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Presowing seed inoculation of wheat (*Triticum aestivum* L. cv. VL 832) for seed yield and quality enhancement in North-West Himalayan agriculture system

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ABSTRACT

To examine the effect of pre-sowing seed inoculation either singly with Pseuodomonas fluorescens and Trichoderma harzianum or combination with both bio-inoculants @ 4g, 6g, 8g per kg seed of singly or @ (2+2) g, (3+3) g, (4+4) g/kg seed with mixture on growth yield and subsequent seed quality of wheat. The results revealed that the effect of pre-sowing seed bio-inoculation was significant to each and every treatment over un-inoculated control for the field germination, 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, root, shoot and seedling length, fresh and dry weight of seedling and vigour index under laboratory experiment. Seed yield and its contributing characters as well as subsequent seed quality were increases as the amount of P. fluorescens and T. harzianum increases 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 seed treated either with P. fluorescens and T. harzianum and both bio-inoculants mixture @ (4+4) g/kg seed had maximum influence on characters studies under field as well as laboratory experiment.

Key Words: Pseudomonas fluorescens, seed inoculation, seed quality and Trichoderma harzianum

The common bread wheat *Triticum aestivum* L. is the world's most widely cultivated food crop. In North-West Himalayan, wheat is a major crop along with rice, barnyard millet, foxtail millet and finger millet. It supports the livelihood and economy of the hilly peasants. Pressure for intensive farming along 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 and also do not fit into the frame work of organic farming. An alternative approach might be use bio-inoculants which are known to augment plant growth and health through increasing the planting value of seed, better germination rate, vigour, increasing the uptake of inorganic phosphate, better seed yield and subsequent seed quality.

Soil microorganisms are very important in the biogeochemical 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 appears by plant growth promoting micro-organism to be a major mechanism of plant growth promotion and in addition to bio-fertilization may contribute to enhance plant growth (Singh *et al*., 1977; Yehia *et al*., 1994; Yeole and Dube, 1997). Several organisms have been successfully used as plant growth promoting and bio-control agents such as *Pseudomonas spp*. (Vidhyaksekaran and Muthamilan, 1995), *Bacillus spp*. (Copper and Campbell, 1986) and *Trichoderma spp.* (Raguchander *et al*., 1997). In this present studies talc based formulation of *Pseuodomonas fluorescens* and *Trichoderma harzianum* have recorded considerable attention and intensity reviewed on seed yield and quality of wheat designated to to asses the influence of pre-sowing seed bio-inoculants on seed yield and subsequent seed quality of wheat seed.

MATERIALS AND METHODS

The present experiments were conducted during 2007-08 and 2008-09 of two consecutive r*abi* season at the Seed Science and Technology Research Block, GBPUA&T, Hill Campus, Ranichauri, Tehri Garhwal, Uttarakhand, India. The experimental site is situated in between 30^0 15' N latitude and 78^0 30' E longitudes and an altitude of 2100 m MSL. The seeds of wheat cv. VL 832 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 also assessed at Seed Science and Technology Laboratory, GBPUA&T, Hill Campus, Ranichauri, Uttarakhand, India.

The talc based formulation of *Pseudomonas fluorescens* (Pf-173) and *Trichoderma harzianum having* 2×10^8 cfu 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 per kg seed, T_2 = *P. f.* (@ 6 g per kg seed, T_3 = *P. f.* (@ 8 g per kg seed, T_4 = *T. h.* @ 4 g per kg seed, T_5 = *T. h.* @ 6 g per kg seed, T_6 = *T. h.* @ 8 g per kg seed, T_7 = *P. f.* +*T. h.* @ (2+2) g per kg seed, T_8 = *P. f.* +*T. h.* @ (3+3) g per kg seed, T_9 = *P. f.* +*T. h.* @ (4+4) g per kg seed. Added bio-agent to the seed were mixed gently so that the bacterial cells in the formulation get

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absorbed on seed surface and subsequently seeds were air dried in shade. The experiments were laid out in a randomized block design with three replications. Each row of 3.5 meter with row to row spacing 23 cm and plant to plant 5 cm were kept.

