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Effect of pre-sowing seed bio-inoculation on seed yield, its contributing characters and subsequent seed quality of barley (*Hordeum vulgare*)

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ABSTRACT

A study was carried out to examine the effect of pre-sowing seed inoculation with *Pseuodomonas fluorescens* and *Trichoderma harzianum* either singly (@ 4g, 6g, 8g per kg seed) or in combination with both bio-inoculants (@ (2+2)g, (3+3)g, (4+4) g/kg seed) on growth, seed yield and subsequent seed quality of barley. There was significant difference among different seed bio-inoculation treatment over un-inoculated control with regard to field emergence, root length, plant height, ear length, number of seeds per ear, 1000-seed weight and seed yield/ha under field trial and also for subsequent seed quality, i e germination, radicle, plumule and seedling length, fresh and dry weight of seedlings and vigour index (I&II) under laboratory experiment. Seed yield and its contributing characters as well as subsequent seed quality were found to be higher as the amount of *P. fluorescens* and *T. harzianum* increased from 4 to 8 g/kg either singly or in combination of both bio-inoculants. The bio-inoculants mixture showed greater influence on seed yield and quality over seeds treated either with *P. fluorescens* and *T. harzianum* and both bio-inoculants mixture @ (4+4) g/kg seeds had maximum influence on characters studies under field as well as laboratory experiment.

Key words: Plant growth, *Pseudomonas fluorescens,* Seed bio-inoculation, Seed quality, Seed yield, *Trichoderma harzianum*

Barley (*Hordeum vulgare* L.) is the major source of food, feed and fodder in cooler, semi arid areas of the world, where wheat and other staple cereals are less well adapted. In India, the chief barley growing regions are mid to higher Himalayas, central parts of eastern Uttar Pradesh, eastern part of Rajasthan and northwestern parts of Bihar. Plant very much resembles to the wheat plant and usually grows 0.75 to 1.0 meter in height and belongs to *Poaceae* family. It has six rows of grain arrangement (Aberg and Wiebe 1946), and has evolved from a wild species *Hordeum spontaneum* L. It requires cool weather during early growth and warm dry weather at maturity, and grows fairly well in temperate as well as in sub-tropical regions. The crop has low water requirement, also drought resistant and is well adapted to areas with scanty rainfall.

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Barley is a major crop along with rice, wheat and small millets, primarily in North-West Himalayan region of India. It supports the livelihood and economy of the hilly peasants. Therefore, barley is the centre pillar of livelihood and economy of North-West Himalayan farmers. Unfortunately, in this region, soils in most farming situations are poor in organic matter, low in pH and phosphorus content. These poor soils do not augment in better yield and disease resistance. Pressure for intensive farming coupled with poor soil condition has played a key role in poor productivity. In order to improve the yield and disease suppression, farmers' resort to inorganic fertilizers and possibly pesticides, which often have hazardous effects on environment and human health and also do not fit into the frame work of organic farming. An alternative approach is the possible use of bio-inoculants which are known to augment plant growth and health through increasing the planting value of seed with better germination and vigour, increasing the uptake of nutrients, better seed yield and subsequent seed quality.

In the era of sustainable agricultural production, the interactions in the rhizosphere play a pivotal role in transformation, mobilization, solubilization etc. from a limited nutrient pool in the soil and subsequent uptake of essential plant nutrients by the crop plants to realize full genetic potential of the crop. Soil microorganisms are very important in the bio-geochemical cycles of both inorganic and organic nutrients in the soil and in the growth promotion of the plant (Jeffries *et al*. 2003). Bio-control of phyto-phathogens by plant growth promoting micro-organism appears to be due to mechanism of plant growth promotion, and in addition to bio-fertilization, enhancing plant growth (Mastouri and Harman 2009, Shoresh *et al*. 2010, Prasad *et al*. 2011). Several organisms have been successfully used as plant growth promoting and bio-control agents such as *Pseudomonas* spp. (Moeinzadeh *et al*. 2010) and *Trichoderma* spp. (Nzanza *et al*. 2011). In this present studies talc based formulation of *Pseuodomonas fluorescens* and *Trichoderma harzianum* have recorded considerable attention and intensely reviewed on seed yield and quality of barley designated to address the following issues:

- To asses the influence of pre-sowing seed bio-inoculants on seed yield and its contributing characters in barley.
- To asses the influence of pre-sowing seed bio-inoculants on subsequent seed quality of barley.

