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Short Communication

Influence of Biopriming with Microbial Inoculants on Seed Quality Parameters in Soybean

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Soybean is known as a world's most important crop due to high content of protein as well as oil content. Soybean crop is most important source of vegetable oil in India that occupies 35-65% of total oilseed crop in country. Soybean is a major oilseed crop grown during *Kharif* season. The improvement in seed quality in Soybean (*Glycine max* L. Merrill) by priming treatments is attributed to primary reduction of lipid peroxidation and quantitative changes in biochemical activities inducing greater amylase activity increasing per cent sugar during seed germination. The seed has consistently been a key factor in agriculture. Modern crop production and the study of agriculture affirm that without seed quality we won't have an effective production.

Seed quality means that the seed has maximum genetic purity, physical purity, optimum moisture content, free of insect pests and is in good physical condition according to the standard set for seed certification. Soybean is grown in different agro-ecological conditions; hence seed germination and vigour are also influenced by various unfavorable environmental factors such as extreme temperature, drought, untimely planting and so forth. Reddy [1] explained bio-priming on bio-control aspects as application of beneficial bacterial inoculum to the seeds and their hydration protects seeds against seed borne diseases. Seed biopriming is being focused as it ensures the entrance of entophytic bacteria into the sides along with avoiding the effect of high temperature. Bio-priming treatment is potentially able to promote quick and even germination as well as better plant growth [2]. Interaction between Trichoderma and Pseudomonas may operate independently or together and their activities can result in the suppression of the plant pathogens [3, 4]. Thus, there is need to improve the effectiveness or significant measure of external inputs by utilizing the best mix of useful micro-organisms by priming of seed for enhancing the planting value, germination, take-up of inorganic phosphate, plant development, seed yield and its ensuing quality. The objective of this study was to assess the influence of seed bio-priming on seed quality parameters.

The experimental material consists of four varieties from which three varieties *viz*. Shilajeet, PK 327 and PS 1092 were taken from Soybean Breeding Station, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar and one variety PRS 1 was obtained from Research Station, Gaja, College of Forestry, Ranichauri and three Bio-agents *viz. Pseudomonas fluorescens* (Psf-173), *Trichoderma harzianum* (Th-14) and Phosphorus Solubilizing Bacteria (PSB) were obtained from Plant Pathology Section, College of Forestry, Ranichauri, Tehri Garhwal.

Four varieties of soybean namely Shilajeet (V1), PK 327 (V2), PS 1092 (V3) and PRS1 (V4) and five treatments consisting of three microbial inoculants *i.e.* phosphorous solubilizing bacteria (PSB) (T1), *Pseudomonas fluorescens* (Psf-173) (T2), *Trichoderma harzianum* (Th-14) (T3) and a combination of *Pseudomonas fluorescens* (Pf-173) and *Trichoderma harzianum* (Th-14) (T4) along with uninoculated control (T5) were taken to conduct the experiment.

Before sowing, seeds were surface sterilized in one per cent sodium hypochlorite solution for three minutes, rinsed with sterilized water and air dried. For seed biopriming, seeds were separately treated with talc formulation of different bio-agent i.e. Phosphorous solubilizing bacteria (PSB), *Pseudomonas fluorescens* (Pf-173), *Trichoderma harzianum* (Th-14) at the recommended dose of 8g/Kg of seeds while the treatment of mixture of *Pseudomonas fluorescens* with *Trichoderma harzianum* was added in 50:50 ratio at the recommended dose of 8g (4g + 4g each)/kg of seeds. Untreated seeds

were considered as a control. Seeds were then kept under warm and moist conditions at 28°C for 24h until prior to radical emergence.

