Original Research Article

Effect of bio-fungicides on seed quality parameters and disease control in chilli seeds infected with *Colletotrichum capsici*

ABSTRACT

Damping off and fruit rot caused by *Colletotrichum capsici* are the major constraints in production and marketability of chilli. Systemic fungicides are commonly used to control this disease. However, continuous use of chemical fungicides leads to negative impact on environment, soil and human health. Therefore, present studies (laboratory and pot experiment) were conducted to explore the bio-fungicides (as an alternative to chemical fungicide) in comparison with carbendazim using chilli seeds infected with *Colletotrichum capsici*.

Experiments were conducted at the CCSHAU, Hisar, India during 2016 in completely randomized design with nine treatments replicated thrice. Results revealed that the seed germination was more than 90 per cent with *Trichoderma viride* treatment and was comparable to that carbendazim treatment both in blotter method and pot culture. The seedling length was significantly superior with *Trichoderma viride* compared to the carbendazim both in blotter and pot culture. However, the seedling dry weight and seedling vigour were significantly high with carbendazim compared to the *Trichoderma viride* treatment or others. However, with equal weightage to all seedling parameters like germination, seedling length and dry weight, the seedling vigour with *Trichoderma viride* was on par to the carbendazim. The disease incidence was significantly least with *Pseudomonas fluorescens* as compared to the *Trichoderma viride* and carbendazim in blotter method. In pot culture, *T. viride* + *P. fluorescens*treatment was on par to that of carbendazim treatment. Therefore, use of *Trichoderma viride* and *Pseudomonas fluorescens* individually or in combinations are suggested in place of carbendazim to control the *Colletotrichum capsici* for better yields of chilli.

Key words: Chilli; *Colletotrichum capsici;* Carbendazim; bio-fungicides.

1. INTRODUCTION

Chilli is a major spice crop in India and India stands 3rd in production (21). The crop is suffered mainly by seedling rot and fruit rot caused by *C. capsici* leading to reduced marketability and yield [14, 15]. To control this disease, a systemic fungicide like carbendazim is commonly used chemical at the recommended dose of 0.2 % [17]. However, continuous use of chemical fungicides has deleterious effects on biodiversity, environment and human health [3].In this direction, several reports show the effect of bio-fungicides like, *Trichoderma viride*, *Pseudomonas fluorescens* etc. on control of *Colletotrichum capsici* and improved the seedling parameters and yield of chilli with decreased fruit rot [8, 11, 22, 23, 24, 25].These studies pertain only to bio-fungicides. Therefore, it is very pertinent to identify a bio-fungicide comparable to that of chemical fungicides in the changing climate scenario. Hence, the present study was undertaken to study the effect of bio-fungicides *viz*., *Trichoderma viride, Pseudomonas fluorescens, Trichoderma asperellum* and their combination in comparison with chemical fungicide (carbendazim) on seedling vigour and disease control.

2. MATERIAL AND METHODS

Two experiments (laboratory and pot culture) were conducted to study the effect of bio-fungicides on seed quality parameters of *Colletotrichum capsici* infected seeds of chilli. These experiments were conducted at the Department of Seed Science and Technology, CCSHAU, Hisar during October-

November, 2016. The seeds used for these experiments were harvested during February – March, 2016 (high yielding popular variety, RCH-1). The seed germination (79 %) was above Indian Minimum Seed Certification Standards.

In blotter method, the 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 rest from local market.The treated seeds (25 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°Cand three replications were maintained for each of the treatment. Distilled water was added whenever the blotter paper appeared nearly to dry. There were three control treatments, namely, untreated infected seed, healthy seed and carbendazim treated infected seed (2g kg^{-1} seed). The other treatments were seed treatment with bio-fungicides to seeds already infected with *Colletotrichum capsici*. After 14 days of incubation, in ten randomly selected seedlings observations were made on seed germination, seedling vigour, disease incidence and disease control.

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 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. Ten seedlings from each replication were taken for observations on 14^{th} day after sowing.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 Seedling vigour index-I and Seedlingvigour index-II were calculated. The following formulas were used for various calculations[1].

Seed Vigour Index I = Seed germination percentage × Seedling length (cm)
Seed vigour Index II = Seed germination percentage × Dry seedling weight (mg)

$$\text{Disease incidence (\%)} = \frac{\text{Number of seedlings affected}}{\text{Total number of seedlings observed}} \times 100$$

$$\text{Disease control (\%)} = \frac{\text{Treatment - Control}}{\text{Treatment}} \times 100$$

$$\text{Seed germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds placed for germination}} \times 100$$

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

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

3. RESULTS AND DISCUSSION

3.1 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. viride* (94.7 %) showed significantly higher germination compared to the carbendazim (92.0 %). While, *Pseudomonas fluorescens* treatment (92.7 %) was on par to the carbendazim treatment (Table 1). Although the differences between the treatments are meagre, the germination percentage was markedly high both in bio-fungicide treatments and carbendazim

treatment compared to the infected seed and healthy seed. The higher seed germination with bio-fungicides could be through inhibition of growth of *C. capsici* [18, 26].

