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Efficiency of Bio-fungicides *(Trichoderma spp and Pseudomonas fluorescens*) on Seedling Emergence, Vigour and Health of Infected Chilli Seeds (*Capsicum annuum*) by *Colletotrichum capsici*

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Authors' contributions

This work was carried out in collaboration among all authors. Author YNPR conducted the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SSJ and OSD designed and supervised the study. Author YNPR managed the literature searches. All authors read and approved the final manuscript.

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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 (blotter 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 three times. Six months old seeds having germination above the Indian Minimum Seed Certification Standard, were infected with *Colletotrichum capsici* and such infected seeds were treated with *Trichoderma asperellum*, *Trichoderma viridae*, *Pseudomonas*

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fluorescens individually and their combinations to control the disease incidence. The infected, un-infected and seed treatment with carbendazim served as controls. Results revealed that the seed germination was significantly higher (94.7%) with *Trichoderma viride* treatment compared to all other treatments including controls in blotter method. However, the seedling emergence in pot culture was significantly superior with Carbendazim treatment, the seed treatment with *Pseudomonas fluorescens* and *Trichoderma viridae* was on par to that of Carbendazim treatment. The seedling length was significantly superior with *Trichoderma viride* compared to the carbendazim and other controls both in blotter and pot culture. The seedling dry weight and seedling vigour were significantly higher with carbendazim as compared to the *Trichoderma viride* treatment or other treatments in both blotter and pot culture. However, the overall seedling vigour obtained with *Trichoderma viride* was similar to that of carbendazim treatment. The disease incidence was significantly lower with *Pseudomonas fluorescens* as compared to the *Trichoderma viride* and carbendazim in blotter method and; *T. viride* + *P. fluorescens* treatment was on par to that of carbendazim treatment in pot culture. Therefore, use of *Trichoderma viride* and *Pseudomonas fluorescens* individually or in combination are suggested as an alternative to carbendazim to control the *Colletotrichum capsici.*

Keywords: Chilli; Colletotrichum capsici; carbendazim; bio-fungicides.

1. INTRODUCTION

Chilli is a major spice crop in India and India stands 3rd in production (1). The crop is suffered mainly by seedling rot and fruit rot caused by *Colletotrichum capsici* leading to reduced marketability and fruit yield [2,3]. To control this disease, systemic fungicides are commonly used, especially the carbendazim at the recommended dose of 0.2% [4]. However, continuous use of chemical fungicides has deleterious effects on biodiversity, environment and human health [5]. 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 a decreased fruit rot [6,7,8,9,10,11]. As most of the studies pertain only to bio-fungicides, it is 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* individually and their combinations in comparison with chemical fungicide (carbendazim), infected and un-infected seed on seed germination, seedling emergence, seedling vigour and disease control in chilli seeds infected with *Colletotrichum capsici*.

2. MATERIALS AND METHODS

Two experiments (blotter and pot culture) were conducted to study the effect of bio-fungicides on seed quality parameters of chilli seeds infected with *Colletotrichum capsici*. These experiments were conducted at the Department of Seed Science and Technology, CCSHAU, Hisar during October-November, 2016. The seeds used in these experiments were six months old which were harvested during February – March, 2016 (high yielding popular variety, RCH-1). The seed germination was above the Indian Minimum Seed Certification Standards.

The experiments were conducted with nine treatments in three replications (Table 1) both in blotter and pot experiments. In blotter method, petri dishes(15 cm diameter) lined with two layers of blotting paper (Whatman No.1) were prepared, adequately watered, 25 seeds in each petri dish were placed and kept in BOD (biological oxygen demand) incubator for 14 days at 25ºC. Sixteen petri plates were used for each replication. These plates were watered as and when the blotter paper appeared nearly to dryness. For pot culture experiment, pots (27.5 cm diameter and 30 cm height) were filled with four kg of oven sterilized soil. The soil is sandy loam with organic carbon (0.15%), pH (8.1) and Ec (0.15 dS/m at 25ºC) [12]. Twenty five seeds were placed at a depth of 1-2 cm in each pot and eight pots replication were maintained. The pots were watered daily up to 14 days. The weeds were uprooted whenever appeared.

In both the experiments, final germination count, disease incidence and disease control was monitored on 14th day, and ten randomly selected seedlings per replication were taken 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±1ºC until they attained a constant dry weight and calculated the seedling vigour. The formulae used for various calculations are given below [13].

