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Sujata Kumari
Department of Seed Science and Technology, SAS, BFIT Group of Institution Dehradun, Uttarakhand, India

Narendra K Bharat
Department of Seed Science and Technology, COH, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

DP Singh
Department of Seed Science and Technology, SAS, BFIT Group of Institution Dehradun, Uttarakhand, India

Correspondence
Sujata Kumari
Department of Seed Science and Technology, SAS, BFIT Group of Institution Dehradun, Uttarakhand, India

Effect of PGPR and BCA on seed quality parameters in bell pepper (*Capsicum annuum* L.)

Sujata Kumari, Narendra K Bharat and DP Singh

Abstract
An investigation was carried out during two consecutive years (2015-16 and 2016-17) at Experimental Farm of Department of Seed Science and Technology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan-273230 (H. P.). The experiment was conducted on effect of plant growth promoting rhizobacteria and biocontrol agent on seed quality parameters of bell pepper. There were nine treatments including control and each treatment was replicated thrice. The data was analysed in factorial randomized block design. The study revealed that all the seed quality parameters like 1000 seed weight (6.63 g), per cent seed recovery (1.58), seed germination (93.26%), seedling length (12.39 cm), seedling dry weight (2.29 mg), seed vigour index- (SVI-L) (1156.03) and seed vigour index- (SVI-M) (213.69) were recorded maximum with treatment [PGPR (seed treatment) + *T. harzianum* (soil application)]. All parameters showed minimum in untreated control as compared to rest of the treatments. The study revealed that all the treatments of bioagents i.e. seed treatment, soil application and their combinations resulted in increase of all the parameters had significant impact on seed quality parameters of bell pepper. The effect of all the treatments was significantly higher during the trial conducted in 2015-16 as compared to the trial conducted in 2016-17.

Keywords: Seed quality parameters, PGPR, BCA, *Capsicum annuum*

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) [8]. Bell pepper contains large amount of vitamin A, C, pungency, colour and is also famous for its pleasant flavour and delicate taste (Nadeem *et al*., 2011) [9]. Pungent bell pepper is used in the formation of balms and carotenoids pigment (colour extract) is used as colour additives in food industry and in poultry and prawn feed industry (Holguin and Glick, 2003) [10].

In India, bell pepper is cultivated in an area of 30,000 ha with a production of 171000 MT (NHB, 2015) [12]. In Himachal Pradesh, it is an important summer and rainy season crop of mid hills and covers an area of 2070 ha with a production of 34,130 MT (NHB, 2014) [13]. In the state, it is cultivated mainly for fresh fruit and also for seed purpose in some areas.

In order to meet the growing demand of burgeoning population, large amounts of herbicides, pesticides and fertilizers are being applied to the fields every year to achieve maximum production. The use of chemicals in Indian agriculture has increased 170 times in last 50 years (FAO, 2010) [13]. This is now a major environmental and health concern because of the deleterious impact of these chemical compounds on terrestrial and aquatic ecosystems.

Plant growth promoting rhizobacteria (PGPRs) are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion and disease suppression. Various isolates of PGPR's have been demonstrated to increase growth and productivity of many crops including capsicum (Saharan and Nehra, 2011) [14]. Another group of beneficial microbes are fungal species *Trichoderma* which are well-known biological control agents (BCAs) and are now formulated and used extensively to prevent several soil borne plant diseases. These fungi not only suppress the plant pathogens but also show some plant growth promotion activities under stress conditions. These antagonistic fungi have shown promise as a bio control agent of many diseases of different plants including chilli (Bunker and Mathur, 2001) [15]. Different mechanisms have been suggested for growth promotion and bio control activity of these beneficial bio-agents, including increased nutrient uptake, competition for space and nutrients, secretion of chitinolytic enzymes, hyper parasitism, production of inhibitory substances, induction of defense related mechanisms etc.

Materials and methods

The present investigation was carried out in 2015-16 and 2016-17 at Pandah Research Farm of Department of Seed Science and Technology, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan, HP. The experiment was laid out in randomised block design (RBD) with nine treatments replicated thrice. The treatment were T_1: PGPR (seed treatment), T_2: *Trichoderma harzianum* (seed treatment), T_3: PGPR (soil application), T_4: *T. harzianum* (soil application), T_5: PGPR (seed treatment) + *T. harzianum* (soil application), T_6: PGPR (soil application) +*T. harzianum* (seed treatment), T_7: PGPR (seed treatment) + PGPR (soil application), T_8: *T. harzianum* (seed treatment) + *T. harzianum* (soil application) and T_9: Untreated control. PGPR and BCA were applied as per the recommendations. Data were statistically analysed as suggested by Cochran and Cox (1964) [4]. Seed quality parameters studied were 1000 seed weight (g), per cent seed recovery, seed germination (%), seedling length (cm), seedling dry weight (mg), seed vigour index-(SVI-L) and seed vigour index-(SVI-M). 100 seeds from all replications of each treatment were used for conducting the germination test as per ISTA (Anonymous, 1985). This was carried out by using paper roll method in the seed germinator at 25^{O}C. The first and final counts were taken after 7 and 10 days, respectively. Seed vigour indexes were calculated as per method of Abdul-Baki and Anderson, (1973) [1].