Table 1. Effect of bio-fungicides on seed germination, seedling length and 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	30.00
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	28.50	30.16
Trichoderma asperellum + *Trichoderma viride*	88.0 (69.7)	85.3 (67.5)	5.66	6.60	27.83	29.86
Pseudomonas fluorescens + *Trichoderma viride*	89.3 (70.9)	89.3 (71.0)	4.90	6.64	33.56	30.20
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	85.3 (67.5)	83.7 (66.1)	5.94	5.22	28.00	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	2.40
SEm±	0.3	0.6	0.13	0.14	0.45	0.80
C.V. (%)	0.7	1.6	4.19	3.87	2.58	4.35

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

In pot culture experiment, carbendazim was superior to all the bio-fungicide treatments. However, the seed germination was above the minimum standards of seed germination in all the treatments except the infected seeds both in blotter and pot culture experiments. Hence, for the purpose of higher seed germination any of the bio-fungicides may be suggested to achieve higher seed quality parameters.

3.2 Seed quality parameters

Both in blotter and pot culture, among the treatments, seedling length was significantly superior with *Trichoderma viride* (7.04 cm in blotter and 7.75 cm in pot culture) as compared to the carbendazim (5.26 and 6.68 cm respectively). In pot culture, bio-fungicides treatments showed significantly higher seedling length as compared to the healthy seed. Similar results of increased seedling length due to application of *Trichoderma viride*, *Trichoderma asperellum* and *Pseudomonas fluorescens* individually or in combination was reported in different species [7,19, 20].

Seedling dry weight among the treatmentsand across the two experiments, was significantly higher in carbendazim treated seeds (36.67 mg in blotter method and 39.33mg in pot culture) compared to all bio-fungicide treatments (Table 1). However, in pot culture, bio-fungicide treatments performed better

over the healthy seed, this could be due to effective control of pre-emergence and post-emergence damping off through decreased colony formation by *C. capsici* [10, 12].In pot culture, (similar to field conditions), lower effect of bio-fungicides could be due to longer time required for perpetuation of bio-fungicides in view of requirement of carbohydrate at early stages, whereas, carbendazim do not depend on seedling for carbohydrate requirement.

3.3 Seedling vigour

Seedling vigour is an important trait in ensuring proper crop establishment and economic yields especially under adverse conditions. Seed borne pathogen like *C. capsici* is known to affect the seedling vigour causing 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* and others on seedling vigour in chilli [2, 9, 18, and 19].However, scanty literature is available with respect to comparison of bio-fungicides with carbendazim which is a popular systemic fungicide [17].Therefore, it is very pertinent to identify a bio-fungicide comparable to that of carbendazim in the changing climate scenario as carbendazim have deleterious effects on biodiversity, environment and human [3].In the present study, seedling vigour index was significantly high with carbendazim treatment compared to all the bio-fungicides both in blotter and pot culture (Table 2). However, superiority of bio-fungicides over the control (healthy seed) was observed for SVI-I in pot culture and SVI-II in blotter technique (Table 2). These differences are due to variations in seed germination, seedling length and seedling dry weights in calculation of seedling vigour indices. However, when the data was normalized by giving equal weightage to unity for all three parameters, seedling vigour with *Trichoderma viride* found on par to the carbendazim treatment (Table 2). Similarly, Choudhary *et al*. [5] reported that *Trichoderma viride* was effective compared to the carbendazim in terms of seedling vigour. Further, all the bio-fungicides were better than the control (healthy seed). Hence, seed treatment with *Trichoderma viride* is suggested to combat the *C. capsici* and thus healthy vigorous seedlings for better yields of chilli.

