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EFFECT OF BIO-FUNGICIDES ON SEED QUALITY PARAMETERS AND DISEASE CONTROL IN CHILLI SEEDS INFECTED WITH COLLETOTRICHUM CAPSICI

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ABSTRACT
Fruit rot caused by Colletotrichum capsici is the major constraint for production and marketability of chilli. Continuous use of chemical fungicides like carbendazim for effective control leads to negative impact on environment, soil and human health. Therefore, we evaluated the efficacy of bio-fungicides in comparison with carbendazim in chilli seeds infected with Colletotrichum capsici for seed germination, seedling vigour and disease incidence.Seed germination was high in Trichoderma viridae and carbendazim treatments in blotter and pot experiments respectively. Seedling length was significantly high in Trichoderma viridae treatment while, the seedling dry weight and seedling vigour index were significantly high in carbendazim treatment both in blotter method and pot culture. However, the seedling vigour with Trichoderma viridae was on par to the carbendazim, with equal weightage to seed germination, seedling length and dry weight in SVI calculations. The disease incidence was significantly low in Pseudomonas fluorescens treatment as compared to the carbendazim in blotter method. While in pot culture, T. viridae + P. fluorescens treatment was effective in controlling the fungal infection. Therefore, Trichoderma viridae or in combination with Pseudomonas fluorescens can be effectively used for seed treatment to control Colletotrichum capsici as an alternative to carbendazim.

INTRODUCTION

Chilli is a major spice crop in India and India stands 3rd in production (Saxena *et al*., 2016). The crop is suffered mainly by seedling rot and fruit rot caused by C. capsici leading to reduced marketability and yield (Pandey and Pandey, 2003; Pakdeevaraporn *et al*., 2005). Colletotrichum capsici alone contributes to more than 70 % infection as compared to other fungal organisms (Santoshreddy *et al*., 2014). To control this fungus, carbendazim a systemic fungicide is commonly used chemical at the recommended dose of 0.2 % (Chauhan *et al*., 2014; Phansawan *et al*., 2015) although the Propiconazole (0.1 %), Tebuconazole (0.1 %) found more effective (Begum *et al*., 2015; Arunakumara and Sathyanarayana, 2016). However, continuous use of chemical fungicides has negative impact on biodiversity, environment and human health (Avinash and Hosmani, 2012). In this regard, positive effect of bio-fungicides like Trichoderma viridae, Pseudomonas fluorescens *etc*. in reducing the infection of Colletotrichum capsici and thus improved seedling vigour and yield with decreased fruit rot have been reported (Jeyalakshmi *et al*., 1998; Sharma *et al*., 2005; Srinivas *et al*., 2005; Intana *et al*., 2007; Tiwari *et al*., 2008; Theerthagiri and Ramanujam, 2009). Although most studies have emphasized the bio-fungicides, little information is available about comparison of bio-fungicides with that of carbendazim on disease incidence. Therefore, the objective of the present study was to compare the effect of bio-fungicides viz., Trichoderma viridae, Pseudomonas fluorescens, Trichoderma asperellum and their combinations in comparison to carbendazim on seed germination, seedling vigour and disease control to identify a bio-fungicide comparable to the carbendazim.

MATERIALS AND METHODS

The study (laboratory and pot culture) was conducted at the Department of Seed Science and Technology, CCSHAU, Hisar during October-November, 2016 to study the effect of bio-fungicides on seed quality parameters of Colletotrichum capsici infected seeds of chilli. The seeds (popular high yielding variety, RCH-1) used for these experiments were collected from the crop harvested during February – March, 2016.

In blotter method, the Colletotrichum capsici infected chilli seeds were treated with Trichoderma viride, Trichoderma asperellum and Pseudomonas fluorescens powder formulations @ 5g kg^{-1} seed and their combinations. Trichoderma asperellum was obtained from Bio-control laboratory, Government of Haryana and the Trichoderma viridae and Pseudomonas fluorescens from the local market. The dressed seeds for 10 minutes (25 seeds) were placed equidistantly in petri plates lined with two layers of blotter paper (Whatman No.1) wetted with distilled water. Each replication had four petri plates and these petri plates in triplicate were placed in BOD incubator maintaining temperature at 25 + 1°C for 14 days. Distilled water was added whenever the blotter paper appeared nearly to dry. The control treatments were untreated infected seed, healthy seed (not infected) and infected seed with carbendazim treatment (2g kg^{-1} seed). After 14 days (both in blotter and pot culture) of incubation, seed germination, disease incidence (the number of seeds having mycelia growth) and disease control (number of seeds without mycelia growth) were recorded. Further, ten randomly selected seedlings from each replication were randomly chosen for measuring the root and shoot length and then these seedlings were oven dried at 70±1ºC until they attained a constant dry weight.