After the completion of harvesting, threshing and winnowing process, seeds were kept into the cloth bag and provided sun drying continuously to maintain the optimum moisture level. These seeds were further used for conducting a laboratory experiment and were evaluated for seed quality characters under the laboratory of Department of Seed Science and Technology, College of Forestry, Ranichauri, Tehri Garhwal, Uttarakhand. The observations were recorded under laboratory condition on nine quality parameters *viz*.; first count, standard germination, root length, shoot length, seedling length, seedling fresh weight, seedling dry weight and vigour index I and II.

The seeds of each variety as 4 x 100 were germinated in between paper (B.P.) media as per the recommendations of ISTA [5]. Then the samples were placed at 25°C in BOD germinator. Only normal seedlings were counted on the 5th day of test and multiply it by 100 as first count per cent. At the end of the germination period, the seedlings were counted on 8th day. The germination test was evaluated as normal seedlings, abnormal seedlings and dead seed and the germination was reported in percentage adopting the following formula as per the standard procedure [5].

$$\text{Germination per cent} = \frac{\text{Normal seedlings}}{\text{Total number of seed}} \times 100$$

Among the normal seedlings ten seedlings were randomly selected on 8th day of germination test from each replication of each treatment and measured with the help of measuring scale for root length and shoot length. The value was obtained by calculating mean of 10 seedlings for each replication in centimeter. The total length of seedlings (cm) was obtained by adding shoot and root length as recorded earlier. The ten normal seedlings were weighed and the seed fresh weight was measured on an electronic balance in gram. For dry weight seedlings were dried in oven at 80°C for 24h. The dried seedling were weighed on an electronic balance and expressed as gram. Based on the results obtained, the vigour index values were computed the seedling vigour index I was calculated as per the following formula: Standard germination (%) × Seedling length (cm) and the seedling vigour index II was calculated as per the following formula: Standard germination (%) × Seedling dry weight (g) [6]) and the values were reported as whole number without unit. The critical difference at 5 per cent level of significance was calculated to compare the mean different treatment.

Under laboratory condition results suggested that seed germination and seed vigour were greatly affected by different bio-agents. Among all the bio-agents, PSB showed positive influence on most of the quality parameter and showed significant differences due to varieties, treatments as well as varieties x treatments. However, other bio-agents had also showed significant effect over control. These results indicated that bio-priming caused considerable influence on different varieties of soybean as given below.

Analysis of variance revealed significant differences for the effect of different bio-agent on germination percentage for first count (Table 1). Phosphorous solubilizing bacteria (74.33%) showed maximum first count followed by Psf-173 (69.16%) and Psf-173 +Th-14 (65.00%) over control (53.33%). Varying varietal mean also exhibited significant

Table 1. Influence of seed bio-priming on first count of soybean varieties

Treatments	First count (%)				
	Shilajeet (V_1)	PK 327 (V_2)	PS 1092 (V_3)	PRS 1 (V_4)	Mean
PSB (T_1)	77.33(61.6)*	73.33(58.9)	72.00(58.1)	74.66(59.8)	74.33(59.6)
Psf -173 (T_2)	74.66(59.8)	70.66(57.2)	68.00(55.6)	63.33(52.8)	69.16(56.3)
Th-14 (T_3)	58.66(50.0)	57.33(49.2)	61.33(51.6)	54.66(47.7)	58.00(49.6)
Psf-173 + Th-14 (T_4)	70.66(57.2)	69.33(56.4)	65.33(54.0)	54.66(47.7)	65.00(53.8)
Control (T_5)	53.33(46.9)	49.33(44.6)	60.00(50.8)	50.66(45.4)	53.33(46.9)
Mean	66.93(54.9)	64.00(53.2)	65.33(54.0)	59.60(50.6)	63.96(53.1)
	Variety	Treatment	Variety × Treatment		
SEm(±)	0.797	0.891	1.782		
CD(p=0.05)	2.278	2.547	5.095		

*Figures in parentheses are the transformed values

effect on first count. Shilajeet variety showed maximum first count (66.93 %) which was statistically at par with PS 1092 (65.33%) while minimum first count was recorded in PRS -1 variety (59.60%). Interaction of both the factor also showed significant difference for first count. The maximum value for first count was recorded in PSB primed Shilajeet variety (77.33%) which was at par with Psf-173 primed Shilajeet varieties, PSB bio-primed Shilajeet variety (74.66 %) and PSB bio-primed PK 327 (73.33%) while minimum was recorded in un-inoculated PK327 (49.33%).