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	0.81
Trichoderma asperellum	430.1	628.9	2533.6	2718.4	0.60
Pseudomonas fluorescens	453.8	584.2	2641.2	2513.8	0.59
Trichoderma asperellum + *Trichoderma viride*	498.1	563.5	2449.3	2544.4	0.60
Pseudomonas fluorescens + *Trichoderma viride*	438.4	593.4	2997.9	2698.9	0.64
Pseudomonas fluorescens + *Trichoderma asperellum*	435.7	603.0	2918.3	3040.0	0.69
Untreated infected seed	329.8	317.5	1805.3	1779.3	0.33
Healthy seed	506.9	436.7	2389.0	2873.0	0.57
Carbendazim treated	647.7	608.5	3373.3	3579.3	0.81
Mean	**471.0**	**548.6**	**2684.1**	**2713.0**	
C.D at 5%	32.83	40.05	127.27	198.24	
SEm±	10.96	13.37	42.50	66.21	
C.V. (%)	4.03	4.22	2.74	4.22	

3.4 Disease infection and disease control

In laboratory experiment, disease incidence was significantly less in *Pseudomonas fluorescens* (5.33 %) compared to the carbendazim (8.00 %) whereas; the *Trichoderma viride* (7.33 %) was comparable to the carbendazim. In pot culture, carbendazim had significantly lower disease incidence (9.0 %) and on par to that of *Trichoderma viride* (11.0%) and *Trichoderma viride* + *Pseudomonas fluorescens* (10.67 %). All bio-fungicide treatments resulted in significantly lower disease incidence or on par to the control (healthy seed)(Table 3).In contrast to disease incidence, the disease control was significantly higher in *Pseudomonas fluorescens* (81.61 %) compared to carbendazim (73.03 %) and *Trichoderma viride* (74.71 %) on par to the carbendazim (73.03 %) in blotter technique (Table 3). In pot culture, disease control (70.65 %) was significantly superior in carbendazim treatment compared to all bio-fungicides except *Trichoderma viride* + *Pseudomonas fluorescens* (64.44 %).

Table 3. Effect of bio-fungicides on disease incidence and 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	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. (%)	2.40	4.59	2.02	5.48

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

Many reports have shown that the bio-fungicides like *Trichoderma viride*, *Pseudomonas fluorescens* and their combinations inhibited the mycelial growth of pathogen and hence disease control caused by *C. capsici* [8, 11, 22, 23, 24, 25]. These studies have not compared the effectiveness of bio-fungicide against the carbendazim which is a popular systemic fungicide. However, a few studies show that chemical fungicides like copper oxychloride is more effective than *Trichoderma viride* in controlling the disease caused by *C. capsici* [16]. The bio-fungicide, *Trichoderma viride* produce antibiotic (trichodermin) and extracellular enzymes (chitinase, cellulose) those inhibit the plant pathogen [19]. Further, it would be effective in combined use of bio-fungicide with carbendazim to reduce the disease incidence, thus higher yield and quality of chilli [6, 13]. Therefore, it was also suggested for both seed treatment and soil treatment for effective control of *C. capsici* [4].

Therefore, the use of *Trichoderma viride* and *Pseudomonas fluorescens* or their combinations are suggested in place of carbendazim against *Colletotrichum capsici* and for better seed quality parameters in chilli.

4. CONCLUSION

Seed treatment with *Trichoderma viride* (5g kg^{-1} seed) and *Pseudomonas fluorescens* (5g kg^{-1} seed) individually or combination can be effectively used in place of carbendazim (0.2 %) treatment for effective control of *Colletotrichum capsici* to achieve higher seedling vigour in chilli.

COMPETING INTERESTS

Authors declare no competing interest exists.