For pot culture experiment, pots (27.5 cm diameter and 30 cm height) were filled with four kg of the oven sterilized soil. The seeds were treated as detailed in the laboratory experiment, and twenty five seeds were placed at a depth of 1-2 cm in each of the pot with 4 pots per replication. The pots were adequately watered daily up to 14 days and the weeds were uprooted as and when weeds appear. After 14 days, the number of normal seedlings without root rot and; abnormal seedlings with root rot was recorded for computing disease control and disease incidence respectively. After 14 days, ten randomly selected seedlings from each replication were used to record the 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±1ºC until they attained a constant dry weight.

In each treatment, the seedling vigour index-I and seedling vigour index-II were calculated using the seed germination percentage, seedling length and seedling dry weights from the germinated seedlings as suggested by Anderson and Baki (1973). The following formulae were used.

$$\text{Seed germination}(\%) = \frac{\text{Number of seed germinated}}{\text{Total number of seed placed for germination}}$$

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

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

Seedling Vigour Index - II = Seed germination percentage × Dry seedling weight (mg)

$$\text{Disease incidence}(\%) = \frac{\text{Number of seedlings infected}}{\text{Total number of seedlings observed}} \text{X100}$$

$$\text{Disease control}(\%) = \frac{\text{Treatment(No.of disease free seeds)} - \text{Control (no.of disease free seeds)}}{\text{Treatment(No.of disease free seeds)}} \text{X100}$$

Wherein the treatment means, it is the number of seeds germinated without mycelia in blotter method and, it was normal seedlings without root rot in pot culture.

The data obtained was statistically analyzed in Completely Randomized Design (CRD) in both the experiments.

RESULTS AND DISCUSSION

Seed germination

The seed germination was significantly superior in blotter method (87.6 %) as compared to the pot experiment (84.5 %) although the differences are marginal (3.5 %). In blotter method among the treatments, only T. viridae (94.7 %) showed significantly higher seed germination as compared to the carbendazim (92.0 %) treatment. The Pseudomonas fluorescens treatment (92.7 %) found on par to the carbendazim treatment (Table 1). The germination percentage was markedly high in both bio-fungicide treatments and carbendazim treatment compared to the infected seed and healthy seed. In pot culture experiment, carbendazim was superior to all the bio-fungicide treatments (Table 1). The higher seed germination with bio-fungicides and carbendazim could be through inhibition of mycelia growth of C. capsici (Raj *et al*., 2008; Yadav, 2008). In overall, the seed germination was above the minimum standards of seed certification in all the treatments except the infected seeds both in blotter and pot culture experiments. Hence, for the purpose of better seed germination, Trichoderma viridae or Pseudomonas fluorescens can be effectively used as an alternative to the carbendazim.

Seed quality parameters

Seedling length (Table 1) was significantly high in Trichoderma viridae treatment (7.04 cm in blotter and 7.75 cm in pot culture) as compared to the carbendazim treatment (5.26 cm in blotter and 6.68 cm in pot culture). In pot culture, bio-fungicide treatments showed significantly higher seedling length as compared to the healthy seed. Similar results of increased seedling length due to application of Trichoderma viride,

Table 1: Effect of bio-fungicides on seed germination, seedling length and seedling dry weight in Colletotrichum capsici infected chilli seeds

Treatments	Germination (%)		Seedling length (cm)		Seedling dry weight (mg/ seedling)	
	Blotter	Pot	Blotter	Pot	Blotter	Pot
Trichoderma viride	94.7 (76.6)	89.0 (70.6)	7.04	7.45	32.21	30
Trichoderma asperellum	86.7 (68.6)	84.3 (66.7)	4.96	6.76	29.23	32.23
Pseudomonas fluorescens	92.7 (74.3)	83.3 (65.9)	4.89	7.01	28.5	30.16
Trichoderma asperellum + Trichoderma viride	88.0 (69.7)	85.3 (67.5)	5.66	6.6	27.83	29.86
Pseudomonas fluorescens + Trichoderma viride	89.3 (70.9)	89.3 (71.0)	4.9	6.64	33.56	30.2
Pseudomonas fluorescens + Trichoderma asperellum	89.3 (70.9)	85.0 (67.2)	4.87	7.09	32.67	35.76
Untreated infected seed	70.3 (57.0)	69.3 (56.4)	4.69	4.58	25.67	25.66
Healthy seed (not infected)	85.3 (67.5)	83.7 (66.1)	5.94	5.22	28	34.33
Carbendazim treated	92.0 (73.5)	91.0 (72.5)	5.26	6.68	36.67	39.33
Mean	87.6 (69.9)	84.5 (67.1)	5.36	6.45	30.48	31.95
C.D at 5%	0.8	1.8	0.38	0.43	1.36	2.4
SEm +	0.3	0.6	0.13	0.14	0.45	0.8
C.V. (%)	0.7	1.6	4.19	3.87	2.58	4.35