MATERIALS AND METHODS

The present experiment were conducted during *rabi* season of 2008–09 and 2009–10 at the Seed Science and Technology Research Block, GBPUA&T, Hill Campus, Ranichauri located at 30° 15’ N latitude and 78° 30’ E longitude at an altitude of 2100 m above MSL. Seeds of barley cv. VLB 1 were obtained from Department of Seed Science and Technology and both the bio-agents, i e *Pseudomonas fluorescens* (Pf-173) and *Trichoderma harzianum* were received from Plant Pathology Division of Hill Campus, Ranichauri, Tehri Garhwal, Uttarakhand, India. The seed quality parameters were assessed at Seed Science and Technology Laboratory, GBPUA&T, Hill Campus, Ranichauri.

The talc based formulation of *Pseudomonas fluorescens* (Pf-173) and *Trichoderma harzianum* having 2×10^8 cfu (colony forming unit) were used for seed inoculation. Seeds to be inoculated were weighed and moistened with distilled water. Any excess water was drained. Besides an uninoculated control, there were nine treatments of seed inoculation with *Pseudomonas fluorescens* (*P.f.*) and *Trichoderma harzianum* (*T.h.*) used either solely or combination of both in different concentration *viz.,* T_1 = *P. f.* (@ 4 g/kg seed, T_2 = *P. f.* (@ 6 g/kg seed, T_3 = *P. f.* (@ 8 g/kg seed, T_4 = *T. h.* @ 4 g/kg seed, T_5 = *T. h.* @ 6 g/kg seed, T_6 = *T. h.* @ 8 g/kg seed, T_7 = *P. f.* +*T. h.* @ (2+2) g/kg seed, T_8 = *P. f.* +*T. h.* @ (3+3) g/kg seed, T_9 = *P. f.* +*T. h.* @ (4+4) g/kg seed. Added bio-agent to the seed were mixed gently so that the microbial cells in the formulation get absorbed on seed surface and subsequently seeds were air dried in shade. Each row of 3.5 m length with row to row spacing of 23 cm was established. The experiments were laid out in a randomized block design (RBD) with three replications.

To know the field emergence (%), proportion of germinated seed to the total number of seed sown in a row was counted after 20 days of sowing. Before harvesting selected plants were uprooted for recording root length, plant height and length of the ear. Number of seed/ear was counted through randomly selected 20 ear head harvested from each row of each plot. Crops were threshed, cleaned, sun dried and weighed to know the yield (q/ha). In case of 1 000-seed weight, random sample of 1000 seed from the bulk produce of each plot was weighed. However, after the field trial, harvested seeds were subjected to laboratory test for evaluating the seed quality parameters in a completely randomized block design (CRBD). In laboratory, seed germination percentage was recorded as per ISTA (2008). Seedling vigour index was evaluated as per the procedure given by AOSA (2009). Vigour Index-I was calculated as a product of germination and seedling length, however, Vigour Index-II was worked out by multiplying germination per cent with seedling dry weight. The two consecutive years of field as well as laboratory data were also pooled and analyzed. Significance difference among means were separated by using least significant difference (LSD: $P< 0.05$) following Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

The enhancement of pre-sowing seed bio-inoculation on seed yield and its contributing attributes for barley (cv. VL1) are presented in Table 1(a) and 1(b), while subsequent seed quality parameters are mentioned in Table 2(a) and 2(b).