To know the field germination per cent, 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 1000-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. Under laboratory to know the seed germination per cent, 100 seeds of three replication for each treatment were tested. The seeds were kept in between paper (BP) and then placed in a germinator at 20 ^{0}C. Normal seedling were recorded on 8th day and germination expressed as the percentage of seed which produced normal seedlings. After the germination count, ten seedlings from every replication of each treatment were used to measure for the root, shoot and seedling length, fresh weight and dry weight of seedlings (ISTA, 1985). Seedling dry weight was measured after subjecting the samples in an air oven at 80^{0}C for 24 h. 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 (Abdul-Baki and Anderson, 1993). The two consecutive years of field as well as laboratory data were also pooled and analyzed following Panse and Sukhatme, (1967).

RESULTS AND DISCUSSION

The enhancement of pre-sowing seed bio-inoculation on seed yield and its contributing attributes for wheat (*Triticum aestivum* L. cv. VL 832) are presented in table 1a and 1b, while subsequent seed quality parameters are mentioned in Table 2a and 2b.

Field parameters

The pooled mean of two successive year for field germination was found also significantly maximum (80.16 %) in seed treated with *P.f. + T.h.* @ (4+4) g/kg seed while, statistically least field germination (64.83%) was recorded for uninoculated control. Seed inoculation with *P. fluorescens* increase in seedling emergence rate in winter wheat was reported by Freitas and Germida (1970) and with *T. harzianum* resulted increased percentage of emergence as compared to control in tomato (Ahmed and Baker, 1988). Data also depicted that pooled mean of two year, the root length as well as plant height (6.73 cm and 64.49 cm) respectively, was significantly greater again for T_9 treatment *i.e.* seed treated with *P. fluorescens + T. harzianum* mixture @ (4+4) g/kg seed while, the result at par with T_8 i.e. *P.f. +T.h.* @ (3+3) g/kg seed for root length (6.68 cm). However, significantly least root length and plant height (6.11 cm and 55.03 cm) respectively were noticed for uninoculated control (Table 1a). The plant growth promoting bio-inoculants stimulate plant growth by a plethora of mechanism. This is in conformity with the findings of Kumar (1998) and Vessey (2004).

Table (1b) denoted that the significantly maximum pooled value for ear length and number of seed per ear (7.40 cm and 62.49) respectively, for T_9 over all the treatments while, the result did not differ with T_8 for number of seed per ear (61.59). However, least pooled value with respect to ear length (6.43 cm) and number of seed per ear (48.68) was observed for control. Maximum 1000-seed weight and yield/ha and was found (44.43 g and 25.44 q/ha) in seed treated with *P.f.+T.h.* @ (4+4) g/kg seed while, results at par (43.76 g and 25.10 q/ha) with T_8 treatment *i.e. P.f.+T.h.* @ (3+3) g/kg seed for both traits. However, lowest pooled value for 1000-seed weight (38.56 g) and yield (19.63 q/ha) was measured for uninoculated control. These findings have also been confirmation of the work with Varshney and Chaube (1997) reported that application of *Pseudomonas fluorescens* and *Trichoderma harzionum* increased the growth and yield of tomato and significantly increased plant height, 100-seed weight, number of seed/ear and seed yield in maize through seed inoculation of *P. fluorescens* (Nezarat and Gholami, 2009).

Laboratory parameters

The pooled data of two years for seed germination (89.66 %), root, shoot and seedling length (17.44, 16.39 and 33.83) cm respectively was found to be significantly maximum for the treatment with *P.f. + T.h.*@ (4+4)g/kg seed, while the results of treatment T_8 did not differ statistically for above traits, however, the results of *P.f. + T.h.*@ (2+2)g/kg seed and *P.f.*@8g/kg seed treatment were *at par* for shoot and seedling length. The significantly least pooled mean for seed germination (81.49%), root, shoot and seedling length (12.84, 12.02 and 24.86 cm) respectively was found in un-inoculated control compare to all the inoculated treatments. Pooled mean indicated that the cumulative effect of both bio-agents had greater influence on fresh and dry weight of seedlings and significantly higher values (3.35g & 0.300g) respectively were weighed for bio-agent mixture *P. f + T. h.*@ (4+4) g/kg seed, results at par (3.23g & 0.295g) with the treatment of bio-agent mixture *P. f + T. h.*@ (3+3) g/kg seed for both

parameters respectively, while the results of pooled values were also at par for fresh weight (3.05g & 3.02g) of treatment bio-agent mixture *P. f* + *T. h.*@ (2+2) g/kg seed and seed treated with *T.h.* @8g/kg seed, respectively (Table 2b). An increased seed germination, shoot length, root length, fresh and dry weight have also been reported by Ashrafuzzaman *et al*., (2009) through seed bacterization in rice and through *T.* v*iride* and *P. fluorescens* in tomato seedling (Manoramhitham *et al*., 2001).