Results and discussion

It is evident from Table 1 that all the treatments of bio agents i.e. seed treatment, soil application and their combinations resulted in increase 1000 seed weight (g), per cent seed recovery, seed germination (%), seedling length (cm), seedling dry weight (mg), seed vigour index-I and seed vigour index-II as compared to untreated control. The effect of all the treatments was significantly higher during the trial conducted in 2015-16 as compared to the trial conducted in 2016-17. The 1000 seed weight, in general ranged between 4.88 to 6.63 g in different treatments as compared to 4.79 g in control. Amongst different treatments, maximum 1000 seed weight of 6.63 g was recorded in T_5 [PGPR (seed treatment) + *T. harzianum* (soil application)] followed by 6.10 in T_6 [PGPR (soil application) + *T. harzianum* (seed treatment)], 5.92 in T_7 [PGPR (seed treatment) + PGPR (soil application)] and 5.72 g inT_8 [*T. harzianum* (seed treatment) + *T. harzianum* (soil application)]. The 1000 seed weight in untreated control was observed minimum, which was statistically different from rest of the treatment. In the present study, higher 1000 seeds weight was recorded with [PGPR (seed treatment) + *T. harzianum* (soil application)] which might be due to increase in availability of nutrient to the plant which caused bold seed thereby resulting higher 1000 seed weight. The present investigation is the first attempt in this regard. Application of PGPR (seed treatment) and *T. harzianum* (soil application)] is also responsible for producing auxins and cytokinins like substances in the treated plants. The growth substances are having positive effect on processes like cell division and cell enlargement which are responsible for producing bolder seeds. The present results are in accordance with the findings of Yadegari and Rahmani (2010) [2] studied that effect of co-inoculation with plant growth-promoting rhizobacteria (PGPR) and *Rhizobium* on yield components in common bean and found that treatment with PGPR significantly increased 100 seed weight (g).

Table 1: Effect of seed treatment and soil application of PGPR and BCA on 1000 seed weight in bell pepper

Treatment	1000 seed weight (g)		
	2016	2017	Mean
T_1: PGPR (seed treatment)	5.79	4.39	5.09
T_2: *Trichoderma harzianum* (seed treatment)	5.91	4.51	5.21
T_3: PGPR (soil application)	5.58	4.18	4.88
T_4: *T. harzianum* (soil application)	6.21	4.81	5.51
T_5: PGPR (seed treatment) + *T. harzianum* (soil application)	7.33	5.93	6.63
T_6: PGPR (soil application) + *T. harzianum* (seed treatment)	6.80	5.40	6.10
T_7: PGPR (seed treatment) + PGPR (soil application)	6.62	5.22	5.92
T_8: *T. harzianum* (seed treatment) + *T. harzianum* (soil application)	6.42	5.02	5.72
T_9: Untreated control	4.49	3.09	3.79
Mean	6.13	4.73	5.43
C.D. (0.05)			
Year :	0.40		
Treatment :	0.92		
Year X Treatment :	NS		

The per cent seed recovery presented in Table 2, in general ranged between 1.38 to 1.58% in different treatments as compared to 1.21% in control. Amongst different treatments, maximum seed recovery of 1.58% was recorded in T_5 [PGPR (seed treatment) + *T. harzianum* (soil application)] followed by 1.50% in T_6 [PGPR (soil application) + *T. harzianum* (seed treatment)], 1.56% in T_7 [PGPR (seed treatment) + PGPR (soil application)] and 1.51% in T_8 [*T. harzianum* (seed treatment) + *T. harzianum* (soil application)]. The per cent seed recovery in untreated control was observed statistically lower from rest of the treatments. In the present study, per cent seed recovery was recorded with [PGPR (seed treatment) + *T. harzianum* (soil application)] which might be due to increase in availability of nutrient to the plant which caused bold seed thereby resulting higher per cent seed recovery. The present results are in accordance with the findings of Yadegari and Rahmani (2010) [2] who studied the effect of co-inoculation with plant growth-promoting rhizobacteria (PGPR) and *Rhizobium* on yield components in common bean and found that treatment with PGPR significantly increased per cent seed recovery.