Field parameters

- The seed inoculated with *Pseudomonas fluorescens* (Pf-173) and *Trichoderma harzianum* and mixture of both bio-inoculants of all combinations had significantly more number of plants in the field as compared to the un-inoculated control (63.75 and 66.25%) respectively in both the years and maximum field germination (81.75 and 84.75%) were observed for seed inoculated with a mixture of both the inoculants @ (4+4) g/kg seed (Table 1). Seed inoculation with *P. fluorescens* and *T. harzianum* increased seedling emergence was reported by Prasad *et al*.(2009) in wheat and seed treatment with *T. harzianum* resulted in increased percentage of emergence as compared to control in tomato (Nzanza *et al.* 2011). Significant influence of seed bio-inoculant in all treatments was observed on root length as well as plant height over un-inoculated control in both the years. The maximum root length (7.25 and 7.33 cm) was found in seed inoculated with both bio-agents mixture @ (4+4) g/kg seed in both the consecutive years. However, least root length (6.21 and 6.22 cm) was recorded for un-inoculated control. Manoranjitham *et al.* (2001) also reported that application of talc based formulation of *T.*

Table 1 Enhancement of field emergence, root length and plant height by pre-sowing microbial seed inoculation in barley cv. VLB 1

Parameters→	Field emergence (%)			Root length (cm)			Plant height (cm)		
Treatments↓	2008–09	2009–10	Pooled	2008–09	2009–10	Pooled	2008–09	2009–10	Pooled
P. f. @ 4 g/kg seed	72.25	73.75	73.00	6.45	6.52	6.48	70.16	71.71	70.93
P. f. @ 6 g/kg seed	74.50	76.50	75.50	6.67	6.79	6.72	73.20	73.51	73.35
P. f. @ 8 g/kg seed	76.25	79.50	77.87	7.11	7.13	7.12	75.00	75.87	75.43
T. h.@ 4 g/kg seed	72.75	74.75	73.75	6.35	6.37	6.36	66.96	68.30	67.63
T. h. @ 6 g/kg seed	77.00	79.75	78.37	6.66	6.63	6.64	68.07	69.23	68.64
T. h. @ 8 g/kg seed	77.50	81.75	79.75	6.75	6.78	6.76	69.00	70.26	69.63
P. f. + *T. h.* @ (2+2) g/kg seed	71.50	74.25	72.87	6.53	6.52	6.52	68.81	68.71	68.76
P. f. + *T. h.* @ (3+3) g/kg seed	79.25	82.50	80.87	7.08	7.14	7.11	73.08	71.87	72.47
P. f. + *T. h.* @ (4+4) g/kg seed	81.75	84.75	83.25	7.25	7.33	7.29	73.73	73.50	73.61
Control (uninoculated)	63.75	66.25	65.00	6.21	6.22	6.21	63.79	64.92	64.35
Grand Mean	74.65	77.37	76.02	6.70	6.74	6.72	70.18	70.79	70.48
Sem(±)	0.77	0.78	0.46	0.03	0.02	0.02	0.11	0.25	0.14
LSD (*P*=0.05)	2.26	2.29	1.33	0.09	0.07	0.06	0.32	0.73	0.41

P. f., *Pseudomonas fluorescens* and; *T.h.*, *Trichoderma harzianum*

Table 2 Enhancement of ear length, no. of seeds/ear, 1000-seed weight and seed yield by pre-sowing microbial seed inoculation in barley cv. VLB 1

Parameters→	Length of ear (cm)			No. of seeds/ear			1000-seed weight (g)			Seed yield (q/ha)		
Treatments↓	2008–09	2009–10	Pooled	2008–09	2009–10	Pooled	2008–09	2009–10	Pooled	2008–09	2009–10	Pooled
P. f. @ 4 g/kg seed	7.34	7.52	7.43	65.87	66.85	66.36	44.26	44.59	44.42	18.92	19.30	19.11
P. f. @ 6 g/kg seed	7.53	7.83	7.68	69.15	69.87	69.51	45.11	45.07	45.09	19.30	20.03	19.66
P. f. @ 8 g/kg seed	7.83	8.00	7.91	71.05	71.92	71.48	45.24	45.53	45.38	20.16	21.57	20.84
T. h. @ 4 g/kg seed	7.19	7.28	7.23	64.50	65.20	64.88	43.46	43.92	43.68	18.20	18.82	18.51
T. h. @ 6 g/kg seed	7.37	7.49	7.43	67.87	67.55	67.71	44.17	44.81	44.49	19.04	19.45	19.24
T. h. @ 8 g/kg seed	7.59	7.60	7.59	69.00	69.00	69.00	44.58	45.52	45.05	19.56	20.18	19.87
P. f. + *T. h.* @ (2+2) g/kg seed	7.31	7.37	7.33	65.97	66.52	66.25	43.36	43.98	43.67	18.72	19.31	19.01
P. f. + *T. h.* @ (3+3) g/kg seed	7.83	8.00	7.91	71.87	72.62	72.24	45.10	45.34	45.22	20.00	21.91	20.95
P. f. + *T. h.* @ (4+4) g/kg seed	8.12	8.17	8.14	73.70	73.92	73.81	45.80	45.92	45.86	21.05	22.57	21.81
Control (uninoculated)	6.56	6.65	6.60	59.10	60.12	59.61	41.37	41.70	41.53	16.75	17.45	17.10
Grand Mean	7.47	7.59	7.53	67.81	68.36	68.08	44.24	44.64	44.43	19.17	20.06	19.61
Sem(±)	0.04	0.03	0.02	0.23	0.28	0.64	0.82	0.11	0.16	0.82	0.11	0.08
LSD (*P*=0.05)	0.12	0.08	0.07	0.67	0.84	1.87	0.23	0.32	0.48	0.24	0.33	0.23

