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Response of seed bio-priming on chilli (*Capsicum annum* L.)

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Abstract
This study was conducted to determine the effect of bio-priming with *Trichoderma viride* and *Pseudomonas fluorescens* on qualitative parameters of chilli. Culture broth of *Trichoderma viride* strain Tv-4 and *Pseudomonas fluorescens* strain Psf- 2 were utilized for the purpose of pre-sowing bio-priming of seeds of five chilli genotypes *viz.*, Ac- 574, Ac- 615, Bcc-62, Hyb(3)2 and Pant C1 in consecutive two years (2012 and 2013). Bio-priming was significantly improved by the capability of these strains and the highest amount germination percentage at first count 77.25% with, Germination percentage at final count (97.25%), seedling length (12.85 cm), root length (9.25cm) and seedling vigour index (1175.00) reached, in comparison with the control. Therefore, we reported that application of *Trichoderma viride* and *Pseudomonas fluorescens* are the effective biological agents on chilli seed germination and their seedling parameters.

Keywords: Bio-priming, *Pseudomonas fluorescens*, *Trichoderma viride*, seedling growth, chilli

Introduction
Chilli is key vegetable crop among Solanaceaeous vegetables which cultivated in sub-tropic and tropics areas where both ripe and unripe fruits are used for different purpose (Ravi Hunje, B. S. *et al*., 2007) [4]. Imbalance use of fertilizers, improper plant protection, poor growth and sub-optimum plant population are the most important factors by which yield reduce. Sub-optimum plant population generally results from poor and erratic germination. In current years, a lot of studies have been done on invigoration of seeds to improve the germination rate and uniformity of growth and reduce the emergence time of many vegetables and some field crops. Seed priming is now a widely used commercial process that accelerates the germination rate and improves seedling uniformity in many crops (Basra et al., 2003) [2]. Seed priming as one of the most important developments to help rapid and uniform germination and emergence of seeds and to increase seed tolerance to adverse environmental conditions Harris *et al*. (1999) [7]. Among the all seed priming techniques, bio-priming using microbial mixture has been practiced for creating priming eco-friendly. Bio-priming is a process of biological seed treatment that refers to combination of seed hydration (physiological aspect of disease control) and inoculation (biological aspect of disease control) of seed with beneficial organism(s) to protect seed. It is an ecological approach using either bacteria or selected fungal antagonists against the soil and seed-borne pathogens. Biological seed treatments may provide an alternative to chemical control of crop diseases. Some bacteria and fungi prevent diseases and enhance plant growth, in which *Pseudomonas fluorescens and Trichoderma viride* are most popular.

Trichoderma viride is a fungus used as bio-agent. It is used for seed and soil treatment for suppression of various diseases caused by fungal pathogens. The increased growth response induced by *Trichoderma* sp. has been reported for many crops such as beans (*Phaseolus vulgaris*) cucumber (*Cucumis sativus*), pepper (*Capsicum annum*), carnation (*Dianthus carophyllus*), maize (*Zea mays*), and wheat (*Tritichum aestivum*) (Lo and Lin, 2002). *Trichoderma* produce phytohormones, such as indol acetic acid (IAA) and ethylene, whose metabolic pathways have been, identified (Osiewacz, 2002) [14] which produce cytokinin-like molecules, e.g. zeatyn and gibberellin GA_3 or GA_3-related have been recently detected (M. Ahsanur Rahman *et al.,* 2012) [9]. The controlled production of these compounds could improve bio-fertilization (Osiewacz, 2002) [14]. *Pseudomonas fluorescens* is an another bio-agent used widely to control diseases and also known as plant growth rizobacteria due to production of plant growth-regulating substances (PGRs), phytohormones, mineralization of organic phosphorus, production of phytoalexins / flavonoids-like compounds, enhancement of mineral uptake, etc. (Mukerji *et al*. 2006) [12]. Increased dry weight and plant height were recorded with *Pseudomonas* sp. MML2212 and *Pseudomonas fluorescens* on rice and green

gram when compared with the control (Mathivanan *et al*., 2005, Shanmugaiah *et al*., 2005, 2007) [15, 16].