Treatment mean was found significant on standard germination (Table 2). The maximum standard germination was recorded for Psf-173 (93.66%) followed by PSB (87.66%) and Psf-173 + Th-14 (84.66%) over control (65.66%). Different varieties also exhibited significant effect on standard germination. The maximum standard germination was recorded in Shilajeet variety (84.80%) followed by PS 1092 (81.60%) and PRS 1 (81.06%) while the minimum was recorded in PK 327 variety (76.80%). Interaction due to varieties and treatment was also showed significant difference for standard germination. The maximum value for standard germination was recorded in Shilajeet variety that was bio-primed with Psf-173 (97.33%) which was statistically at par with Shilajeet variety bio-primed with Psf-173 +Th-14, PS 1092 bio-primed with Pf (93.33%) and PK 327 bio-primed with Psf-173 (92.00 %) while minimum was recorded in uninoculated PK327 (49.33%).

Treatment mean was found significant for seedling length (Table 3). The maximum seedling length was recorded in PSB (18.98cm) followed by Psf-173 + Th-14 (18.05cm) and Psf-173 (16.39cm) over control (14.29cm). Varietal mean showed significant difference for seedling length. The maximum seedling length was found in Shilajeet variety (18.43cm) then other three varieties of soybean. Interaction due to varieties and treatment was also showed significant difference for seedling length. The

Table 2. Influence of seed bio-priming on standard germination of soybean varieties

Treatments	Standard germination (%)				
	Shilajeet (V_1)	PK 327 (V_2)	PS 1092 (V_3)	PRS 1 (V_4)	Mean
PSB (T_1)	90.66(72.2)	89.33(71.0)	85.33(67.5)	85.33(67.5)	87.66(69.5)
Psf -173 (T_2)	97.33(80.6)	92.00(73.6)	93.33(75.1)	92.00(73.6)	93.66(75.5)
Th-14 (T_3)	77.33(61.6)	72.00(58.1)	74.66(59.8)	70.66(57.2)	73.66(59.2)
Psf-173 + Th-14 (T_4)	93.33(75.1)	70.66(57.2)	86.66(68.6)	88.00(69.8)	84.66(67.0)
Control (T_5)	65.33(54.0)	60.00(50.8)	68.00(55.6)	69.33(56.4)	65.66(54.2)
Mean	84.80(67.1)	76.80(61.2)	81.60(64.6)	81.06(64.2)	81.06(64.2)
	Variety	Treatment	Variety × Treatment		
SEm(±)	1.123	1.256	2.512		
CD(p=0.05)	3.211	3.590	7.180		

*Figures in parentheses are the transformed values

Table 3. Influence of seed bio-priming on seedling length of soybean varieties

Treatments	Seedling length (cm)				
	Shilajeet (V_1)	PK 327 (V_2)	PS 1092 (V_3)	PRS 1 (V_4)	Mean
PSB (T_1)	21.50	18.19	19.38	16.85	18.98
Psf -173 (T_2)	17.85	17.26	16.58	13.89	16.39
Th-14 (T_3)	16.91	14.80	15.14	13.54	15.10
Psf-173 + Th-14 (T_4)	19.64	17.84	18.62	16.10	18.05
Control (T_5)	16.27	13.90	14.50	12.50	14.29
Mean	18.43	16.39	16.84	14.57	16.56
	Variety	Treatment	Variety × Treatment		
SEm(±)	0.787	0.906	0.181		
CD(p=0.05)	0.231	0.258	0.517		

highest seedling length was recorded in Shilajeet when bio-primed with bio-agent PSB (21.50cm) than the other interactions.