P. f., *Pseudomonas fluorescens* and; *T.h.*, *Trichoderma harzianum*

viride and *P. fluorescens* increased the roots of tomato seedlings. However, the maximum height of plant (75.00 and 75.87cm) was found in seeds inoculated with *P. fluorescens* @ 8 g/kg seed in both the successive years which was significantly superior from all other treatments. The plant growth promoting bio-inoculants stimulate plant growth by a plethora of mechanism. This is in conformity with the findings of Nezarat and Gholami (2009) and Vessey (2004).

All the treatments induced significant increase in length of the ear and number of seeds per ear over un-inoculated control (Table 2). A mixture of *P. fluorescens* + *T. harzianum* @ (4+4) g/kg seed inoculation was most effective on ear length enhancement (8.12 and 8.17 cm) in both the years which was significantly higher when compared to other treatments. However, least ear length (6.56 and 6.65 cm) was recorded in un-inoculated control in both the years, respectively. Number of seeds per ear was also influenced and positively correlated with ear length. Therefore, maximum number of seeds per ear (73.70 and 73.92) was counted for bio-inoculants mixture @ (4+4) g/kg seed in both the successive years, respectively. However, the pooled value for number of seeds per ear was similar with treatment mixture @ (3+3) g/kg seed. While statistically lowest number of seeds per ear (59.10 and 60.12) was counted for control in both the years. 1 000-seed weight is prime qualitative and quantitative factors that determine the inherent seed quality and yield of the crop. The effect of treatments was found to be significant for 1 000-seed weight over un-inoculated control. The significantly higher 1 000-seed weight (45.80 and 45.92 g) was weighed for bio-inoculants mixture @ (4+4) g/kg seed in both the years over other treatments. However, significantly lowest 1 000-seed weight (41.37 and 41.70 g) was recorded in control in both the years. Seed yield is ultimately important economic parameter that depends on other yield attributing characters. The other attributing characters were influenced significantly with the bio-inoculants treated lonely or in combination together. In the consequences the seed yield was also significantly improved with seed inoculation and maximum seed yield (21.05 and 22.57 q/ha) was harvested for bio-inoculants mixture (*P. f* + *T. h.*)@ (4+4) g/kg seed in both the years respectively, while significantly lowest seed yield (16.75 and 17.45 q/ha) was obtained for un-inoculated control in both the years, respectively. These findings is also in confirmation with the work of Varshney and Chaube (1997) who reported that application of *Pseudomonas fluorescens* and *Trichoderma harzionum* increased the growth and yield of tomato. Further, significant increase in plant height, 100-seed weight, number of seed/ear and seed yield in maize through seed inoculation of *P. fluorescens* (Nezarat and Gholami 2009).