Table 2: Effect of seed treatment and soil application of PGPR and BCA on per cent seed recovery in bell pepper (Seed crop)

Treatment	Per cent seed recovery		
	2016	2017	Mean
T_1: PGPR (seed treatment)	1.78 (1.33)*	0.98 (0.99)	1.38 (1.17)
T_2: *Trichoderma harzianum* (seed treatment)	1.81 (1.35)	1.01 (1.01)	1.41 (1.19)
T_3: PGPR (soil application)	1.78 (1.33)	0.98 (0.99)	1.38 (1.18)
T_4: *T. harzianum* (soil application)	1.81 (1.35)	1.01 (1.01)	1.41 (1.19)
T_5: PGPR (seed treatment) + *T. harzianum* (soil application)	1.98 (1.41)	1.08 (1.48)	1.58 (1.26)
T_6: PGPR (soil application) + *T. harzianum* (seed treatment)	1.90 (1.38)	1.20 (1.10)	1.55 (1.23)
T_7: PGPR (seed treatment) + PGPR (soil application)	1.82 (1.35)	1.30 (1.01)	1.56 (1.25)
T_8: *T. harzianum* (seed treatment) + *T. harzianum* (soil application)	1.82 (1.34)	1.20 (1.10)	1.51 (1.23)
T_9: Untreated control	1.61 (1.27)	0.81 (0.90)	1.21 (1.10)
Mean	1.81 (1.35)	1.01 (1.01)	1.14 (1.07)
*Figures in parentheses represent square root transformation			
C.D. (0.05)			
Year	:	0.02	
Treatment	:	0.04	
Year X Treatment	:	NS	

The germination% depicted in Table 3, in general ranged between 88.58 to 93.26% in different treatments as compared to 85.25% in control. Amongst different treatments, maximum germination of 93.26% was recorded in T_5 [PGPR (seed treatment) + *T. harzianum* (soil application)] followed by 92.92% in T_6 [PGPR (soil application) + *T. harzianum* (seed treatment)], 91.25% in T_7 [PGPR (seed treatment) + PGPR (soil application)] and 90.42% in T_8 [*T. harzianum* (seed treatment) + *T. harzianum* (soil application)]. In the present study, maximum germination of harvested seed was recorded with application of [PGPR (seed treatment) + *T. harzianum* (soil application)] which may be due to optimum availability of nutrients at all stages of plant growth and thus gave bold, good quality and vigorous seeds resulting ultimately maximum germination. The present findings are also in accordance with the findings of Kanchana *et al*. (2014) [3] who have reported that combined inoculation of PGPR recorded the highest germination of harvested seed in chilli. Gupta *et al*. (2015) [5] also reported increased germination of harvested seed in capsicum with the inoculation of PGPR. This treatment might have provided optimum availability of nutrients to the inoculated plants at all growth stages and thus gave bold, good quality and vigorous seeds resulting ultimately in higher germination.

Table 3: Effect of seed treatment and soil application of PGPR and BCA on seed germination of harvested crop in bell pepper (Seed crop)

Treatment	Germination per cent (%)		
	2016	2017	Mean
T_1: PGPR (seed treatment)	90.43 (9.51)*	88.73 (9.42)	89.58 (9.46)
T_2: *Trichoderma harzianum* (seed treatment)	89.77 (9.47)	88.07 (9.38)	88.92 (9.43)
T_3: PGPR (soil application)	89.43 (9.45)	87.73 (9.36)	88.58 (9.41)
T_4: *T. harzianum* (soil application)	91.17 (9.55)	89.47 (9.46)	90.32 (9.50)
T_5: PGPR (seed treatment) + *T. harzianum* (soil application)	94.11 (9.70)	92.41 (9.61)	93.26 (9.66)
T_6: PGPR (soil application) + *T. harzianum* (seed treatment)	93.77 (9.68)	92.07 (9.59)	92.92 (9.64)
T_7: PGPR (seed treatment) + PGPR (soil application)	92.10 (9.60)	90.40 (9.51)	91.25 (9.55)
T_8: *T. harzianum* (seed treatment) + *T. harzianum* (soil application)	91.27 (9.55)	89.57 (9.46)	90.42 (9.51)
T_9: Untreated control	86.10 (9.28)	84.40 (9.19)	85.25 (9.23)
Mean	90.90 (9.53)	89.20 (9.44)	90.05 (9.49)
C.D. (0.05)			
Year	:	0.07	
Treatment	:	0.15	
Year X Treatment	:	NS	

