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Evaluation of bio-control agents for management of fruit rot and its effect on seed quality in chilli

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ABSTRACT

The fruit rot/anthracnose disease by *Colletotrichum capsici* (Sydow.) is a wide spread problem, limiting the profitable cultivation and seed production throughout the major chilli growing regions of India. The present investigation was carried out to evaluate the effect of fruit rot on seed quality parameters as well as on eco-friendly management options in-vitro and in-vivo during 2018-2020. Seeds from the bio-agents treated plots exhibited good germination percentage, seedling vigour indices and electrical conductivity compared to control. The seeds from control (diseased) plots exhibited poor germination, which is less than IMSC standards (<60%). This clearly elucidates that there is a huge loss in all the seed quality parameters due to fruit rot in chilli. Among the three bio-agents evaluated for their efficacy in inhibiting the mycelial growth of *C. capsici* in dual culture technique, *Trichoderma viride* and *T. harzianum* were having better antagonistic effect due to their aggressiveness and fast growth on the fruit rot pathogen as compared to *Talaromyces* sp. Therefore, bio-agent can be a better ecofriendly option for the management of fruit rot disease with better quality of seeds in chilli.

Key words: *Capsicum annuum* L., *Colletotrichum capsici* (Sydow.), seed quality, *Trichoderma viride, T. harzianum, Talaromyces.*

INTRODUCTION

Chilli (*Capsicum annuum* L.) is one of the important spice/vegetable/cash crop grown in India and belongs to family Solanaceae. Chilli suffers from many diseases caused by fungi, bacteria and viruses. Among the fungal diseases damping off, anthracnose or fruit rot, powdery mildew and leaf spots are the most prevalent ones. The anthracnose or ripe fruit rot caused by *Colletotrichum capsici* (Sydow.) Butler and Bisby is a wide spread disease throughout the major chilli growing regions of India (Akhtar *et al*., 2). The disease has been observed to occur in three phases *viz.,* (i) seedling blight or damping off stage, prevalent in the nursery, (ii) leaf spotting and die back stage which is initiated at different stages of growth and (iii) fruit rot stage in which the ripe fruits are infected. The last phase causes extensive damage to the fruits since the lesions on the fruits considerably reduce the market value of the produce. The disease is both seed borne and airborne and affects seed germination and vigour to a greater extent. There lies an urgent need to develop an efficient bio-control/ biological management strategy keeping in concern the different environmental factors and pathogenic resistance, driving the successful colonization of the pathogen in the host tissues. The market value and nutritive value is degraded in infected fruits resulting in poor quality seed. Another adverse effect of seed borne pathogen is that it will contaminate the areas which were disease free previously. So, it necessitates the eradication of seed borne inoculum through various seed treatments. There is not much research work that is being done on seed borne aspects of anthracnose of chilli. There is very little information available on management strategies like use of effective bio-agents. Management of the disease as far as eco-friendly approach is concerned is also not available. The objective of the present study was to understand the effects on seed quality deterioration by fruit rot in chilli and establishment of an eco-friendly management options against fruit rot.

MATERIALS AND METHODS

The present investigation was carried out both in the laboratory and field at ICAR- Indian Agricultural Research Institute, New Delhi during 2018-20 using a resistant variety, IIVRC 452 and a susceptible variety, Pusa Jwala (Fig. 1) with the following experimental details.

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Fig. 1. Pusa Jwala (Susceptible variety) plant.

T_1 = Seed treatment with *Talaromyces sp.* @10gm $kg^{-1;}$ T_2 = Seed treatment with *T. harzianum* @10gm kg^{-1}; T_3 = Seed treatment with *T. viride* @10gm kg^{-1}; T_4 = Seed treatment with *T. viride* + *Talaromyces sp.;* T_5 = *T. harzianum* + *Talaromyces sp.;* T_6 = T_1 + Seedling dip in *Talaromyces sp.* @10gm L^{-1}; T_7 = T_2 + Seedling dip in *T. harzianum* @10gm L^{-1}; T_8 = T_3 + Seedling dip in *T. viride* @10gm L^{-1}; T_9 = T_6 + One spray of *Talaromyces sp.*@10gm L^{-1}; T_{10} = T_7 + One spray of *T. harzianum* @10gm L^{-1}; T_{11} = T_8 + One spray of *T. viride* @10gm L^{-1}; T_{12} = Control (untreated). The seeds of Pusa Jwala and IIVRC 452 were obtained from the Division of Vegetable Science, ICAR-IARI, New Delhi and ICAR-Indian Institute of Vegetable Research, Varanasi and the bioagents, like *Talaromyces sp., T. harzianum* and *T. viride* were procured from Department of Plant Pathology ICAR-IARI. Four isolates of *C. capsici* (CC-DSST-05, CC-DSST-13, CC-DSST-11 and CC-DSST-04) were used in dual culture test.

