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In vitro screening and efficacy of plant growth promoting rhizobacteria and biocontrol agents in bell pepper (*Capsicum annuum* L.)

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Abstract
An investigation was carried out during (2015-16) at laboratory and experimental farm of Department of Seed Science and Technology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan-273230 (H. P.). *In vitro* screening of plant growth promoting rhizobacteria (PGPR) and biocontrol agents was done with three bacterial (Kt_6, Kl_5 and A_3) and three fungal (*Trichoderma viride*, *T. harzianum* and *T. hamatum*) strains. Among all, one best from both bacterial and fungal strains were evaluated for their effect on seed germination and seedling growth of bell pepper under field conditions. The studies revealed that all the seed quality parameters viz. speed of germination (49.60), germination percentage (9.79), seedling length (13.58), seedling dry weight (2.66), seedling vigour index-length (12.86.66) and seedling vigour index-mass (252.14) were found maximum with treatment T_3 (PGPR-3) under in vitro conditions. However under nursery conditions, speed of germination (38.23), germination percentage (9.26), seedling length (10.77), seedling dry weight (3.91), seedling vigour index-length (912.17) and seedling vigour index-mass (331.32) were recorded maximum with treatment T_5 PGPR (seed treatment) + *Trichoderma* (soil application).

Keywords: bell pepper, PGPR, germination and *Trichoderma*

Introduction
Bell pepper (*Capsicum annuum* L.) commonly known as sweet pepper, capsicum, green pepper or Shimla mirch, belongs to family solanaceae. It has attained a status of high value vegetable crop in India in recent years because of its delicacy and pleasant flavour coupled with rich content of ascorbic acid, other vitamins and minerals (Sreedhara *et al*., 2013) [1]. In India, bell pepper is cultivated in an area of 30000 ha with a production of 171000 MT (NHB, 2015) [2]. In Himachal Pradesh, it is an important summer and rainy season crop of mid hills which covers an area of 2070 ha and having production of 34130 MT (NHB, 2014) [3] and has about 50% share in the country's area and production. In order to meet the growing demand of burgeoning population, large amounts of insecticides, pesticides and fertilizers are being applied to the fields to achieve higher production leading to deleterious environmental effects.
Plant growth promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion and disease suppression. The PGPR have been demonstrated to increase growth and productivity of many commercial crops (Saharan and Nehra, 2011) [4]. In addition, *Trichoderma* species are well-organized biocontrol agents that are used to prevent development of several soil pathogenic fungi. Different mechanisms have been suggested as being responsible for their biocontrol activity, which include competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism and production of inhibitory compounds. The antagonistic fungus like *Trichoderma harzianum* has shown promise as a biocontrol agent of *Rhizoctonia solani* in chilli (Bunker and Mathur, 2001) [5].

Materials and Methods
The experiment was conducted at laboratory of Department of Seed Science and Technology, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan Himachal Pradesh (India) during 2016 in capsicum variety Solan Bharpur. *In vitro* screening of plant growth promoting rhizobacteria (PGPR) and biocontrol agents was done as seed treatment by using roll paper towel method in the seed germinator at 25^{O}C as per ISTA (Anonymous, 1985). The treatments were T_1 (PGPR-1), T_2 (PGPR-2), T_3 (PGPR-3), T_4 (*Trichoderma*-1), T_5 (*Trichoderma*-2), T_6 (*Trichoderma*-3), T_7 (Untreated Control), T_8 (Hot water treatment + PGPR-1), T_9 (Hot water treatment + PGPR-2), T_{10} (Hot water treatment + PGPR 3), T_{11} (Hot water treatment + *Trichoderma*-1), T_{12} (Hot water treatment + *Trichoderma*-2), T_{13}