Note: Values in parenthesis are arc sign transformed values

Table 2: Effect of bio-fungicides on seedling vigour index in Colletotrichum capsici infected chilli seeds

Treatments	SVI-I		SVI-II		Overall SVI
	Blotter	Pot	Blotter	Pot	Pooled
Trichoderma viride	498.6	601.6	3048.8	2670	0.81
Trichoderma asperellum	430.1	628.9	2533.6	2718.4	0.6
Pseudomonas fluorescens	453.8	584.2	2641.2	2513.8	0.59
Trichoderma asperellum + Trichoderma viride	498.1	563.5	2449.3	2544.4	0.6
Pseudomonas fluorescens + Trichoderma viride	438.4	593.4	2997.9	2698.9	0.64
Pseudomonas fluorescens + Trichoderma asperellum	435.7	603	2918.3	3040	0.69
Untreated infected seed	329.8	317.5	1805.3	1779.3	0.33
Healthy seed (not infected)	506.9	436.7	2389	2873	0.57
Carbendazim treated	647.7	608.5	3373.3	3579.3	0.81
Mean	471	548.6	2684.1	2713	
C.D at 5%	32.83	40.05	127.27	198.24	
SEm +	10.96	13.37	42.5	66.21	
C.V. (%)	4.03	4.22	2.74	4.22	

Table 3: Effect of bio-fungicides on disease incidence and disease control in Colletotrichum capsici infected chilli seeds

Treatments	Disease incidence (%)		Disease control (%)	
	Blotter	Pot	Blotter	Pot
Trichoderma viride	7.33 (15.70)	11.00 (19.36)	74.71 (59.80)	63.33 (52.71)
Trichoderma asperellum	13.33 (21.41)	15.67 (23.31)	54.02 (47.29)	47.78 (43.71)
Pseudomonas fluorescens	5.33 (13.34)	16.67 (24.08)	81.61 (64.60)	44.44 (41.79)
Trichoderma asperellum + Trichoderma viride	12.00 (20.26)	14.67 (22.47)	58.62 (49.94)	51.11 (45.63)
Pseudomonas fluorescens + Trichoderma viride	10.67 (19.05)	10.67 (18.98)	63.22 (52.65)	64.44 (53.46)
Pseudomonas fluorescens + Trichoderma asperellum	10.67 (19.05)	15.00 (22.77)	63.22 (52.65)	50.00 (44.98)
Untreated infected seed	29.67 (32.99)	30.67 (33.61)	0.00 (0.00)	0.00 (0.00)
Healthy seed (not infected)	14.67 (22.51)	16.33 (23.83)	50.57 (45.31)	46.74 (43.11)
Carbendazim treated	8.00 (16.42)	9.00 (17.45)	73.03 (58.69)	70.65 (57.17)
Mean	12.41 (15.52)	20.08 (22.87)	57.67 (47.88)	48.72 (42.51)
C.D at 5%	0.83	1.82	1.67	4.03
SEm +	0.28	0.61	0.56	1.34
C.V. (%)	2.4	4.59	2.02	5.48

Note: Values in parenthesis are arc sign transformed values

Trichoderma asperellum and Pseudomonas fluorescens individually or in combination was reported in different species (Ekefan *et al*., 2009; Rehman *et al*., 2012; Rohini *et al*., 2016).

Seedling dry weight was significantly high in carbendazim treated seeds (36.67 mg in blotter method and 39.33mg in pot culture) as compared to all bio-fungicide treatments (Table 1). However, in pot culture, bio-fungicide treatments performed better compared to the healthy seed. Probably in pot culture (similar to field conditions) conditions, bio-fungicides may take longer time for perpetuation in addition to the requirement of energy sources at early stages, whereas, carbendazim do not depend on seedling for energy source. Further, the quantity of bio-fungicide for seed treatment (5 g Kg^{-1} seed) may be insufficient at early stages it requires 15 days for it multiplication in soil. However, the increased seedling length and seedling dry weight in both bio-fungicides or carbendazim may be attributed to the effective control of pre-emergence and post-emergence damping off through decreased colony formation by C. capsici (Manoranjitham *et al*., 2000; Jayaraj *et al*., 2006).