2.1 Isolation of *Colletotrichum capsici* and Seed Infection

The infected chilli fruit portion was sterilized and cultured on potato dextrose agar (PDA) medium in a petri plate. The pure culture of *Colletotrichum capsici* was identified, isolated, sub-cultured and multiplied again on PDA and used for seed infection. The multiplication has taken 7-9 days.

Twenty gram of chilli seeds were taken in a beaker. The *Colletotrichum capsici* was scrapped into the beaker containing the seeds using a scrapper in the laminar air flow. The beaker was closed with para-film tape, shaken for 15 minutes and left undisturbed for 24 h. Such infected seeds with *Colletotrichum capsici* were used further for treatment with bio-fungicides or carbendazim.

2.2 Seed Treatment with Bio-fungicide or Carbendazim

The *Colletotrichum capsici* infected seeds were treated with different bio-fungicides, *Trichoderma asperellum*, *Trichoderma viridae* and *Pseudomonas fluorescens* (200 mg/ 20 g seed) individually and in combinations (100mg + 100mg) or with carbendazim (40 mg/ 20 g seed) in a beaker, shaken gently to cover the seed uniformly with bio-fungicide or carbendazim (Table 1). The control treatments were, infected seed (not treated with any bio-fungicide or Carbendazim), uninfected seed (six months old seed which was not treated with any bio-fungicide or Carbendazim) and Carbendazim treatment (infected seed treated with Carbendazim).

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

Seedling emergence (%) in pot = (Number of seedlings emerged / Total number of seeds placed for seedling emergence) × 100

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

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

Seed Vigour Index I = Seedling emergence percentage × Seedling length (cm) in pot culture

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

Seed vigour Index II = Seed emergence percentage × Dry seedling weight (mg) in pot culture

Disease incidence (%) = (Number of seedlings affected either in blotter or pot/ Total number of seedlings either in blotter or pot) × 100

Disease control (%) = ((Treatment – Infected)/ Treatment) × 100

Where, treatment refers to all the eight treatments including two controls namely, un-infected and Carbendazim treatments.

Table 1. Treatment details involved in the experiment

No.	Treatments
T_1	*Trichoderma viride* (200 mg/ 20 g seed)
T_2	*Trichoderma asperellum* (200 mg/ 20 g seed)
T_3	*Pseudomonas fluorescens* (200 mg/ 20 g seed)
T_4	*Trichoderma asperellum* (100 mg/ 20 g seed)+ *Trichoderma viride* (100 mg/ 20 g seed)
T_5	*Pseudomonas fluorescens*(100 mg/ 20 g seed) + *Trichoderma viride* (100 mg/ 20 g seed)
T_6	*Pseudomonas fluorescens*(100 mg/ 20 g seed) + *Trichoderma asperellum* (100 mg/ 20 g seed)
T_7	Infected seed (*Colletotrichum capsici* infected seed but not treated with bio-fungicide or Carbendazim)
T_8	Un-infected seed (Six months old seed which was not-infected with *Colletotrichum capsici* and not treated with bio-fungicide or Carbendazim)
T_9	Carbendazim treated (*Colletotrichum capsici* infected seed treated with Carbendazim (40 mg/ 20 g seed)

Note: (1) Dose:10 g kg^{-1} alone and in combination @ 5 + 5 g kg^{-1} seed, (2) Except the controls (T_7 and T_8), all the bio-fungicides and Carbendazim treatments were given to the seeds that are infected with Colletotrichum capsici

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 seedling emergence in 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 seed germination compared to all other treatments including the carbendazim treatment (92.0%). While, *Pseudomonas fluorescens* treatment (92.7%) was on par to the carbendazim treatment (Table 2). Although the differences between the treatments are meagre, the germination percentage was markedly high both in the bio-fungicide treatments and carbendazim treatment compared to the controls (infected seed and un-infected seed). The higher seed germination with bio-fungicides could be through inhibition of growth of *C. capsici* [14,15].

In pot culture experiment, the seedling emergence was significantly superior with carbendazim as compared to all the bio-fungicide treatments and other control treatments. However, the seed germination was above the minimum standards of seed germination in all the treatments except the infected seeds (absolute control) 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 germination or seedling emergence of chilli seeds. Both in blotter and pot culture, infected seed maintained showed significantly lower seed germination and seedling emergence respectively as compared to the un-infected control or carbendazim treatments (Table 2).

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-fungicide treatments showed significantly higher seedling length as compared to the un-infected 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 [16,17,18].