The pooled values of two years for vigour index I and II were also influenced with microbial seed inoculation and significantly maximum influence (3034.47 AND 26.90) was calculated in treatment *P.f.* + *T.h.*@ (4+4)g/kg seed for both the vigour index respectively, while the pooled mean for vigour index I & II were at par (2963.62 and 26.67) respectively for seed inoculated with *P.f.* + *T.h.*@ (3+3)g/kg seed treatment. However, the value of vigour index I for treatment *P.f.* + *T.h.*@ (2+2)g/kg seed (2823..35) and *P.f.*@ 8g/kg seed (2821.81) was statistically similar and did not differ significantly. In pertaining to uninoculated control treatment had the significantly lowest pooled value (2026.50 and 17.11) for vigour index I and II respectively.

The paper can be concluded that seed treated with combination of these two bio-inoculants results better than used a lonely either with *P. fluorescens* or *T. harzianum* for most of the field as well as laboratory parameters. Increases 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 stimulation of growth of plant by nutrients secreted by the plant root.

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Table 1(a): Enhancement of field germination, root length and plant height by pre-sowing seed inoculation in wheat

Parameters→	Field germination(%)			Root length (cm)			Plant height (cm)		
Treatments↓	2007-08	2008-09	Pooled	2007-08	2008-09	Pooled	2007-08	2008-09	Pooled
P. f. @ 4 g / kg seed	75.00 (60.01)	71.33 (57.63)	73.16 (58.82)	6.46	6.33	6.39	62.10	60.13	61.11
P. f. @ 6 g / kg seed	75.66 (60.44)	71.66 (57.84)	73.66 (59.14)	6.46	6.43	6.44	65.46	62.66	64.06
P. f. @ 8 g / kg seed	78.66 (62.51)	76.66 (61.11)	77.66 (61.81)	6.60	6.50	6.55	67.36	65.46	66.41
T. h. @ 4 g / kg seed	73.66 (59.12)	69.66 (56.58)	71.66 (57.85)	6.26	6.23	6.24	59.50	57.33	58.41
T. h. @ 6 g / kg seed	73.66 (59.12)	71.33 (57.62)	72.49 (58.37)	6.40	6.30	6.35	59.76	58.20	58.98
T. h. @ 8 g / kg seed	74.00 (59.35)	72.33 (58.26)	73.16 (58.80)	6.56	6.46	6.51	61.70	59.56	60.63
P. f. + *T. h.* @ (2+2) g / kg seed	74.33 (59.56)	73.66 (59.13)	73.99 (59.34)	6.50	6.36	6.43	63.33	60.30	61.81
P. f. + *T. h.* @ (3+3) g / kg seed	80.00 (63.46)	76.33 (60.90)	78.16 (62.18)	6.70	6.66	6.68	64.50	62.33	63.41
P. f. + *T. h.* @ (4+4) g / kg seed	81.00 (64.19)	79.33 (62.96)	80.16 (63.57)	6.80	6.66	6.73	65.43	63.56	64.49
Control (uninoculated)	66.00 (54.33)	63.66 (52.93)	64.83 (53.63)	6.13	6.10	6.11	56.26	53.80	55.03
Average	60.21	58.50	59.35	6.49	6.40	6.44	62.54	60.33	61.43
SEm (±)	**0.74**	**0.69**	**0.37**	**0.06**	**0.07**	**0.23**	**0.75**	**0.66**	**0.22**
LSD (0.05)	**2.20**	**2.06**	**1.20**	**0.19**	**0.21**	**0.07**	**2.24**	**1.96**	**0.72**
CV (%)	**2.13**	**2.06**	**0.90**	**1.73**	**1.96**	**0.51**	**2.09**	**1.90**	**0.52**

P. f. = *Pseudomonas fluorescens* and *T. h.* = *Trichoderma harzianum,* are given in parentheses

Table 1(b): Enhancement of ear length, No. of seed/ ear, 1000-seed weight and seed yield by pre-sowing seed inoculation in wheat