Seedling vigour

Seedling vigour is an important trait in ensuring the proper crop establishment and economic yields especially under adverse conditions. Seed borne pathogen like C. capsici affect the seedling vigour resulting in fruit rot and reduced yield. Under such conditions, application of chemical fungicide or bio-fungicide would help to combat the effects of C. capsici. Several reports have shown the positive influence of bio-fungicides like Trichoderma *etc*. on seedling vigour in chilli (Raj *et al*., 2008; Asaduzzaman *et al*., 2010; Islam *et al*., 2011; Rehman *et al*., 2012). However, scanty literature is available for comparing the bio-fungicides with carbendazim, a popular systemic fungicide (Phansawan *et al*., 2015). Therefore, it is very pertinent to identify a bio-fungicide comparable to that of carbendazim as carbendazim has deleterious effects on biodiversity, environment and human (Avinash and Hosmani, 2012). In the present study, seedling vigour index was significantly high with carbendazim treatment as compared to all the bio-fungicides in both blotter and pot culture (Table 2). The bio-fungicides performed better over the control (healthy seed) for SVI-I in pot culture and SVI-II in blotter technique (Table 2). These differences in SVI are due to variations in seed germination, seedling length and seedling dry weights. However, when the data were normalized by giving equal weightage to unity for all three parameters, seedling vigour index with Trichoderma viridae found on par to the carbendazim treatment (Table 2). Similarly, Choudhary *et al*. (2013) reported that Trichoderma viride was effective when compared to the carbendazim in terms of seedling vigour. Hence, seed treatment with Trichoderma viridae is suggested for effective control of C. capsici, in addition to enhancement of seedling vigour for better yields of chilli.

Disease infection and disease control

In laboratory experiment, disease incidence (number of seeds with mycelia) was significantly less in Pseudomonas fluorescens (5.33 %) as compared to the carbendazim (8.00 %) and the Trichoderma viridae (7.33 %) was comparable to the carbendazim treatment (Table 3). In pot culture, carbendazim treatment recorded significantly lower disease incidence (9.0 %) and was on par to that of Trichoderma viridae (11.0%) and Trichoderma viridae + Pseudomonas fluorescens (10.67 %). All bio-fungicide treatments resulted in significantly lower disease incidence or on par to the control (healthy seed) (Table 3).

The disease control (seed without mycelia) was significantly higher in Pseudomonas fluorescens (81.61 %) as compared to the carbendazim (73.03 %) and Trichoderma viridae (74.71 %) in blotter technique (Table 3). In pot culture, disease control (normal seedlings without root rot) was significantly superior in carbendazim treatment (70.65 %) as compared to all the bio-fungicides treatments except the Trichoderma viridae + Pseudomonas fluorescens treatment (64.44 %). In this regard, many reports showed that the bio-fungicides, Trichoderma viridae or Pseudomonas fluorescens and their combinations inhibited the mycelia growth of C. capsici (Jeyalakshmi *et al*., 1998; Sharma *et al*., 2005; Srinivas *et al*., 2005; Intana *et al*., 2007; Tiwari *et al*., 2008; Theerthagiri and Ramanujam, 2009). The bio-fungicide, Trichoderma viridae known to produce antibiotic (trichodermin) and extracellular enzymes (chitinase, cellulose) those inhibit the plant pathogen (Rehman *et al*., 2012). However, these studies have not compared the effectiveness of bio-fungicides vis-à-vis carbendazim, which is a popular systemic fungicide. A few studies show that the chemical fungicide, the copper oxychloride is more effective than Trichoderma viridae in controlling the disease caused by C. capsici (Patel *et al*., 2014). It would be more effective in combination of bio-fungicide with carbendazim to reduce the disease incidence, and for higher yield and quality of chilli (Ekbote, 2005; Mesta *et al*., 2009). Further, combined application as seed treatment and soil treatment will be more effective in controlling the C. capsici (Chang *et al*., 2002).

These experimental results suggests that, the Trichoderma viridae and Pseudomonas fluorescens or their combinations are alternatives to carbendazim to control Colletotrichum capsici incidence and for better seed quality parameters in chilli.

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