The thousand seed weight (grams) was recorded in each treatment as per the procedure given by ISTA (7). Seed germination tested, as per the International Seed Testing Association Rules (ISTA, 7) taking, four hundred seeds of each variety by employing standard blotter method with four replications. Seedling length (cm) was observed taking, 10 normal seedlings selected randomly from the germination by recording, shoot and root length measurement. The mean value of shoot length was calculated and expressed in centimetres. For seedling dry weight (mg), randomly selected ten normal seedlings were placed in a butter paper bag and dried for 24 hours in a hot air oven maintained at 70°C. The dry weight of seedling was recorded in an electronic balance and average weight was computed and expressed in milligram per ten seedlings. For electrical conductivity (milli Siemens), five grams of seeds were taken at random from each treatment. Then the seeds were soaked in 25 ml of distilled water for 24 hr at 20°C. The seed leachate was collected by decanting, the electrical conductivity of seed leachate was measured at room temperature with a conductivity bridge and expressed as milli Siemens (Presley, 11). For vigour indices, the vigour index (V I) was calculated by adopting the method suggested by Abdul-Baki and Anderson (1) and was expressed as pure number.

Vigour Index (V I) I = Germination (%) x Total seedling length (cm)

Vigour Index (V I) II = Germination (%) x Seedling dry weight (mg)

In dual culture technique, 20 ml of sterilized potato dextrose agar was poured into sterile Petri plates and allowed to solidify. The pathogen at one side of Petriplate and the antagonist inoculated at exactly opposite side of the same plate by leaving 3-4 cm gap. Each treatment was replicated four times. These plates were incubated at 25±2°C for seven days and colony diameter was recorded. Per cent inhibition over control was worked out according to formula given by Vincent (15).

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

Where, I = per cent inhibition of mycelium; C = Colony diameter (cm) in control, and T = Colony diameter (cm) treatment.

Anthracnose of chilli incidence was recorded by scoring five plants in each microplot using 0-9 scale. Further, the PDI was calculated with the above scales using the formula.

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of numerical values of disease ratings}}{\text{Number of fruit observed} \times \text{Maximum disease rating}} \times 100$$

Crop was harvested at red fruit stage and after harvest seed weight of each replication in kilograms was recorded and yield per hectare was computed by using net plot yield data and it was then converted to quintals per hectare. The analysis of variance was done using single and two factor analysis in OPSTAT sheet. Statistical significance was tested using the "F" test. Critical difference (CD) was also used to test the difference between any two means.

RESULTS AND DISCUSSION

Seed size varied among genotypes. 1000 seed weights of IIVRC 452 and all treatment of Pusa Jwala were recorded after harvesting of red ripe fruits and extraction of seeds. It was observed that there was significant difference among the two genotypes. Pusa Jwala seeds were having 1000-seed weight more than IIVRC 452. It was observed that 1000- seed weight was statistically *at par* among the seeds of all the biological treatments and the control seeds of Pusa Jwala (Table 1). The genotypes with higher 1000 seed weight are likely to have bolder and well filled seeds with more viability as compared to those

Table 1. Seed test wt, electrical conductivity of IIVRC 452 of Biological treatments of Pusa Jwala recorded during 2019 and 2020.

