry weight. The Seed vigour index-I (SVI-I) and Seed vigour index-II (SVI-II) were calculated as suggested by Anderson and Baki (1973). The following formulae were used.

Seed Vigour Index I = Seed germination percentage × Seedling length (cm)

Seed vigour Index II = Seed germination percentage × Dry seedling weight (mg)

Disease incidence (%) = (Number of seedlings affected/ Total number of seedlings observed) × 100

Disease control (%) = [(Treatment – Control)/ Treatment] × 100

Seed germination (%) = (Number of seeds germinated/ Total number of seeds placed for germination)× 100

Seedling length (cm) = Seedling shoot length + Seedling root length

The data obtained was statistically analysed in Completely Randomised Design (CRD) in both the experiments.

Results and discussion

Mean seed germination in blotter method was high (90.81 %) as compared to the pot culture (88.52 %). In blotter method, the germination percentage was significantly high in seeds treated with bio-fungicides *viz.*, *T. viride* (96.3 %), *T. viride* +*T. asperellum* (96.0 %) and *T. viride* +*P. fluorescens* (96.0 %) as compared to the carbendazim treatment (95.0 %). However, in pot culture, these bio-fungicides (*T. viride*, *T. viride* + *T. asperellum* and *T. viride* + *P. fluorescens*) were at par with carbendazim treatment. However, these treatments showed higher germination (> 10 %) as compared to the healthy seed (Table 1). Similar to our results, several reports show that treating seeds with these bio-fungicides preferably *T. viride* suppresses the radius of the pathogen colony like *P. vexans* and thus enhanced the seed germination (87.7 %) as compared to the carbendazim (86.2 %) (Raj *et al*., 2008; Ekefan *et al*., 2009; Islam *et al*., 2011; Rehman *et al*., 2012). Hence, these bio-fungicides can be used in place of carbendazim for achieving higher seed germination in brinjal.

The seedling length in blotter method was significantly high in all the bio-fungicides except the *P. fluorescens* as compared to the carbendazim treatment (Table 1). In pot culture, the *T. viride* produced significantly longer seedlings (7.95 cm) over the carbendazim (6.87 cm). Similar results of increased seedling length due to application of *T. viride*, *T. asperellum* and *P. fluorescens* individually or in combinations were reported in different species including brinjal (Ekefan *et al*., 2009; Rehman *et al*., 2012; Rohini *et al*., 2016a).

The seedling dry weight in blotter method with *T. viride* + *P. fluorescens* was at par to the carbendazim treatment while, *T. viride* and *T. viride* + *P. fluorescens* were significantly superior to healthy seed (18.5 mg/ seedling). In practical terms, the performance of seedling under field conditions is more important than under laboratory. In this context, in the present study, in pot culture, all the bio-fungicides (Table 1) except the combination of *P. fluorescens* + *T. asperellum* resulted in significantly higher seedling dry weight compared to the carbendazim (28.0 mg/ seedling).

The SVI-I in blotter method was significantly higher in all bio-fungicide treatments due to higher seedling length except for the *P. fluorescens*. While the SVI-II was significantly higher in carbendazim compared to all the bio-fungicide treatments due to lower seedling dry weights. However, the treatments *T. viride* and *T. viride* + *P. fluorescens* were at par to the carbendazim treatment (Table 2). In pot culture, the SVI-I was significantly higher in *T. viride* (758.0) over the carbendazim (650.4). The SVI-II was significantly higher in the *T. viride* (3209.3), *P. fluorescens* (3014.5) and *T. viride* + *T. asperellum* (3141.4) as compared to the carbendazim (2650.7). The mean SVI over the two experiments was markedly high in *T. viride* as compared to the carbendazim treatment (Table 2). Such higher SVI in bio-fungicides, specifically in *T. viride* could be due to production of antibiotic compounds (Trichodermin), extracellular enzymes (chitinase, cellulose), unsaturated monobasic acids (Dermadine) and peptides which damage plant pathogens and therefore increase seedling weight and seedling vigour (Rehman *et al*., 2012).

The disease incidence in untreated infected control was between 27.67 and to 29.33 per cent in blotter and pot culture respectively (Table 3). Similar percentage of disease incidence was reported by Jayaramaiah *et al*. (2013). The disease incidence in blotter

Table 1. Effect of bio-fungicides on seed germination, seedling length and dry weight in *P. vexans* infected brinjal seeds

Treatments	Germination (%)		Seedling length (cm)		Seedling dry weight (mg/seedling)	
	Blotter method	Pot culture	Blotter method	Pot culture	Blotter method	Pot culture
T. viridae	96.33 (78.95)	95.33 (77.51)	6.54	7.95	20.17	33.67
T. asperellum	90.67 (72.19)	87.67 (69.42)	5.27	6.99	17.77	30.83
P. fluorescens	92.00 (73.54)	92.00 (73.54)	4.79	6.52	19.57	32.77
T. asperellum + T viride	96.00 (78.43)	93.33 (75.02)	5.26	5.04	19.77	33.67
P. fluorescens+T. viride	96.00 (78.43)	94.67 (76.80)	5.78	6.43	21.33	29.97
P. fluorescens + T. asperellum	93.33 (75.02)	84.00 (66.40)	5.13	5.92	18.57	27.47
Untreated infected seed	72.33 (58.24)	70.67 (57.19)	3.42	3.96	12.80	24.00
Healthy seed	85.67 (67.73)	84.33 (66.66)	4.02	6.36	18.50	26.30
Carbendazim (2 g kg^{-1} seed)	95.00 (77.05)	94.67 (76.63)	4.42	6.87	21.90	28.00
Mean	90.81 (73.29)	88.52 (71.02)	4.96	6.23	18.93	29.63
CD at 5 %	0.80	1.84	0.44	0.34	1.41	1.38

Note: Values in parenthesis are arc sign transformed values for statistical analyses

method was significantly low in *T. viride* and their combination treatments with *P. fluorescens* and *T. asperellum* as compared to the carbendazim. However, in pot culture experiment, these treatments were at par to the carbendazim treatment (Table 3). In this context, Chakravarthy and Kalita (2012) reported that wilt disease was least in brinjal seeds treated with *P. fluorescens* and a negative correlation between per cent wilt incidence and population density of *P. fluorescens* was observed indicating the role of *P. fluorescens* in controlling the wilt of brinjal.