Hence, research on response of different chilli genotypes towards bio-priming with *Pseudomonas fluorescens* and *Trichoderma viride* in respect to growth, yield, and quality attributes will have immense use to the farmers and seed industry.

Materials and methods

Seed treatment: Seeds of five genotypes of chilli, viz., V_1-Ac- 574, V_2-Ac- 615, V_3-Bcc-62, V_4-Hyb(3)2 and V_5-Pant C1 were bio-primed with *T. viride* (5×10^7) and *P. fluorescens* (9×10^6) individually for two hours and the seeds soaked in distilled water for the same duration were used as control. After the treatment, seeds were shade dried for 30 minutes under Laminar Air Flow, and then those seeds were utilized for laboratory experiments. Hence, treatments carried out for this experiment were as:- T_0- Untreated control, T_1- Treated with *T. viride*, T_2-. Treated with *P. fluorescence*

Laboratory experiments

For *in vitro* experiment, 100 seeds with four replications for individual treatments and genotypes were placed in petriplates lines with two layers of Whatman filter paper soaked in sterilized distilled water and incubated at 25 ± 1^0C. Germination percentage was recorded at both first count (7DAS) and final count (14DAS) as per ISTA. Paper plate method used to measure root and shoot length of seedling and they were recorded after the 14DAS (Final count) by random selection of ten seedlings from each replication

Results and Discussion

Seeds produced after pre-sowing bio-priming of seeds were subjected to laboratory testing for its quality assessment through germination, root length, shoot length, seedling length and vigour index.

Germination at first count

Significantly highest germination percentage at first count was recorded by V_5 followed by V_2, V_1 and V_4 where as the lowest was recorded by genotype V_3 in both the year of experiments. Average influences of both the bio-inoculants as well as its influence on performance of individual varieties were also found to be significant in all the situations for expression of this character over the control. Both the bio-inoculants exerted significant influence for production of higher germination at first count over control in all the situations, of which influence of *Trichoderma viride* and *Pseudomonas fluorescence* was insignificant with each other in first year whereas significant differences recorded in second year i.e. influence of *Trichoderma viride* was superior then *Pseudomonas fluorescence*. Highest enhancement was recorded in V_5 with *Pseudomonas fluorescence* and *Trichoderma viride* respectively in the years of experimentation where as slightly enhancement was recorded in second year over the first year.

Germination percentage at final count

It could be revealed from data that first position was occupied by V_1 for higher germination percentage at final count in all the situations followed by V_5, V_2, V_4 whereas lowest was recorded by genotype V_3 in both the years of experimentation. Average influence of *Pseudomonas fluorescens* was found to be greater on this character in comparison to that of *Trichoderma viride in* both the years. Response of individual varieties towards pre-sowing bio-priming of seed germination at final count varied: influence of both the bio-inoculants was same for V_1, V_3 and V_4 all situations excepting V_2 and V_5 in first year where as V_5 only for second year. Influence of *Pseudomonas fluorescence* was more for genotype V_1 and V_5 whereas in V_1 germination percentage was also increased by *Trichoderma viride* in all condition (Hanson, 2000; Mishra and Sinha, 2000, Mukhtar I 2008) [6, 11, 12].

Average Shoot Length

Significantly longest seedlings were produced by genotypes V_2 and V_5 in both the years, when overall performance of the individual varieties were considered followed by genotypes V_1, V_3 and V_4 respectively in all the conditions while V_1 and V_3 both were statistically insignificant with each other in first year only. Average influence of both *Trichoderma viride* and *Pseudomonas fluorescens* were found to be statistically insignificant with each other and positive over unprimed control over the years, while greater influence of *Trichoderma viride* could be noted in noticed in both the years of experimentation. The trend in response of individual varieties varied: V_1, V_3, V_4 and V_5 responded in similar way towards priming with both *Trichoderma viride* and *Pseudomonas fluorescens* separately in all the situations, excepting V_2 for first year.