Treatments	Seed quality parameters					
	Test wt (g)			EC milli Siemens		
	2019	2020	Pooled	2019	2020	Pooled
RV	4.378	4.379	4.378	1.060	1.110	1.085
T_1	4.492	4.492	4.492	2.247	2.316	2.282
T_2	4.488	4.489	4.488	2.119	2.343	2.231
T_3	4.491	4.491	4.491	1.980	1.843	1.911
T_4	4.491	4.489	4.490	1.796	1.695	1.746
T_5	4.489	4.493	4.491	1.737	1.837	1.787
T_6	4.492	4.489	4.490	1.629	1.516	1.572
T_7	4.490	4.492	4.491	1.547	1.465	1.506
T_8	4.489	4.490	4.489	1.439	1.542	1.490
T_9	4.490	4.493	4.491	1.316	1.258	1.287
T_{10}	4.489	4.489	4.489	1.254	1.143	1.199
T_{11}	4.493	4.491	4.492	1.163	1.296	1.230
T_{12}	4.487	4.489	4.488	2.372	2.604	2.488
C.D.	0.003	0.003	0.003	0.099	0.260	0.198
SE(m)	0.001	0.001	0.001	0.032	0.084	0.064
CV (%)	0.034	0.034	0.027	2.69	4.99	4.405

with lower 1000 seed weights as reported by Pandit and Adhikary (10).

Electrical conductance of IIVRC 452 is less than Pusa Jwala. Electrical conductance from seed leachates increased with increased disease infection. Among biological treatments T_{11} (Seed treatment, Seedling dip and one spray of *T. viride*) and T_{10} (Seed treatment, Seedling dip and one spray of *T. harzianum*) were recorded less electrical conductance and differed significantly over rest of the treatments and the highest electrical conductance was recorded with the treatments, T_1 (seed treatment with *Talaromyces sp*. @10gm kg^{-1}) and followed by T_2 (seed treatment with *T. harzianum* @10gm kg^{-1}). Untreated seeds (control) exhibited highest electrical conductance. All the treatments were found to produce significantly less electrical conductance compared to the control in both years (Table: 1). Due to the infection by fruit rot pathogen in seeds, the fungal inoculums causes rupture of cell membrane by penetrating it leading to excess release of electrolytes caused higher electrical conductivity. Hence the deterioration level in these seeds was more which is evident with higher EC values.

Germination percentage of IIVRC 452 was more than Pusa Jwala seeds. Among the biological treatments T_{11} (Seed treatment, Seedling dip and one spray of *T. viride*) and T_{10} (Seed treatment, Seedling dip and one spray of *T. harzianum*) were recorded highest germination percentage and differed significantly over rest of the treatments and the lowest germination was recorded with the treatments, T_1 (seed treatment with *Talaromyces sp*. @10gm kg^{-1}) and followed by T_2 (seed treatment with *T. harzianum* @10gm kg^{-1}). Untreated seeds (control) exhibited lowest germination percent. All the treatments were found significantly superior to the control in both years. The results on seed germination revealed that the fruit rot pathogen significantly reduced the seed germination. The highly infected control seeds gave poor germination and failed to reach the minimum required standard as per the Indian Minimum Seed Certification Standards (IMSCS, 6) i.e. ≥60% (Table: 2 and Fig. 2). The germination in diseased seed sample might be low due to proliferation of pathogenic fungal species on germinating seedlings and resulting in seed and seedling death.

Seedling growth of IIVRC 452 is more than Pusa Jwala seeds. Among the biological treatments T_{11} (Seed treatment, Seedling dip and one spray of *T. viride*) and T_{10} (Seed treatment, Seedling dip and one spray of *T. harzianum*) recorded highest seedling growth, highest seedling dry weight, highest seedling vigour indices and differed significantly

Fig. 2. Germination test- normal chilli seedlings.

Table 2. Germination, seedling length of IIVRC 452 of Biological treatments of Pusa Jwala recorded during 2019 and 2020.