Parameters→	Length of ear (cm)			No. of seed/ ear			1000- seed weight (g)			Seed yield (q/ ha^{-1})		
Treatments↓	**2007-08**	**2008-09**	**Pooled**	**2007-08**	**2008-09**	**Pooled**	**2007-08**	**2008-09**	**Pooled**	**2007-08**	**2008-09**	**Pooled**
P. f. @ 4 g / kg seed	6.96	6.73	6.84	55.93	55.30	55.61	42.27	41.09	41.68	23.02	21.71	22.36
P. f. @ 6 g / kg seed	7.06	7.03	7.04	59.03	56.46	57.74	42.64	41.93	42.28	23.69	22.91	23.30
P. f. @ 8 g / kg seed	7.16	7.10	7.13	61.96	57.43	59.69	42.96	42.81	42.88	24.88	23.79	24.33
T. h. @ 4 g / kg seed	7.06	6.83	6.94	54.53	53.43	53.98	41.34	40.89	41.11	22.40	21.16	21.78
T. h. @ 6 g / kg seed	7.10	7.06	7.08	58.13	56.76	57.44	42.27	42.87	42.57	23.65	22.14	22.89
T. h. @ 8 g / kg seed	7.13	7.10	7.11	61.00	57.76	59.38	42.59	42.73	42.66	24.20	23.17	23.68
P. f. + T. h. @ (2+2) g / kg seed	7.23	7.03	7.13	60.93	56.10	58.51	43.01	42.77	42.89	24.30	23.53	23.91
P. f. + T. h. @ (3+3) g / kg seed	7.30	7.23	7.26	62.96	60.23	61.59	44.22	43.31	43.76	25.62	24.58	25.10
P. f. + T. h. @ (4+4) g / kg seed	7.40	7.40	7.40	63.46	61.53	62.49	44.89	43.97	44.43	25.94	24.94	25.44
Control (uninoculated)	6.46	6.40	6.43	48.86	48.50	48.68	39.23	37.89	38.56	20.15	19.11	19.63
Average	7.08	6.99	7.03	58.68	56.35	57.91	42.54	42.02	42.28	23.78	22.70	23.24
SEm (±)	**0.07**	**0.07**	**0.04**	**0.65**	**0.69**	**0.77**	**0.29**	**0.40**	**0.30**	**0.21**	**0.55**	**0.11**
LSD (0.05)	**0.22**	**0.21**	**0.14**	**1.95**	**2.05**	**2.47**	**0.88**	**1.19**	**0.97**	**0.65**	**1.64**	**0.36**
CV (%)	**1.83**	**1.81**	**0.89**	**1.93**	**2.12**	**1.90**	**1.21**	**1.65**	**1.02**	**1.59**	**4.22**	**0.68**

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

Table 2(a): Enhancement of subsequent seed quality (germination, root and shoot length) by pre sowing seed inoculation in wheat

Parameters→ / Treatments↓	Germination(%)			Root length (cm)			Shoot length (cm)		
	2008	2009	Pooled	2008	2009	Pooled	2008	2009	Pooled
P. f. @ 4 g / kg seed	88.00 (69.82)	87.00 (68.92)	87.50 (69.37)	16.05	15.04	15.54	14.98	14.13	14.55
P. f. @ 6 g / kg seed	88.33 (70.05)	87.66 (69.48)	87.99 (69.76)	16.41	15.46	15.93	15.37	14.52	14.94
P. f. @ 8 g / kg seed	89.00 (70.43)	88.00 (69.21)	88.50 (69.82)	17.05	15.81	16.43	16.04	14.86	15.45
T. h. @ 4 g / kg seed	87.66 (69.46)	87.33 (69.16)	87.49 (69.31)	15.81	14.89	15.35	14.82	13.98	14.40
T. h. @ 6 g / kg seed	88.00 (69.90)	87.66 (69.48)	87.83 (69.69)	16.22	15.48	15.85	15.20	14.56	14.88
T. h. @ 8 g / kg seed	88.66 (70.39)	88.33 (70.05)	88.49 (70.22)	16.58	15.89	16.23	15.54	14.94	15.24
P. f. + *T. h.* @ (2+2) g / kg seed	89.00 (70.66)	88.00 (69.97)	88.50 (70.31)	16.80	15.92	16.36	15.78	15.32	15.55
P. f. + *T. h.* @ (3+3) g / kg seed	89.66 (71.37)	89.00 (70.64)	89.33 (71.00)	17.28	16.85	17.06	15.97	15.90	15.93
P. f. + *T. h.* @ (4+4) g / kg seed	90.00 (71.68)	89.33 (70.96)	89.66 (71.32)	17.75	17.13	17.44	16.62	16.16	16.39
Control (uninoculated)	81.66 (64.66)	81.33 (64.40)	81.49 (64.53)	12.9 4	12.74	12.84	12.22	11.82	12.02
Average	69.84	69.23	69.53	16.29	15.52	15.90	15.25	14.62	14.93
SEm (±)	**1.40**	**1.17**	**0.40**	**0.17**	**0.23**	**0.40**	**0.18**	**0.26**	**0.35**
LSD (0.05)	**4.13**	**3.46**	**1.07**	**0.52**	**0.68**	**1.29**	**0.55**	**0.78**	**1.10**
CV (%)	**3.47**	**2.94**	**0.69**	**1.88**	**2.60**	**3.64**	**2.12**	**3.14**	**3.31**