(Hot water treatment + *Trichoderma*-3) and T_{14} (Hot water treatment alone). The first and final counts were taken after 7 and 14 days, respectively. One best PGPR and one *Trichoderma* strains were further evaluated as seed treatment and soil application under nursery conditions. The treatment combinations were T_1 PGPR (seed treatment), T_2 *Trichoderma* (seed treatment), T_3 PGPR (soil application), T_4 *Trichoderma* (soil application), T_5 PGPR (seed treatment) + *Trichoderma* (soil application), T_6 PGPR (soil application) + *Trichoderma* (seed treatment), T_7 PGPR (seed treatment) + PGPR (soil application), T_8 *Trichoderma* (seed treatment) + *Trichoderma* (soil application) and T_9 Untreated Control. Seed quality parameters studied were germination percentage (%), speed of germination (days), seedling height (cm), seedling vigour index-length (SVI-L) and seedling vigour index-mass (SVI-M). Speed of germination was worked out (Maguire, 1962) [22] by counting the number of seeds that geminated on daily basis up to the day of final count, shoot length, root length, seedling dry weight and vigour index was calculated as per the formula suggested (Abdul-Baki and Anderson, 1973) [23]. The data of the *in vitro* experiment were statistically analyzed by adopting completely randomized design and of nursery experiment by using randomized block design as per Gomez and Gomez (1984).

Results and Discussion

Data recorded under *in vitro* experiment presented in Table 1 revealed that maximum speed of germination was recorded with treatment T_3 (49.60 days) which was statistically at par with T_2 (48.15 days), T_1 (46.93 days) and T_4 (37.02 days) followed by T_5 (33.75 days). Minimum speed of germination was recorded with T_{14} (16.42 days). Maximum germination percentage was recorded with treatment T_3 (94.75%) which was statistically at par with T_2 (92.25%), T_1 (91.00%) followed by T_6 (91.25%) and minimum was recorded with treatment T_{14} (84.25%). Maximum seedling length was recorded with treatment T_3 (13.58 cm) followed by T_2 (12.35 cm) and T_6 (12.19 cm) whereas minimum was found with treatment T_{14} (7.25cm). The maximum seedling dry weight was recorded with treatment T_3 (2.66 mg) which was statistically at par with T_2 (2.52 mg), T_6 (2.39 mg), T_5 (2.30 mg), T_4 (2.23 mg) and T_{10} (2.17 mg) whereas minimum was found with treatment T_{14} (1.71 mg). The maximum SVI-L was found in treatment T_3 (1286.66) followed by T_2 (1139.21) and T_6 (1112.51). Minimum SVI-L was recorded with treatment T_{14} (610.81). Maximum SVI-M was recorded with treatment T_3 (252.14) which was statistically at par with T_2 (232.00), T_6 (218.32) and T_5 (208.97) Minimum SVI-L was with treatment T_{14} (147.99). This present work revealed that under in vitro conditions, seed treatment with PGPR strains improved speed of germination, seed germination, seedling vigour, seedling emergence and seedling stand over the control. Similar improvement of seed germination parameters by rhizobacteria has been reported in other cereals such as sorghum (Raju *et al*, 1999) [6] and pearl millet (Niranjan *et al*, 2004) [7] These findings may be due to the increased synthesis of hormones like gibberellins, which would have triggered the activity of specific enzymes that promoted early germination. Beside, significant increase in seedling vigour would have occurred by better synthesis of auxins. (Bharathi *et al*, 2004) [15].

The data recorded under nursery experiment presented in Table 2 showed that maximum speed of germination was recorded with treatment T_5 (38.23 days) which was statistically at par with T_6 (38.16 days) and T_7 (36.00 days) followed by T_4 (26.73 days). Minimum speed of germination was recorded with T_9 (15.68 days). Maximum germination percentage was recorded with treatment T_3 (74.33%) which was statistically at par with T_2 (77.00%), T_1 (76.33%) followed by T_6 (82.33%) and minimum was recorded with treatment T_9 (71.00%). Improvement of speed of germination and seed germination parameters by rhizobacteria has been reported in other plants such as pearl millet (Niranjan *et al*., 2003, 2004) [9, 7], maize (Egamberdiyeva, 2007) [10] sugar beet (Cakmakc *et al*., 2006) [11] and wheat and sunflower (Salanture *et al*., 2006; Shaukat *et al*., 2006) [12, 13], where it was found that some PGPR induced increases in seed emergence, in some cases achieving increases up to 100% greater than controls (Nezarat and Gholami, 2009) [14]. These findings may be due to the increased synthesis of hormones like gibberellins, which would have triggered the activity of specific enzymes that promoted early germination, such as a-amylase, which have brought an increase in availability of starch assimilation. Beside, significant increase in seedling vigor would have occurred by better synthesis of auxins (Bharathi *et al*., 2004) [8].