Seed quality parameters

Seed germination was significantly influenced by the seed bio-inoculation and it revealed that highest germination (92.00 and 89.75) per cent was recorded when seeds were treated with mixture of *P. fluorescens* and *T. harzianum* @ (4+4) g each/kg seed during both the years, which was equal with treatment mixture of *P. fluorescens* and *T. harzianum* @ (3+3) g/kg seed for 2009. However, significantly minimum germination (83.25 and 82.00) per cent was recorded for un-inoculated control in both the respective years. The subsequent growth of seedlings, i e radicle, plumule and total seedling length were also significantly increased by the mixture of bio-inoculants of seed and significantly maximum radicle, plumule as well as seedling length was noticed for *P. fluorescens* and *T. harzianum* mixture @ (4+4) g/kg seed followed by treatment *P. f* @ 8 g/kg seed, however, statistically least value was also observed for un-inoculated control in both the years (Table 3). The effect of pre-sowing seed inoculation on fresh and dry weight of seedling was significantly reflected positive over un-inoculated control. Significantly higher fresh weight (8.25 and 8.24 g) and dry weight of 20 seedlings (0.66 and 0.69 g) was recorded in seeds treated with bio-inoculants mixture @ (4+4) g/kg seed over other treatments except bio-inoculants (*P. f* + *T. h.*) @

Table 3 Enhancement of subsequent seed quality, ie germination, radical, plumule and seedling length by pre sowing microbial seed inoculation in barley cv. VLB 1

Parameters→	Germination (%)			Radicle length (cm)			Plumule length (cm)			Seedling length (cm)		
Treatments↓	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled
P. f. @ 4 g/kg seed	88.00	86.00	87.00	16.19	16.08	16.13	15.02	15.29	15.15	31.21	31.38	31.29
P. f. @ 6 g/kg seed	89.75	86.50	88.12	16.62	16.42	16.52	15.21	15.49	15.35	31.84	31.91	31.87
P. f. @ 8 g/kg seed	90.00	88.50	89.25	16.80	16.81	16.80	15.45	15.80	15.62	32.26	32.61	32.43
T. h.@ 4 g/kg seed	87.50	86.50	87.00	15.51	15.81	15.66	14.85	15.16	15.00	30.37	30.97	30.67
T. h. @ 6 g/kg seed	88.25	87.00	87.62	15.86	16.09	15.97	15.15	15.68	15.41	31.01	31.77	31.37
T. h. @ 8 g/kg seed	88.50	88.00	88.25	16.10	16.18	16.14	15.25	15.78	15.51	31.35	31.97	31.66
P. f. + *T. h.* @ (2+2) g/kg seed	90.25	86.75	88.50	15.98	16.32	16.14	15.01	15.55	15.28	31.00	31.87	31.43
P. f. + *T. h.* @ (3+3) g/kg seed	92.00	88.25	90.12	16.25	16.69	16.47	15.28	15.87	15.57	31.53	32.56	32.04
P. f. + *T. h.* @ (4+4) g/kg seed	92.00	89.75	90.87	16.95	17.32	17.11	15.77	16.20	15.98	32.72	33.52	33.12
Control (uninoculated)	83.25	82.00	82.62	14.15	14.30	14.23	12.32	13.08	12.70	26.48	27.39	26.93
Grand mean	88.95	86.92	87.93	16.04	16.20	16.12	14.93	15.39	15.16	30.97	31.59	31.28
Sem(±)	0.55	0.47	0.37	0.51	0.52	0.40	0.58	0.55	0.43	0.76	0.82	0.60
LSD (*P*=0.05)	1.60	1.37	1.08	0.14	0.15	0.11	0.16	0.16	0.12	0.21	0.23	0.17

P. f., *Pseudomonas fluorescens* and; *T. h., Trichoderma harzianum*

Table 4 Enhancement of subsequent seed quality, i e fresh weight and dry weight of seedling and vigour index by pre sowing microbial seed inoculation in barley cv. VLB 1