Treatments	Seed quality parameters					
	Germination %			Seedling length (in cm)		
	2019	2020	Pooled	2019	2020	Pooled
RV	77.50(61.66)*	77.00(61.32)*	77.25(61.48)*	9.800	9.750	9.775
T_1	66.50(54.61)	66.00(54.31)	66.25(54.46)	7.750	7.700	7.725
T_2	67.00(54.91)	66.00(54.31)	66.50(54.61)	8.050	7.900	7.975
T_3	68.00(55.53)	67.50(55.23)	67.75(55.37)	8.200	8.250	8.225
T_4	68.50(55.83)	66.50(54.62)	67.50(55.22)	7.900	8.000	7.950
T_5	68.50(55.84)	68.00(55.52)	68.25(55.68)	8.150	8.200	8.175
T_6	69.50(56.45)	71.00(57.40)	70.25(56.92)	8.650	8.600	8.625
T_7	70.50(57.08)	72.50(58.34)	71.50(57.71)	8.850	8.700	8.775
T_8	71.50(57.71)	73.00(58.67)	72.25(58.19)	9.050	9.150	9.100
T_9	72.50(58.34)	73.50(58.99)	73.00(58.67)	9.350	9.300	9.325
T_{10}	73.50(58.99)	73.50(59.00)	73.50(58.99)	9.450	9.400	9.425
T_{11}	75.50(60.30)	76.50(60.97)	76.00(60.64)	9.700	9.650	9.675
T_{12}	55.50(48.14)	55.50(48.13)	55.50(48.13)	6.950	7.000	6.975
C.D.	2.109	4.479	1.739	0.452	0.428	0.127
SE(m)	0.677	1.438	0.563	0.145	0.138	0.041
CV (%)	1.37	2.91	1.144	2.38	2.26	0.675

*Angular transformed values

over rest of the treatments and the lowest seedling growth, the lowest seedling dry weight and the lower seedling vigour indices was recorded with the treatments, T_1 (seed treatment with *Talaromyces sp*. @10 g kg^{-1}) and followed by T_2 (seed treatment with *T. harzianum* @10 g kg^{-1}). Untreated seeds (control) exhibited lowest seedling growth. All the treatments were found significantly superior to the control in reducing the disease severity of fruit rot in both years. The results revealed that the fruit rot pathogen significantly hampered the seedling growth (plumule and radical length) and seedling vigour indices (Table 2 & 3). The damage caused by fungi and toxic metabolites that have hindered the growth of seedling resulted in reduced seedling length. The gradual decline in mobilization efficiency due to disease infection, which inturn also resulted in deterioration of seed, decrease in the testweight and seedling dry weight. Studies on the effect of seed borne fungal infections of chilli on vigour index revealed that seed germination and vigour index decreased with increase in seed infection, as reported by Priya *et al*. (12).

The results of dual culture with *T. viride* depicted that, among the four strains of *Colletotrichum capsici*, the CC-DSST-13 showed maximum inhibition upto 70.47% of fungal colony inhibition, followed by the CC-DSST-11 showed 55.23% of inhibition, the CC-DSST-04 showed 49.43% of inhibition and the CC-DSST-05 showed the least inhibition (45.56%) at seven days of incubation compared to control (Table: 4, Fig. 3a and 4). The results of dual culture with *T. harzianum* depicted that, among the four strains of *Colletotrichum capsici*., the CC-DSST-13 showed maximum inhibition upto 61.64% of fungal colony inhibition, followed by the CC-DSST-05 showed 59.21% of inhibition, the CC-DSST-04 showed 48.32% of inhibition and the CC-DSST-11 showed the least inhibition (39.54%) at seven days of incubation compared to contol (Table: 4, Fig. 3b and 4). The results of dual culture with *Talaromyces sp.* depicted that, among the four strains of *Colletotrichum capsici*, the CC-DSST-04 showed maximum inhibition upto 37.55% of fungal colony inhibition, followed by the CC-DSST-05 showed 36.14% of inhibition, the CC-DSST-11 showed 34.16% of inhibition and the CC-DSST-13 showed the least inhibition (32.91%) at ten days of incubation compared to contol (Table 4, Fig. 3c and 4). Among the three bioagents, *T. viride, T. harzianum* were having better antagonistic effect due to its aggressive and fast growth on the fruit rot pathogen and also was found to be significantly

superior compared to *Talaromyces sp. Talaromyces* sp. takes double the time that of *Trichoderma* spp. to over grow on the pathogen in dual culture. Supiriority of *Trichoderma sp.* over other bioagents was proved by Ramamoorthy *et al.* (13). *Trichoderma* sp. grew over the pathogen and caused hyphal coiling, hyphal abnormalities, reduction in sclerotial production, lysis of hyphae and sclerotia, mutually intermingling growth

Table 3. Seedling dry wt, vigour index (SV I & SV II) of IIVRC 452 and Biological treatments of Pusa Jwala recorded during 2019 and 2020.