Treatment mean was found significant for seedling fresh weight (Table 4). The maximum fresh weight was recorded in PSB (8.00g) followed by Psf-173 + Th-14 (6.95g) over control (5.14g). Varietal mean showed significant difference for seedling fresh weight. The maximum fresh weight was found in Shilajeet variety (7.93g) followed by PS 1092 (7.00g) and PK 327 (5.82g) whereas the minimum value for seedling fresh weight was observed in PRS 1 (5.42g). Interaction due to varieties and treatment was also showed significant difference for seedling fresh weight. The highest fresh weight was recorded in Shilajeet when bio-primed with bio-agent PSB (9.45g) whereas, minimum was recorded in un-inoculated PK327 (4.30g).

Treatment mean was found significant for seedling dry weight (Table 5). The maximum dry weight was recorded in PSB (1.31g) which was statistically at par with Psf-173 + Th-14 (1.23g), Psf-173 (1.17g) and Th-14 (1.11g) over control (0.96g). Varietal mean showed significant difference for seedling dry weight. The maximum dry weight was found in Shilajeet variety (1.32g) which was statistically at par with PS 1092 (1.24g) than the other three varieties. Interaction due to varieties and treatment was also showed significant difference for seedling dry weight. The highest fresh weight was recorded in Shilajeet when bio-primed with bio-agent PSB (1.47g) whereas, minimum was recorded in un-inoculated PRS 1 (0.72g).

Treatment mean was found significant for vigour index I (Table 6). The highest vigour index I was recorded in PSB treatment (1666.80) followed by Psf-173 (1538.12), Psf-173 + Th-14 (1531.31) over control (937.33). Varietal

Table 4. Influence of seed bio-priming on seedling fresh weight of soybean varieties

Treatments	Seedling fresh weight (g)				
	Shilajeet (V_1)	PK 327 (V_2)	PS 1092 (V_3)	PRS 1 (V_4)	Mean
PSB (T_1)	9.45	7.46	8.75	6.34	8.00
Psf -173 (T_2)	8.15	5.69	7.02	5.28	6.53
Th-14 (T_3)	7.90	5.46	6.01	5.05	6.10
Psf-173 + Th-14 (T_4)	8.33	6.23	7.41	5.82	6.95
Control (T_5)	5.81	4.30	5.84	4.62	5.14
Mean	7.93	5.82	7.00	5.42	6.54
	Variety	Treatment	Variety × Treatment		
SEm(±)	0.448	0.500	0.100		
CD(p=0.05)	0.128	0.143	0.286		

Table 5. Influence of seed bio-priming on seedling dry weight of soybean varieties

Treatments	Seedling dry weight (g)				
	Shilajeet (V_1)	PK 327 (V_2)	PS 1092 (V_3)	PRS 1 (V_4)	Mean
PSB (T_1)	1.47	1.18	1.36	1.25	1.31
Psf -173 (T_2)	1.37	1.11	1.25	0.97	1.17
Th-14 (T_3)	1.32	1.04	1.22	0.85	1.11
Psf-173 + Th-14 (T_4)	1.43	1.14	1.29	1.06	1.23
Control (T_5)	1.03	1.02	1.08	0.72	0.96
Mean	1.32	1.09	1.24	0.97	1.16
	Variety	Treatment	Variety × Treatment		
SEm(±)	0.754	0.844	0.168		
CD(p=0.05)	0.215	0.241	0.482		