Parameters→	Fresh weight of 20 seedling (g)			Dry weight of 20 seedlings (g)			Vigour Index I			Vigour Index II		
Treatments↓	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled
P. f. @ 4 g/kg seed	7.29	7.51	7.40	0.58	0.60	0.59	2 746.47	2 698.70	2 722.58	51.37	50.22	50.79
P. f. @ 6 g/kg seed	7.47	7.77	7.62	0.61	0.62	0.62	2 858.00	2 760.79	2 809.39	55.35	53.97	54.66
P. f. @ 8 g/kg seed	7.84	8.08	7.96	0.63	0.66	0.64	2 903.63	2 885.83	2 894.73	57.08	58.88	57.98
T. h.@ 4 g/kg seed	7.10	7.24	7.17	0.56	0.58	0.57	2 657.92	2 679.63	2 668.77	49.66	50.49	50.07
T. h. @ 6 g/kg seed	7.31	7.44	7.37	0.60	0.60	0.60	2 736.87	2 764.41	2 750.64	52.97	53.03	53.00
T. h. @ 8 g/kg seed	7.69	7.70	7.69	0.61	0.62	0.62	2 774.55	2 813.43	2 793.99	54.57	55.13	54.85
P. f. + *T. h.* @ (2+2) g/kg seed	7.62	7.85	7.73	0.60	0.61	0.61	2 797.96	2 764.86	2 781.41	54.60	53.69	54.14
P. f. + *T. h.* @ (3+3) g/kg seed	8.02	8.14	8.08	0.63	0.66	0.64	2 901.30	2 873.97	2 887.63	58.44	58.52	58.48
P. f. + *T. h.* @ (4+4) g/kg seed	8.25	8.24	8.24	0.66	0.69	0.67	3 010.52	3 009.04	3 009.70	60.79	62.04	61.41
Control (uninoculated)	5.99	6.20	6.09	0.45	0.49	0.47	2 204.66	2 246.34	2 225.49	37.59	40.20	38.90
Grand mean	7.46	7.62	7.53	0.59	0.61	0.60	2 759.19	2 749.70	2 754.43	53.24	53.62	53.43
Sem(±)	0.42	0.03	0.02	0.49	0.51	0.37	19.58	16.04	14.30	0.51	0.81	0.46
LSD (*P*=0.05)	0.12	0.11	0.08	0.01	0.01	0.01	56.55	46.35	41.30	1.48	2.35	1.34

P. f. = *Pseudomonas fluorescens* and *T. h.* = *Trichoderma harzianum*

(3+3) g/kg during second year of experiment. However, significantly least value for fresh weight (5.99 and 6.20 g) and dry weight (0.45 and 0.49 g) were noticed for un-inoculated control. An increased seed germination, shoot length, root length, seedling fresh and dry weight have also been reported by Ashrafuzzaman *et al*. (2009) through seed bacterization in rice and through *T. viride* and *P. fluorescens* in tomato seedling (Manoranjitham *et al*. 2001).

Seed Vigour Index (germination per cent × seedling length) and (germination per cent × seedling dry weight) is a actual mirror of seed quality of any seed sample or seed lot. *P. fluorescens* and *T. harzianum* mixture @ (4+4) g/kg seed resulted in the significant maximum value of Vigour Index - I & II over other treatments (Table 4). However, significant lowest Vigour Index-I (2 204.66 and 2 246.34) and Vigour Index II (37.59 and 40.20) was recorded for un-inoculated control in both the consecutive years.

Seed coating with biological agent is the most common strategy used in a number of crops for a variety of purposes. Bio-fertilizers and bio-fungicides include many type of bacteria and fungi. Out of these *P. fluorescens* and *T. harzianum* are group of each bacteria and fungi respectively extensively investigated in plant growth promoting activities with various agricultural crops. It is concluded that pre-sowing seed inoculation with *P. fluorescens* and *T. harzianum* or combination of both bio-inoculants @ 4 to 8 g/kg seed talc powder induced field germination, better seed yield, yield attributing characters and further seed quality in barley cv. VLB 1, while these parameters were increases as the seed bio-inoculants increases from 4 to 8 g/kg seed. However, seed treated with combination of these two bio-inoculants yielded better than when used lonely either with *P. fluorescens* or *T. harzianum* for most of the field as well as laboratory parameters. Increase in these characters by seed inoculation of studied bio-inoculants, as found in this study, might be due to plethora of mechanism, i e activities of plant growth promotion, biological control of various diseases, activation of disease resistance in host plant and direct or indirect way of resulting in growth stimulation of plant by nutrients secreted, by the plant root.

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