The disease control, in blotter method was significantly higher in *T. viride* and its combinations with *P. fluorescens* and *T. asperellum* as compared to the carbendazim while, in pot culture, these treatments were at par to that of carbendazim (Table 3). Similar to the present study, treatment with of *T. viride* and *P. fluorescens* effectively controlled the pre-emergence and post-emergence damping off up to 74 % in solanaceous crops and increased the shoot length, root length and dry matter production (Manoranjitham *et al*., 2000; Jayaraj *et al*., 2006). Such control was attributed to inhibition of mycelial growth to the extent of 69.4 per cent, with *T. viride* (Gholve *et al.,* 2014).

Jayaramaiah *et al*. (2013) reported that disease incidence in brinjal due to *P. vexans* may go up to 30 per cent. *T.* spp. is the most commonly used fungal bio-control agents against *P. vexans* (Kaewchai *et al*., 2009; Adan *et al*., 2015). However, the seed treatment with *T. viride* is cost effective and efficient by the way of mycoparasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites like defensin production protect the seeds from wide range of pests and diseases and additional plant extracts might decrease the pathogen infections effectively (Mancini and Romanazzi, 2014**).** With respect to the quantity of *T. viride*, the dry formulation of 3 to 10 g kg^{-1} of seeds was effective to control seed and soil borne pathogens (Ramanujam *et al.,* 2010) and also *P. vexans* (Islam *et al.,* 2011). The bio-fungicides have beneficial effects on seedling length and seedling vigour although chemical fungicide, the bavistin, controled the mycoflora to a higher extent in case of cowpea (Mogle and Maske, 2012). Subbiah and Kumaravel (2015) reported that the use of *T. viride* at 4g/kg of seeds and neem oil (5 %) spray controlled the pest and diseases of brinjal effectively.

Our study with seed treatment was found effective in controlling the disease caused by *P. vexans* in addition to improve early seedling growth which is responsible for higher seedling vigour. Therefore, *T. viride* or combination with *P. fluorescens* and *T. asperellum* @ 5 g/kg seed can be effectively used in place of carbendazim to control *P. vexans* in brinjal.

Table 2. Effect of bio-fungicides on seedling vigour index in *P. vexans* infected brinjal seeds

Treatments	SVI-I		SVI-II		Mean
	Blotter method	Pot culture	Blotter method	Pot culture	
T. viridae	630.3	758.0	1942.4	3209.3	1635
T. asperellum	477.5	612.9	1610.9	2703.0	1351
P. fluorescens	440.7	600.0	1800.1	3014.5	1464
T. asperellum + T viride	505.0	470.2	1897.6	3141.4	1504
P. fluorescens + T. viride	555.2	608.3	2054.4	2840.0	1514
P. fluorescens + T. asperellum	478.7	497.0	1732.6	2307.2	1254
Untreated infected seed	247.6	280.0	925.9	1696.0	787
Healthy seed	344.2	536.3	1584.7	2217.8	1171
Carbendazim (2g kg^{-1}seed)	419.9	650.4	2080.5	2650.7	1450
Mean	455.5	557.0	1736.6	2642.2	
CD at 5 %	42.0	27.9	222.7	283.9	

Table 3. Effect of bio-fungicides on disease incidence and disease control in *P. vexans* infected brinjal seeds

Treatments	Disease incidence (%)		Disease control (%)	
	Blotter method	Pot culture	Blotter method	Pot culture
T. viridae	3.67 (11.01)	4.67 (12.46)	86.73 (68.66)	84.06 (66.49)
T. asperellum	9.33 (17.78)	12.33 (20.55)	66.23 (54.46)	57.93 (49.55)
P. fluorescens	8.00 (16.42)	8.00 (16.42)	71.08 (57.44)	72.72 (58.48)
T. asperellum + T viride	4.00 (11.53)	6.67 (14.95)	85.54 (67.62)	77.24 (61.50)
P. fluorescens + T. viride	4.00 (11.53)	5.33 (13.16)	85.54 (67.62)	81.76 (65.02)
P. fluorescens + T. asperellum	6.67 (14.95)	16.00 (23.57)	75.88 (60.58)	45.44 (42.37)
Untreated infected seed	27.67 (31.72)	29.33 (32.78)	0.00 (0.00)	0.00 (0.00)
Healthy seed	14.33 (22.24)	15.67 (23.31)	48.19 (43.95)	46.59 (43.03)
Carbendazim (2g kg^{-1}seed)	5.00(12.92)	5.33 (13.34)	81.92 (64.81)	81.84 (64.76)
Mean	9.19 (16.68)	11.48 (18.95)	66.79 (53.90)	60.84 (50.13)
CD at 5 %	0.80	1.84	1.87	3.86

Note: Values in parenthesis are arc sign transformed values for statistical analyses

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