Table 2. Effect of bio-fungicides on seed germination, seedling length and dry weight in *chilli* seeds infected with *Colletotrichum capsici*

Treatments	Seed germination (%)	Seedling emergence (%)	Seedling length (cm)		Seedling dry weight (mg/ seedling)	
	Blotter	Pot	Blotter	Pot	Blotter	Pot
Trichoderma viride	94.7 (76.6)^{g}	89.0 (70.6)^{c}	7.04^{e}	7.45^{e}	32.21^{d}	30.00^{bc}
Trichoderma asperellum	86.7 (68.6)^{c}	84.3 (66.7)^{b}	4.96^{ab}	6.76^{cd}	29.23^{c}	32.23^{cd}
Pseudomonas fluorescens	92.7 (74.3)^{f}	83.3 (65.9)^{b}	4.89^{ab}	7.01^{cd}	28.50^{bc}	30.16^{bc}
Trichoderma asperellum + *Trichoderma viride*	88.0 (69.7)^{d}	85.3 (67.5)^{b}	5.66^{c}	6.60^{c}	27.83^{b}	29.86^{b}
Pseudomonas fluorescens + *Trichoderma viride*	89.3 (70.9)^{e}	89.3(71.0)^{cd}	4.90^{ab}	6.64^{c}	33.56^{d}	30.20^{bc}
Pseudomonas fluorescens + *Trichoderma asperellum*	89.3 (70.9)^{e}	85.0 (67.2)^{b}	4.87^{a}	7.09^{de}	32.67^{d}	35.76^{e}
Infected seed	70.3 (57.0)^{a}	69.3 (56.4)^{a}	4.69^{a}	4.58^{a}	25.67^{a}	25.66^{a}
Un-infected seed	85.3 (67.5)^{b}	83.7 (66.1)^{b}	5.94^{dc}	5.22^{b}	28.00^{bc}	34.33^{de}
Carbendazim treated seed	92.0 (73.5)^{f}	91.0 (72.5)^{d}	5.26^{b}	6.68^{cd}	36.67^{e}	39.33^{f}
Mean	87.6 (69.9)	84.5 (67.1)	5.36	6.45	30.48	31.95
C.D (P< 0.05)	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

Seedling dry weight among the treatments and across the two experiments was significantly higher in carbendazim treated seeds (36.67 mg in blotter method and 39.33 mg in pot culture) as compared to all bio-fungicide treatments (Table 2). However, in pot culture, bio-fungicide treatments performed better over the un-infected seed, this could be due to effective control of pre-emergence and post-emergence damping off through decreased colony formation by *C. capsici* [19,20]. 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 carbo-hydrate 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 reduces the 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 [14,17,21,22]. However, scanty literature is available with respect to comparison of bio-fungicides with carbendazim which is a popular systemic fungicide [4]. 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 [5]. In the present study, seedling vigour index-I and II were significantly high with carbendazim treatment compared to all the bio-fungicides and control treatments in both blotter and pot culture (Table 3). Further, all the bio-fungicide treatments found superior over the un-infected seed for SVI-I in pot culture and SVI-II in blotter method (Table 3). 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 3). Similarly, Choudhary et al. [23] reported that *Trichoderma viride* was effective as compared to the carbendazim in terms of seedling vigour. Further, all the bio-fungicides were better than the control (un-infected seed). Hence, seed treatment with *Trichoderma viride* is suggested to combat the *C. capsici* and thus to achieve healthy vigorous seedlings for better yields of chilli.

3.4 Disease Infection and Disease Control

In blotter experiment, disease incidence was significantly less in *Pseudomonas fluorescens* (5.33%) as compared to the carbendazim (8.00%), whereas, the *Trichoderma viride* (7.33%) was comparable to the carbendazim (Table 4). In pot culture, carbendazim showed significantly lower disease incidence (9.0%) but was 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 un-infected seed (control) (Table 4). In contrast to disease incidence, the disease control was significantly higher in *Pseudomonas fluorescens* (81.61%) as compared to carbendazim (73.03%) and *Trichoderma viride* (74.71%) in blotter technique (Table 4). In pot culture, disease control was significantly superior in carbendazim (70.65%) treatment compared to all bio-fungicides except *Trichoderma viride* + *Pseudomonas fluorescens* (64.44%).