REFERENCES- Can include more recent year references

1. Anderson JD, Baki AA. Vigour determination in soybean seed by multiple criteria. Crop Science. 1973; 13 (6):630-633.
2. Asaduzzaman M, Alam MJ, Islam MM. Effect of Trichodermaon seed germination and seedling parameters of chilli. Journal of Science Foundation. 2011; 8 (1&2):141-150.
3. Avinash VS, Hosmani SP. Effect of carbendazim on morphological and biochemical parameters of *Sorghum bicolor*. Periplex. Indian Journal of Research.2012; 1(10): 12-14.
4. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis. 2002; 10: 178-182.
5. Choudhary CS, Jain SC, Kumar R, Choudhary JS. Efficacy of different fungicides, biocides and botanical extract seed treatment for controlling seed borne *Colletotrichum* sp. in chilli (*Capsicum annuum* L.). The Bioscan. 2013; 8 (1): 123-126.
6. Ekbote S. Effect of Pseudomonas fluorescens on anthracnose of chilli caused by *Colletotrichum capsici*. Karnataka Journal of Agricultural Sciences. 2005; 18 (1): 162-165.
7. Ekefan EJ, Jama A, Gowen SR. Potential of *Trichoderma harzianum* isolates in for prevalence of major diseases. Vegetable science. 2009; 30 (2): 128-134.
8. Intana W, Suwanno T, Chamswarng C, Chantrapromma K, Ngamriabsakul C. Increased efficacy for controlling anthracnose of chili using antifungal metabolites from mutant strains of Trichoderma harzianum. Thai Journal of Agricultural Science. 2007; 40 (1-2): 65-72.
9. Islam MS, Rahman MA, Bulbul SH, Alam MF. Effect of *Trichoderma* on seed germination and seedling parameters in chilli. Journal of Experimental Agriculture International. 2011; 2 (1): 21-26.
10. Jayaraj J, Radhakrishnan NV, Velazhahan R. Development of formulations of *Trichoderma harzianum* strain M1 for control of damping-off of tomato caused by *Pythium aphanidermatum*. Achieves of Phytopathology and Plant Protection. 2006; 39 (1): 1-8.
11. Jeyalakshmi C, Durairaj P, Seetharaman K, Sivaprakasam K. Bio-control of fruit rot and die-back of chilli using antagonistic microorganisms. Indian Phytopathology. 1998; 51(2): 180-183.
12. Manoranjitham SK, Prakasan V, Rajappan K, Amutha G. Effect of two antagonists on damping off disease of tomato. Indian Phytopathology.2000; 53 (4): 441-443.
13. Mesta RK, Mohankumar SA, Aspapupre M, Shivaprasad M, Tatagar MH. Management of fruit rot and powdery mildew of chilli through *Pseudomonas fluorescence* along with fungicides. National workshop on current trends and future prospects in production and export of spice crops with special to chilli. 2009; 123.
14. Pakdeevaraporn P, Wasee S, Taylor PWJ, Mongkolporn O. Inheritance of resistance to anthracnose caused by *Colletotrichum capsici* in *Capsicum*. Plant Breeding. 2005; 124 (2): 206-208.
15. Pandey KK, Pandey PK. Survey and surveillance of vegetable growing areas for prevalence of major diseases.Vegetable Science. 2003; 30 (2):128-134.
16. Patel MD, Lal AA, Singh PP. Efficacy of certain bio agents and fungicides against root rot of chilli (*Capsicum annum* L.). The Bioscan. 2014; 9 (3):1273-1277.
17. Phansawan B, Prapamontol T, Thavornyutikarn P, Chantara S, Mangklabruks A, Santasup C. A sensitive method for determination of carbendazim residue in vegetable samples using HPLC-UV and its application in health risk assessment. Chiang Mai Journal of Science. 2015; 42 (3): 681-690.

18. Raj ST, Christopher DJ, Rajakumar RS, Usharani S. Effect of organic amendments and *Trichoderma viride* on growth and root rot incidence of sunflower. Annals of Plant Protection Sciences. 2008; 16: 242-244.
19. Rehman SU, Lawrence R, Kumar EJ, Badri ZA. Comparative efficacy of *Trichoderma viride*, *T. harzianum* and carbendazim against damping-off disease of cauliflower caused by *Rhizoctonia solani*. Kuehn Journal of Biopesticides. 2012; 5 (1): 23-27.
20. Rohini HGG, Hariprasad P, Singh SB, Niranjana SR. Biological control of Phomopsis leaf blight of brinjal (*Solanum melongena* L.) with combining phylloplane and rhizosphere colonizing beneficial bacteria. Biological Control.2016; 101: 123-129
21. Saxena A, Raghuwanshi R, Gupta VK, Singh HB. Chilli anthracnose: The epidemiology and management, Frontiers in Microbiology.2016; 7: 1527.
22. Sharma PN, Kaur M, Sharma OP, Sharma P, Pathania A. Morphological, pathological and molecular variability in *Colletotrichum capsici*, the cause of fruit rot of chillies in the subtropical region of north-western India.Journal of Phytopathology. 2005; 153 (4):232–237.
23. Srinivas C, Niranjana SR, Kumar P, Chandra L, Nayaka S, Shetty MS. Effect of chemicals and biological agents on seed quality of chilli (*Capsicum annum* L.). Indian Phytopathology. 2005; 59: 62-67.
24. Anand T, Bhaskaran R. Exploitation of plant products and bio-agents for eco-friendly management of chilli fruit rot disease. Journal of Plant Protection Science.2009; 49 (2): 196-197.
25. Tiwari PK, Kasyap A, Awadhiya GK, Thrimurty VS. Efficacy of bio-agents, neem based plant products and plant extracts Against *Colletotrichum capsici*. Indian Journal of Plant Protection. 2008; 36 (1): 97-97.
26. Yadav VK. Organic package of practices for chilli from Uttaranchal. Organic Farming Newsletter. 2008; 4 (4): 3-8.