P. f. = *Pseudomonas fluorescens* and *T. h.* = *Trichoderma harzianum,* Transformed values are given in parentheses

Table 2(b): Enhancement of subsequent seed quality (length, fresh & dry weight of seedling and vigour index) by pre sowing seed inoculation in wheat

Parameters→	Seedling length (cm)			Fresh weight (g)			Dry weight (g)			Vigour index I			Vigour index II		
Treatments↓	2008	2009	Pooled	2008	2009	Pooled	2008	2009	Pooled	2008	2009	Pooled	2008	2009	Pooled
P. f. @ 4 g / kg seed	31.03	29.17	30.10	2.99	2.67	2.83	0.24	0.25	0.245	2732.12	2538.15	2635.13	21.70	22.02	21.86
P. f. @ 6 g / kg seed	31.78	29.99	30.88	3.08	2.82	2.95	0.26	0.26	0.260	2808.28	2630.59	2719.43	22.97	22.80	22.88
P. f. @ 8 g / kg seed	33.09	30.67	31.88	3.20	2.96	3.08	0.27	0.27	0.270	2945.08	2698.54	2821.81	24.31	24.04	24.17
T. h. @ 4 g / kg seed	30.63	28.75	29.69	2.93	2.72	2.82	0.24	0.25	0.245	2684.90	2521.89	2603.39	21.04	21.83	21.43
T. h. @ 6 g / kg seed	31.42	30.05	30.73	3.12	2.73	2.92	0.25	0.25	0.250	2766.38	2634.20	2700.29	21.97	22.20	22.08
T. h. @ 8 g / kg seed	32.12	30.84	31.48	3.09	2.95	3.02	0.27	0.27	0.270	2848.04	2724.44	2786.24	23.93	23.84	23.88
P.f+*T.h.*@(2+2)g/kg seed	32.58	31.24	31.91	3.08	3.02	3.05	0.27	0.28	0.275	2899.60	2747.11	2823.35	24.61	23.97	24.29
P.f+*T.h.*@(3+3)g/kg seed	33.59	32.75	33.17	3.26	3.21	3.23	0.30	0.29	0.295	3011.05	2916.20	2963.62	26.93	26.41	26.67
P.f+*T.h.*@(4+4)g/kg seed	34.38	33.29	33.83	3.45	3.25	3.35	0.30	0.30	0.300	3094.69	2974.26	3034.47	27.01	26.79	26.90
Control (uninoculated)	25.16	24.56	24.86	2.51	2.35	2.43	0.21	0.20	0.205	2056.04	1996.96	2026.50	17.41	16.81	17.11
Average	31.58	30.13	30.85	3.07	2.86	2.96	0.26	0.26	0.261	2784.62	2638.23	2711.42	23.19	23.07	23.13
SEm (±)	**0.35**	**0.49**	**0.76**	**0.06**	**0.06**	**0.11**	**0.006**	**0.006**	**0.003**	**61.39**	**55.71**	**77.39**	**0.67**	**0.60**	**0.22**
LSD (0.05)	**1.03**	**1.45**	**2.42**	**0.19**	**0.19**	**0.35**	**0.19**	**0.018**	**0.011**	**181.12**	**164.35**	**243.79**	**1.98**	**1.79**	**0.70**
CV (%)	**1.92**	**2.83**	**3.52**	**3.69**	**4.05**	**5.40**	**4.26**	**4.08**	**1.91**	**3.81**	**3.65**	**4.03**	**5.02**	**4.55**	**1.36**

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