*Figures in parentheses represent square root transformation

The seedling length presented in Table 4, in general ranged between 9.11 to 12.39 cm in different treatments as compared to 7.43 cm in control. Amongst different treatments, maximum seedling length of 12.39 cm was recorded in T_5 [PGPR (seed treatment) + *T. harzianum* (soil application)] followed by 11.50 cm in T_6 [PGPR (soil application) + *T. harzianum* (seed treatment)], 11.00 cm in T_7 [PGPR (seed treatment) + PGPR (soil application)] and 10.78 cm in T_8 [*T. harzianum* (seed treatment) + *T. harzianum* (soil application)]. The seedling length in untreated control was observed minimum, which was statistically lower than rest of the treatments. In the present study, application of [PGPR (seed treatment) + *T. harzianum* (soil application)] recorded increased seedling length which may be due to accumulation of storage food material which resulted better seedling length. The present findings are in agreement with the findings of Gupta *et al*. (2015) [5] and Kanchana *et al*. (2014) [3] who have reported that the seed quality characters were significantly influence by bio agents. This might be due to optimum availability of nutrients at all stages of germination and development for seedling length.

Table 4: Effect of seed treatment and soil application of PGPR and BCA on seedling length of harvested crop in bell pepper (Seed crop)

Treatment	Seedling length (cm)		
	2016	2017	Mean
T_1: PGPR (seed treatment)	10.53	8.70	9.62
T_2: *Trichoderma harzianum* (seed treatment)	10.37	9.27	9.82
T_3: PGPR (soil application)	9.48	8.73	9.11
T_4: *T. harzianum* (soil application)	11.23	9.46	10.35
T_5: PGPR (seed treatment) + *T. harzianum* (soil application)	13.07	11.71	12.39
T_6: PGPR (soil application) + *T. harzianum* (seed treatment)	12.07	10.93	11.50
T_7: PGPR (seed treatment) + PGPR (soil application)	11.40	10.60	11.00
T_8: *T. harzianum* (seed treatment) + *T. harzianum* (soil application)	11.85	9.70	10.78
T_9: Untreated control	8.46	6.40	7.43
Mean	10.94	9.50	10.22
C.D. (0.05)			
Year	:	0.77	
Treatment	:	1.63	
Year X Treatment	:	NS	

The seedling dry weight presented in Table 5, in general ranged between 2.05 to 2.29 mg in different treatments as compared to 1.85 mg in control. Amongst different treatments, maximum seedling dry weight of 2.29 mg was recorded in T_5 [PGPR (seed treatment) + *T. harzianum* (soil application)] followed by 2.26 mg in T_6 [PGPR (soil application) + *T. harzianum* (seed treatment)], 2.20 mg in T_7 [PGPR (seed treatment) + PGPR (soil application)] and 2.15 mg in T_8 [*T. harzianum* (seed treatment) + *T. harzianum* (soil application)]. The seedling dry weight in untreated control was observed minimum, which was significantly lower than rest of the treatments. In the present study, maximum seedling dry weight was recorded with [PGPR (seed treatment) + *T. harzianum* (soil application)]. It might be due to more vigorous and healthy seedling produce by combined application of PGPR and *T. harzianum* that play important role in plant growth promotion as well as act as strong bio-control agent, respectively. Similar to present findings Mandyal *et al*. (2012) [6] also reported that bell pepper inoculated with a *Bacillus* isolate increased plant biomass and root biomass.

Table 5: Effect of seed treatment and soil application of PGPR and BCA on seedling dry weight of harvested crop in bell pepper (Seed crop)

Treatment	Seedling dry weight (mg)		
	2016	2017	Mean
T_1: PGPR (seed treatment)	2.26	2.01	2.14
T_2: *Trichoderma harzianum* (seed treatment)	2.22	2.00	2.12
T_3: PGPR (soil application)	2.20	1.90	2.05
T_4: *T. harzianum* (soil application)	2.24	1.97	2.11
T_5: PGPR (seed treatment) + *T. harzianum* (soil application)	2.45	2.13	2.29
T_6: PGPR (soil application) + *T. harzianum* (seed treatment)	2.38	2.13	2.26
T_7: PGPR (seed treatment) + PGPR (soil application)	2.31	2.09	2.20
T_8: *T. harzianum* (seed treatment) + *T. harzianum* (soil application)	2.25	2.04	2.15
T_9: Untreated control	1.90	1.80	1.85
Mean	2.25	2.01	2.13
C.D. (0.05)			
Year	:	0.06	
Treatment	:	0.14	
Year X Treatment	:	NS	