Average Root Length

Average root length at final count was recorded as maximum as 8.74 cm and 8.60 cm, followed by V_4, V_2, and V_5 in both the years, when average was made over treatments, though V_1 and V_4 performed statistically at par for this character. Significantly shortest root length was recorded for V_3 in both the years, may be due to its unique genetic expression. Significant positive influence of *Trichoderma viride* bio-inoculant over control as well as *Pseudomonas fluorescence*, was noted for expression for this character whereas *Pseudomonas fluorescence* statistically insignificant with control in the years of experimentation. Though interaction effects were insignificant in both the years, maximum root length was produced by V_1 in both the years when bio-priming was made with *Trichoderma viride*. Similar result found by Lo, C.T. and Lin, C.Y. (2002) regarding screened *Trichoderma* strains root growth of bitter gourd, loofah and cucumber.

Average Seedling Length

Significantly longest seedlings were produced by V_2 and V_1 in both the years, when overall performances of the individual varieties were considered followed by V_4, V_5 and V_3 respectively. Statistically similar performance was noted for V_1 and in all the conditions. V_3 produced seedlings of the smallest type irrespective of the years of experiment. Average influence of both *Trichoderma viride* and *Pseudomonas fluorescens* were found to be significant positive over unprimed control over the years, while greater influence of *Trichoderma viride* could be noted in both years (Dubey et al., 2007). Similar trend was recorded for all the individual varieties and it was of higher magnitude after bio-priming of seeds with *Trichoderma viride*, which may also be assessed through table on change over control due to priming.