Treatments	Seed quality parameters								
	dry wt (mg)			SVI- I			SVI- II		
	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled
RV	31.270	30.665	30.968	759.50	750.90	755.200	2,422.97	2,360.73	2,391.85
T_1	18.595	20.065	19.330	515.40	508.00	511.700	1,236.84	1,324.90	1,280.87
T_2	19.975	19.410	19.693	539.40	521.40	530.400	1,339.08	1,281.68	1,310.38
T_3	20.715	21.295	21.005	557.50	557.25	557.375	1,408.48	1,437.35	1,422.91
T_4	21.275	22.000	21.638	541.25	531.75	536.500	1,457.38	1,464.02	1,460.70
T_5	22.300	22.160	22.230	557.75	557.60	557.675	1,528.03	1,506.88	1,517.45
T_6	23.135	25.345	24.240	601.15	610.20	605.675	1,608.18	1,800.88	1,704.53
T_7	24.735	27.580	26.158	623.90	630.85	627.375	1,744.06	1,999.97	1,872.02
T_8	25.765	26.485	26.125	647.10	667.95	657.525	1,842.52	1,933.40	1,887.96
T_9	27.925	28.910	28.418	677.80	683.60	680.700	2,024.70	2,124.58	2,074.64
T_{10}	29.830	30.365	30.098	694.50	690.75	692.625	2,192.55	2,231.20	2,211.87
T_{11}	30.520	30.640	30.580	732.35	738.30	735.325	2,304.31	2,343.98	2,324.14
T_{12}	16.890	16.635	16.763	385.95	388.55	387.250	937.54	923.31	930.42
C.D.	1.087	2.080	1.844	28.752	47.312	14.815	93.166	191.103	160.38
SE(m)	0.349	0.668	0.597	9.229	15.186	4.800	29.905	61.341	51.95
CV (%)	2.05	3.81	3.46	2.16	3.56	1.12	2.49	4.96	4.26

Table 4. Effect of *T. viride T. harzianum* and *Talaromyces* sp. on growth of *Colletotrichum capsici* isolates in dual culture

Fungal isolate	Growth inhibition (%)								
	T. viride			*T. harzianum*			*Talaromyces* sp.		
	3 days	7 days	Mean	3 days	7 days	Mean	3 days	7 days	Mean
CC-DSST-11	37.57 (37.61)*	55.23 (47.99)	46.40 (42.80)	34.37 (35.69)	39.54 (38.87)	36.96 (37.28)	22.68 (28.42)	34.16 (35.74)	28.42 (32.08)
CC-DSST-13	45.97 (42.67)	70.47 (57.06)	58.22 (49.86)	33.93 (35.58)	61.64 (51.71)	47.78 (43.65)	16.58 (24.01)	32.91 (34.98)	24.74 (29.50)
CC-DSST-04	25.00 (29.76)	49.43 (44.65)	37.21 (37.20)	19.00 (25.76)	48.32 (44.01)	33.66 (34.89)	23.72 (28.74)	37.55 (37.77)	30.63 (33.26)
CC-DSST-05	25.58 (30.29)	45.56 (42.43)	35.57 (36.36)	31.10 (32.59)	59.21 (50.29)	45.16 (41.44)	18.87 (25.70)	36.14 (36.92)	27.51 (31.31)
Mean	33.53 (35.08)	55.17 (48.03)		29.60 (32.41)	52.18 (46.22)		20.46 (26.72)	35.19 (36.35)	
Factors	C.D.	SE(d)	SE(m)	C.D.	SE(d)	SE(m)	C.D.	SE(d)	SE(m)
Factor (A)	12.482	5.330	3.769	N/A	8.460	5.982	N/A	3.474	2.456
Factor (B)	8.826	3.769	2.665	14.009	5.982	4.230	5.752	2.456	1.737
Factor (A × B)	N/A	7.538	5.330	N/A	11.965	8.460	N/A	4.913	3.474