Maximum seedling length was recorded with treatment T_5 (10.77 cm) followed by T_6 (10.15 cm) and T_7 (9.91 cm) whereas minimum was found with treatment T_9 (8.09 cm). The maximum seedling dry weight was recorded with treatment T_5 (3.91 mg) which was statistically at par with T_6 (3.82 mg) followed by T_7 (3.73 mg) whereas minimum was found with treatment T_9 (2.67 mg). Increases of in root length of *Piper nigra* plants was noticed due to inoculation with PGPR over control and are comparable with results of Vikram (2007). Similarly, promotion in growth parameters of various crop plants in response to inoculation with PGPR were reported by other workers (Kozdroja *et al*., 2004; Shaharoona *et al*., 2006; Gravel *et al*., 2007) [17-19]. In a study by Akbari *et al*. (2007) [20], the roots of wheat seedling responded positively to the several bacteria inoculations by an increase in root length, dry weight.

The maximum SVI-L was found in treatment T_5 (912.17) followed by T_6 (835.39) and T_7 (789.76). Minimum SVI-L was recorded with treatment T_9 (574.24). Maximum SVI-M was recorded with treatment T_5 (331.32) followed by T_6 (314.80) and T_7 (297.17). Minimum SVI-L was recorded with treatment T_9 (189.84). It has also been shown that inoculation of plants with PGPR could resulted in significant changes in various growth parameters, such as increase in plant biomass, nutrient uptake, tissue N content root length of cereals (Bashan *et al*., 2004).

Table 1: Effect of PGPR and bio control agents on seed quality parameters under *in vitro* conditions

Treatment	Characters					
	Speed of germination (days)	Germination percentage (%)	Seedling length (cm)	Seedling dry weight (mg)	Seedling Vigour Index – Length (SVI-L)	Seedling Vigour Index – Mass (SVI-M)
T_1	46.93	91.00 (9.59)	11.70	2.20	1064.39	200.34
T_2	48.15	92.25 (9.66)	12.35	2.52	1139.21	232.00
T_3	49.60	94.75 (9.79)	13.58	2.66	1286.66	252.14
T_4	37.02	90.25 (9.55)	11.71	2.23	1057.23	201.25

T_5	33.75	90.75 (9.58)	11.01	2.30	999.31	208.97
T_6	23.87	91.25 (9.60)	12.19	2.39	1112.51	218.32
T_7	28.39	85.25 (9.29)	8.94	1.81	762.06	154.31
T_8	30.72	87.25 (9.39)	9.17	1.92	799.77	167.48
T_9	28.47	88.00 (9.43)	9.42	1.95	829.65	171.61
T_{10}	20.46	89.75 (9.53)	10.36	2.17	930.52	194.57
T_{11}	26.01	86.25 (9.34)	9.52	1.71	820.48	148.12
T_{12}	29.15	86.00 (9.33)	9.58	1.86	823.94	160.05
T_{13}	28.02	89.00 (9.49)	9.88	2.00	879.79	177.75
T_{14}	16.42	84.25 (9.23)	7.25	1.76	610.81	147.99
C.D. (0.05)	12.83	0.13	0.90	0.49	86.48	43.26

Table 2: Effect of PGPR and bio control agents on seed quality parameters under nursery conditions

Treatment	Characters					
	Speed of germination (days)	**Germination percentage (%)**	**Seedling length (cm)**	**Seedling dry weight (mg)**	**Seedling Vigour Index – Length (SVI-L)**	**Seedling Vigour Index – Mass (SVI-M)**
T_1	23.26	76.33 (8.79)	9.20	3.47	702.25	265.09
T_2	24.37	77.00 (8.83)	8.15	3.14	627.47	241.52
T_3	21.99	74.33 (8.68)	8.14	2.90	604.88	215.55
T_4	26.73	78.33 (8.91)	8.87	3.33	694.57	260.55
T_5	38.23	84.67 (9.26)	10.77	3.91	912.17	331.32
T_6	38.16	82.33 (9.13)	10.15	3.82	835.39	314.80
T_7	36.00	79.67 (8.98)	9.91	3.73	789.76	297.17
T_8	23.39	78.67 (8.93)	9.22	3.58	725.05	281.91
T_9	15.68	71.00 (8.49)	8.09	2.67	574.24	189.84
C.D. (0.05)	11.05	0.10	0.41	0.14	34.37	11.37

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