The seed vigour index-length (SVI-L) depicted in Table 6, in general ranged between 806.81 to 1156.03 in different treatments as compared to 634.28 in control. Amongst different treatments, maximum Seed Vigour Index -Length (SVI-L) of 1156.03 was recorded in T_5 [PGPR (seed treatment) + *T. harzianum* (soil application)] followed by 1069.03 in T_6 [PGPR (soil application) + *T. harzianum* (seed treatment)], 1004.09 in T_7 [PGPR (seed treatment) + PGPR (soil application)] and 995.19 in T_8 [*T. harzianum* (seed treatment) + *T. harzianum* (soil application)]. The seed vigour index-length (SVI-L) in untreated control was recorded minimum, which was statistically lower than rest of the treatments.

Table 6: Effect of seed treatment and soil application of PGPR and BCA on seed vigour index - length (SVI-L) of harvested crop in bell pepper (Seed crop)

Treatment	Seed Vigour Index -Length (SVI-L)		
	2016	2017	Mean
T_1: PGPR (seed treatment)	952.26	771.98	862.12
T_2: *Trichoderma harzianum* (seed treatment)	930.91	816.41	873.66
T_3: PGPR (soil application)	847.76	765.85	806.81
T_4: *T. harzianum* (soil application)	1023.84	846.39	935.11
T_5: PGPR (seed treatment) + *T. harzianum* (soil application)	1229.97	1082.08	1156.03
T_6: PGPR (soil application) + *T. harzianum* (seed treatment)	1131.76	1006.29	1069.03

T_7: PGPR (seed treatment) + PGPR (soil application)	1049.94	958.24	1004.09
T_8: *T. harzianum* (seed treatment) + *T. harzianum* (soil application)	1101.55	888.83	995.19
T_9: Untreated control	728.41	540.16	634.28
Mean	999.60	852.91	926.26
C.D. (0.05)			
Year :	76.65		
Treatment :	162.61		
Year X Treatment :	NS		

Data presented in Table 7 revealed that the Seed Vigour Index-Mass (SVI-M), in general ranged between 181.71 to 213.69 in different treatments as compared to 157.76 in control. Amongst different treatments, maximum seed vigour index-mass (SVI-M) of 213.69 was recorded in T_5 [PGPR (seed treatment) + *T. harzianum* (soil application)] followed by 209.63 in T_6 [PGPR (soil application) + *T. harzianum* (seed treatment)], 200.84 in T_7 [PGPR (seed treatment) + PGPR (soil application)] and 198.04 in T_8 [*T. harzianum* (seed treatment) + *T. harzianum* (soil application)]. The Seed vigour index-length (SVI-M) in untreated control was recording minimum, which was statistically lower than rest of the treatments. Seed vigour index is very important character as it determines the actual ability of seed to germinate even under adverse conditions. In the present findings, higher seed vigour index was recorded with PGPR (seed treatment) and *T. harzianum* (soil application) which might be due to adequate availability of nutrients as influenced by these bioagents in the increased quantity that might have helped the plants to produce bolder and heavier seeds. Similar results have been reported by Kanchana *et al*. (2014) [3] and Mandyal *et al*. (2012) [6] who have also observed that combined application of bio agents produced vigorous seeds in capsicum. Rahman *et al.* (1996) [7] have found same results in tomato.

Table 7: Effect of seed treatment and soil application of PGPR and BCA on seed vigour index-mass (SVI-M) of harvested crop in bell pepper (Seed crop)

Treatment	Seed vigour index-mass (SVI-M)		
	2016	2017	Mean
T_1: PGPR (seed treatment)	204.38	178.35	191.37
T_2: *Trichoderma harzianum* (seed treatment)	199.29	176.14	187.71
T_3: PGPR (soil application)	196.74	166.68	181.71
T_4: *T. harzianum* (soil application)	204.22	176.26	190.24
T_5: PGPR (seed treatment) + *T. harzianum* (soil application)	230.56	196.83	213.69
T_6: PGPR (soil application) + *T. harzianum* (seed treatment)	223.16	196.10	209.63
T_7: PGPR (seed treatment) + PGPR (soil application)	212.75	188.94	200.84
T_8: *T. harzianum* (seed treatment) + *T. harzianum* (soil application)	209.36	186.72	198.04
T_9: Untreated control	163.59	151.92	157.76
Mean	204.89	179.77	192.33
C.D. (0.05)			
Year :	7.79		
Treatment :	16.53		
Year X Treatment :	NS		

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