Many reports have shown that the bio-fungicides like *Trichoderma viride*, *Pseudomonas fluorescens* and their combinations inhibited the mycelia growth of pathogen and hence disease control caused by *C. capsici* [6,7,8,9,10,11]. 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* [24]. The bio-fungicide, *Trichoderma viride* produce antibiotic (trichodermin) and extracellular enzymes (chitinase, cellulose) those inhibit the plant pathogen [17]. Further, it was effective with combined use of bio-fungicide and carbendazim in reducing the disease incidence, thus higher yield and quality of chilli was achieved [25, 26]. Further, both seed treatment and soil treatment are suggested for effective control of *C. capsici* [27].

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.

Table 3. Effect of bio-fungicides on seedling vigour index in *chilli* seeds infected with *Colletotrichum capsici*

Treatments	SVI-I		SVI-II		Overall SVI
	Blotter	Pot	Blotter	Pot	Pooled
Trichoderma viride	498.6^{c}	601.6^{cde}	3048.8^{f}	2670.0^{bc}	0.81
Trichoderma asperellum	430.1^{b}	628.9^{e}	2533.6^{cd}	2718.4^{cd}	0.60
Pseudomonas fluorescens	453.8^{b}	584.2^{cd}	2641.2^{d}	2513.8^{b}	0.59
Trichoderma asperellum + *Trichoderma viride*	498.1^{c}	563.5^{c}	2449.3^{bc}	2544.4^{bc}	0.60
Pseudomonas fluorescens + *Trichoderma viride*	438.4^{b}	593.4^{cde}	2997.9^{ef}	2698.9^{bcd}	0.64
Pseudomonas fluorescens + *Trichoderma asperellum*	435.7^{b}	603.0^{cde}	2918.3^{e}	3040.0^{e}	0.69
Infected seed	329.8^{a}	317.5^{a}	1805.3^{a}	1779.3^{a}	0.33
Un-infected seed	506.9^{c}	436.7^{b}	2389.0^{b}	2873.0^{de}	0.57
Carbendazim treated seed	647.7^{d}	608.5^{de}	3373.3^{g}	3579.3^{f}	0.81
Mean	471.0	548.6	2684.1	2713.0	
C.D (P< 0.05)	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	

Table 4. Effect of bio-fungicides on disease incidence and disease control in *chilli* seeds infected with *Colletotrichum capsici*

Treatments	Disease incidence (%)		Disease control (%)	
	Blotter	Pot	Blotter	Pot
Trichoderma viride	7.33 (15.70)^{b}	11.00 (19.36)^{b}	74.71 (59.80)^{f}	63.33 (52.71)^{c}
Trichoderma asperellum	13.33(21.41)^{e}	15.67 (23.31)^{c}	54.02(47.29)^{c}	47.78 (43.71)^{b}
Pseudomonas fluorescens	5.33 (13.34)^{a}	16.67 (24.08)^{c}	81.61(64.60)^{g}	44.44 (41.79)^{b}
Trichoderma asperellum + *Trichoderma viride*	12.00(20.26)^{d}	14.67 (22.47)^{c}	58.62(49.94)^{d}	51.11 (45.63)^{b}
Pseudomonas fluorescens + *Trichoderma viride*	10.67(19.05)^{c}	10.67(18.98)^{ab}	63.22(52.65)^{e}	64.44 (53.46)^{cd}
Pseudomonas fluorescens + *Trichoderma asperellum*	10.67(19.05)^{c}	15.00 (22.77)^{c}	63.22(52.65)^{e}	50.00 (44.98)^{b}
Infected seed	29.67(32.99)^{g}	30.67 (33.61)^{d}	0.00 (0.00)^{a}	0.00 (0.00)^{a}
Un-infected seed	14.67(22.51)^{f}	16.33 (23.83)^{c}	50.57(45.31)^{b}	46.74 (43.11)^{b}
Carbendazim treated seed	8.00 (16.42)^{b}	9.00 (17.45)^{a}	73.03 (58.69)^{f}	70.65 (57.17)^{d}
Mean	12.41 (15.52)	20.08 (22.87)	57.67 (47.88)	48.72 (42.51)
C.D (P< 0.05)	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

4. CONCLUSION

Seed treatment with *Trichoderma viride*(10 g kg^{-1} seed) and *Pseudomonas fluorescens*(10 g kg^{-1} seed) individually or combination (*Trichoderma viride, 5* g kg^{-1} seed + *Pseudomonas fluorescens, 5* g kg^{-1} seed) can be effectively used in place of carbendazim (0.2%) treatment for effective control of *Colletotrichum capsici* to achieve higher seedling vigour.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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