*Angular transformed values

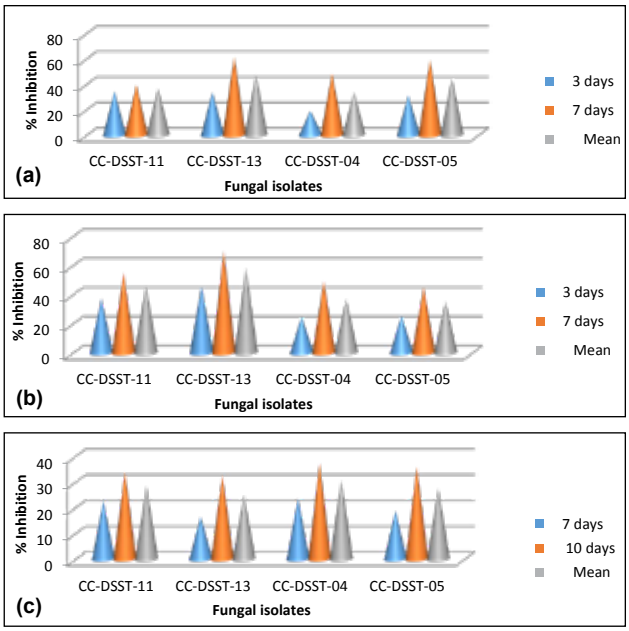

Fig. 3. Effect of *T. viride* (a), *T. harzianum* (b) and *Talaromyces* sp. (c) on growth of *Colletotrichum capsici* isolates.

and mutual inhibition (Malathi, 9). *Trichoderma sp*. have been considered as good model of biological control because of its ubiquitous nature, easy to isolate, rapid growth on many substrates affects wild range of plant pathogens acts as mycoparasite competes well for food and site, has enzyme system capable of attacking many plant pathogens and easy in application (Machenahalli *et al*., 8). The mechanisms involved have been attributed to be mycoparasitim, antibiosis, competition for nutrients and space along with its ability to induce systemic resistance in the plants against the pathogens (Hermosa *et al*., 5). This property has been further attributed due to the secretion of extracellular enzymes, including glucanases, chitinases etc., that degrade the pathogenic mycelia there by restricting its growth and further colonization in the host tissue (Singh *et al*., 14).

In the field trials, there was a significant variation in fruit rot severity and seed yield among all the the biological treatments and the control plants of Pusa Jwala (Susceptible variety) (Table: 5 and Fig. 5). The minimum disease severity percentage and highest seed yield q ha^{-1} were recorded with the treatments, T_{11} (Seed treatment, Seedling dip and one spray of *T. viride*) and followed by T_{10} (Seed treatment, Seedling dip and one spray of *T. harzianum*) and the maximum disease severity percentage and the lowest seed yield were recorded with the treatments, T_1 (Seed treatment with *Talaromyces* @10gm kg^{-1}) and followed by T_2 (Seed treatment with *T. harzianum* @10gm kg^{-1}) and T_3 (Seed treatment with *T. viride* @10gm kg^{-1}). All the treatments were

Table 5. Effect of biological treatments of biological treatments on Seed yield and fruit rot severity of Pusa Jwala during 2019 and 2020.

	Seed yield (q ha^{-1})			Fruit rot severity (%)		
Treatments	2019	2020	Pooled	2019	2020	Pooled
RV	2.550	2.575	2.563			
T_1	1.430	1.335	1.383	33.50(35.35)*	32.00(34.43)*	32.75(34.89)*
T_2	1.505	1.460	1.483	33.00(35.04)	30.50(33.50)	31.75(34.27)
T_3	1.575	1.575	1.575	32.00(34.43)	33.00(35.03)	32.50(34.74)
T_4	1.695	1.645	1.670	31.00(33.82)	32.00(34.42)	31.50(34.12)
T_5	1.820	1.715	1.768	29.00(32.56)	28.00(31.93)	28.50(32.25)
T_6	1.890	1.835	1.863	26.50(30.96)	28.50(32.23)	27.50(31.61)
T_7	1.975	1.965	1.970	23.50(28.97)	26.00(30.62)	24.75(29.81)
T_8	2.100	2.055	2.078	20.00(26.54)	21.00(27.25)	20.50(26.90)
T_9	2.185	2.145	2.165	18.50(25.46)	19.50(26.19)	19.00(25.83)
T_{10}	2.260	2.240	2.250	17.00(24.33)	18.00(25.06)	17.50(24.71)
T_{11}	2.345	2.295	2.320	15.50(23.17)	13.50(21.54)	14.50(22.36)
T_{12}	1.300	1.265	1.283	50.50(45.26)	48.50(44.12)	49.50(44.69)
C.D.	0.145	0.075	0.055	2.714	4.723	2.717
SE(m)	0.047	0.024	0.018	0.862	1.499	0.862
CV	4.43	5.69	1.330	4.057	2.92	4.432