Table 1: Effect of seed bio-priming on seed quality of chilli genotypes

	Germination %		Germination % at 1st count		Shoot length(cm)		Root length (cm)		Seedling length(cm)		V.I.	
V	**1st yr**	**2nd yr**	**1st yr**	**2nd yr**	**1st yr**	**2nd yr**	**1st yr**	**2nd yr**	**1st yr**	**2nd yr**	**1st yr**	**2ndyr**
V1	95.917(79.47)	95.417(78.95)	72.083(58.45)	73.583 (59.41)	2.95	3.07	8.74	8.60	11.69	11.66	1121.00	1112.00
V2	84.833(67.57)	84.583(67.38)	74.5 (60.02)	75.167 (60.46)	3.82	4.08	8.37	8.24	12.20	12.32	1034.00	1042.00
V3	69.667(56.91)	67.083(55.34)	65.083(54.09)	67.083 (55.31)	2.90	2.97	6.45	6.44	9.34	9.41	650.41	628.99
V4	75.333(60.59)	74.417(60.00)	68.25 (56.04)	70.333 (57.33)	2.59	2.63	8.70	8.62	11.28	11.24	850.99	837.69
V5	92.25 (74.85)	91.083(73.83)	75.667(60.82)	77.333 (61.94)	3.55	3.63	7.27	7.11	10.82	10.74	999.16	978.99
SEM (±)	0.179	0.272	0.143	0.102	0.044	0.045	0.108	0.161	0.049	0.066	11.966	15.230
CD AT 5%	0.509	0.776	0.406	0.292	0.124	0.127	0.309	0.459	0.140	0.187	34.084	43.380
T												
T0	81.25 (65.64)	80.15 (64.92)	69.75 (57.01)	71.35 (58.02)	3.05	3.11	7.63	7.50	10.67	10.61	871.57	852.58
T1	84.1 (68.35)	82.95 (67.48)	71.8 (58.31)	73.65 (59.49)	3.27	3.36	8.19	8.12	11.46	11.48	970.73	960.37
T2	85.45 (69.65)	84.45 (68.91)	71.8 (58.33)	73.1 (59.15)	3.17	3.35	7.89	7.78	11.06	11.13	951.38	947.06
SEM (±)	0.107	0.163	0.086	0.061	0.056	0.058	0.140	0.208	0.029	0.039	15.448	19.662
CD AT 5%	0.305	0.466	0.244	0.175	0.160	0.165	0.399	0.592	0.084	0.112	44.002	56.00
V X T												
T0 X V1	94.00 (76.69)	93.25(76.20)	70.75(57.60)	72.25(58.54)	2.79	2.86	8.31	8.14	11.10	11.00	1044.00	1023.00
T0 X V2	82.75(65.91)	83.50(66.51)	73.50(59.36)	74.00(59.69)	3.92	3.96	8.08	7.90	12.00	11.85	991.20	987.88
T0 X V3	68.25(56.02)	65.50(54.40)	64.50(53.74)	65.75(54.50)	2.80	2.87	6.30	6.44	9.10	9.31	619.24	602.01
T0 X V4	73.00(59.03)	72.50(58.76)	66.00(54.65)	68.25(56.02)	2.31	2.39	8.47	8.31	10.78	10.70	787.94	776.10
T0 X V5	88.25(70.52)	86.00(68.75)	74.00(59.71)	76.50(61.36)	3.41	3.46	6.98	6.72	10.39	10.18	915.86	873.51
T1 X V1	96.50(80.21)	96.00(79.51)	73.50(59.36)	75.00(60.33)	2.94	3.16	9.25	9.14	12.19	12.30	1175.00	1180.00
T1 X V2	85.00(67.67)	84.25(67.11)	74.25(59.84)	75.50(60.68)	4.21	4.27	8.64	8.56	12.85	12.83	1093.00	1081.00
T1 X V3	69.75(56.96)	67.25(55.43)	65.00(54.04)	68.00(55.86)	2.94	3.01	6.62	6.57	9.55	9.58	666.30	644.21
T1 X V4	75.75(6086)	74.50(60.07)	70.50(57.45)	72.25(58.54)	2.70	2.69	8.93	8.85	11.63	11.54	880.64	859.57
T1 X V5	93.50(76.04)	92.75(75.27)	75.75(60.85)	77.50(62.04)	3.57	3.69	7.54	7.48	11.10	11.17	1039.00	1037.00
T2 X V1	97.25(81.51)	97.00(81.15)	72.00(58.39)	73.50(59.35)	3.12	3.18	8.66	8.51	11.78	11.69	1145.00	1134.00
T2 X V2	86.75(69.13)	86.00(68.53)	75.75(60.85)	76.00(61.02)	3.34	4.01	8.40	8.26	11.74	12.27	1019.00	1057.00
T2 X V3	71.00(57.74)	68.50(56.18)	65.75(54.49)	67.50(55.55)	2.96	3.03	6.42	6.32	9.38	9.35	665.70	640.75
T2 X V4	77.25(61.88)	76.25(61.23)	68.25(56.02)	70.50(57.42)	2.75	2.81	8.70	8.69	11.45	11.49	884.38	877.40
T2 X V5	95.00(77.99)	94.50(77.48)	77.25(61.91)	78.00(62.42)	3.69	3.74	7.29	7.11	10.98	10.85	1043.00	1027.00
SEM (±)	0.536	0.817	0.428	0.307	0.098	0.100	0.242	0.360	0.148	0.197	26.765	34.056
CD AT 5%	1.527	2.328	1.219	0.875	0.278	0.285	0.690	1.026	0.420	0.561	76.214	97.001

V_1- Ac- 574, V_2- Ac- 615, V_3- Bcc-62, V_4- Hyb(3)2 and V_6- Pant C_1, T0- Control, T1- *Trichoderma viride*, T2- *Pseudomonas Fluo rescens.*

Vigour Index

Critical analysis on average performance of individual varieties for this derived seedling parameter may lead to identify V1 and V2 as the superior most varieties for expression of this important character, followed by V5 and V4 in both the years. Unlike seedling length, significantly lowest magnitude of vigour index was derived for V3 may be due to inherited potential of its seedling length as well as greater influence of its seed germination potential. Significant influence of bio-priming for enhancement in vigour index over control could be noticed in both the years (Asaduzzaman, M., *et al.*, 2010, Mishra, D.S., and Sinha, A.P. 2000) [1, 11]. Superior influence of *Trichoderma viride* for determination of average vigour index was observed over *Pseudomonas fluorescens* in all condition, while it was statistically at par. Response of individual varieties towards bio-priming for production of seeds with enhanced vigour over control followed the similar trend: significantly similar influence of both the bio-inoculants on individual varieties was recorded in all the situations, exception was noted for V4 and V5 in first year, and only V5 in second year, for which positive superiority in influence of *Pseudomonas fluorescens* could be noticed over that of *Trichoderma viride.*

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