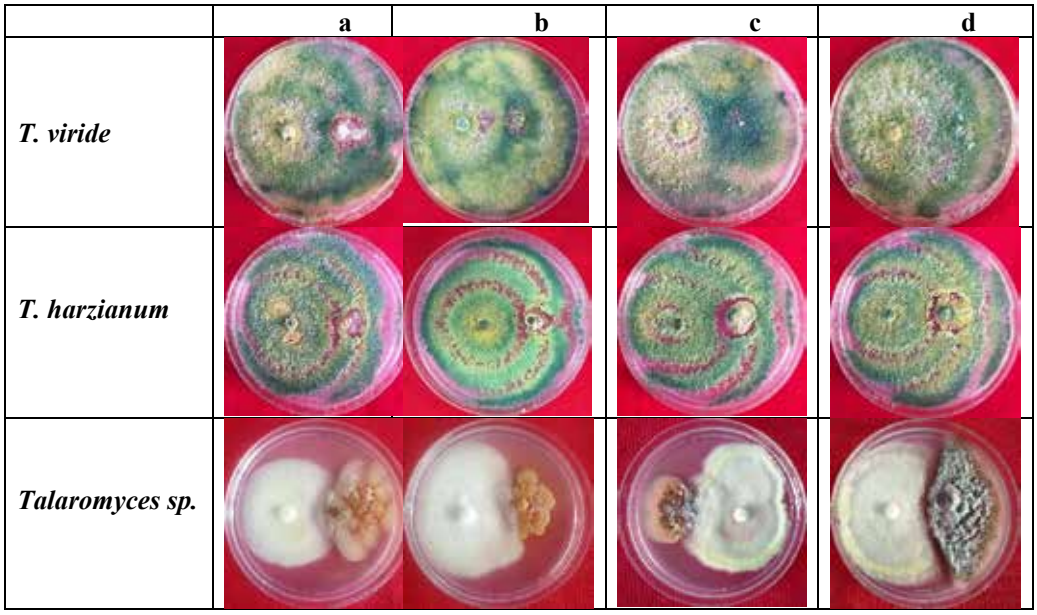

Fig. 4. Bioefficacy of *T. viride*, *T. harzianum* and *Talaromyces* sp. against *Colletotrichum capsici* isolates (a- CC-DSST-05, b- CC-DSST-11, c- CC-DSST-13, d- CC-DSST-04)

Fig. 5: Effect of biological treatments on fruit rot on Pusa Jwala

found significantly superior to the control in reducing the disease severity of fruit rot and in producing seed yield in both the years. Seed treatment with *T. harzianum* recorded least damping off incidence followed by *T. viride* compared to untreated control in chilli (Deshmukh *et al*., 4). Choudhary *et al*. (3) reported that seed treatment with *T. viride* effectively managed seedling rot caused by *C. capsici*. The major advantages of biological control are, they are natural, low cost, and eco-friendly, which is the better option in the present day climate, change scenario.

AUTHORS' CONTRIBUTION

Conceptualization of research (AK, JA, GPM); Designing of the experiments (AK, JA, SKL BG); Contribution of experimental materials (AK SKT); Execution of field/lab experiments and data collection (BG AK SJ); Analysis of data and interpretation (BG SJ RK); Preparation of the manuscript (BG, AK, JA).

DECLARATION

The authors declare that there is no conflict of interest.

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