Table 6. Influence of seed bio-priming on vigour index I of soybean varieties

Treatments	Vigour index I				
	Shilajeet (V_1)	PK 327 (V_2)	PS 1092 (V_3)	PRS 1 (V_4)	Mean
PSB (T_1)	1951.62	1625.18	1652.40	1438.02	1666.80
Psf -173 (T_2)	1737.91	1588.07	1548.13	1278.36	1538.12
Th-14 (T_3)	1308.11	1065.52	1130.60	956.79	1115.25
Psf-173 + Th-14 (T_4)	1833.49	1259.74	1615.38	1416.62	1531.31
Control (T_5)	1063.24	834.20	985.40	866.48	937.33
Mean	1578.87	1274.54	1386.38	1191.25	1357.76
	Variety	Treatment	Variety × Treatment		
SEm(±)	21.413	23.941	47.882		
CD(p=0.05)	61.206	68.430	136.861		

Table 7. Influence of seed bio-priming on vigour index II of soybean varieties

Treatments	Vigour index II				
	Shilajeet (V_1)	PK 327 (V_2)	PS 1092 (V_3)	PRS 1 (V_4)	Mean
PSB (T_1)	133.17	105.54	115.97	107.55	115.56
Psf -173 (T_2)	133.30	102.22	116.54	89.17	110.31
Th-14 (T_3)	102.05	74.80	91.05	60.78	82.17
Psf-173 + Th-14 (T_4)	133.45	80.54	112.73	94.16	105.22
Control (T_5)	67.29	61.21	73.52	50.66	63.17
Mean	113.85	84.86	101.96	80.46	95.28
	Variety	Treatment	Variety × Treatment		
SEm(±)	1.478	1.652	3.305		
CD(p=0.05)	4.224	4.723	9.446		

mean showed significant difference for vigour index I. The Shilajeet variety showed maximum vigour index I (1578.87) followed by PS 1092 (1386.38) and PK 327 (1274.54) whereas, minimum was recorded in PRS 1 (1191.25). Interaction due to varieties and treatment was also showed significant difference for vigour index I. The highest vigour index I was found when Shilajeet bio-primed with PSB (1951.62) which was statistically at par with Shilajeet bio-primed with Psf-173 + Th-14 (1833.49). However, minimum was found in un-inoculated PK 327 (834.20).

Treatment mean was found significant for vigour index II (Table 7). The highest vigour index II was recorded in PSB treatment (115.56) followed by Psf-173 (110.31) and Psf-173 + Th-14 (105.22) over control (63.17). Varietal mean showed significant difference for vigour index II. The Shilajeet variety showed maximum vigour index II (113.85) followed by PS 1092 (101.96) and PK 327 (84.86) whereas, minimum was recorded in PRS 1 (80.46). Interaction due to varieties and treatment was also showed significant difference for vigour index II. The highest vigour index II was found when Shilajeet bioprimed with Psf-173 +Th-14 (133.45) which was statistically at par with Shilajeet bio-primed with Psf-173 (133.30) and Shilajeet bio-primed with PSB (133.17). However, minimum was found in un-inoculated PRS 1 (50.66).

Seed priming is an important process for the reduction of germination time. It reduces up to 50% time of seedling emergence. Primed seeds enhance seed germination and stand establishment in the field condition due to metabolic enzyme activity. Seed priming techniques are being used to reduce the germination time, synchronize germination, improved germination rate and increase plant stand [7]. The promoting effects of the different treatments on speed of emergence and field

establishment may be due to enhanced hydration of all seed parts and thus reducing the damage of embryonic axis [8].

In the present study seed bio-priming enhanced first count, germination percentage, root and shoot length, seedling length, seedling fresh weight, seedling dry weight, vigour index I and II. Seed priming was also reduced time from sowing to germination. Biopriming of seeds with *Trichoderma harzianum* and *Pseudomonas fluorescence* 40% concentration for 4 hours enhance the seed quality parameters [9]. Bio-primed seeds with different bio-agent showed higher first count and standard germination over control. This might be due to biostimulants and phytohormones produced by microbial inoculants. These findings were in close agreement with [10] in soybean and [11] in pea.

In the present study seed bio-primed with PSB showed highest seedling fresh and dry weight. Result of present study also revealed that primed seed increase the root and shoot length. Similar finding was also reported in Soybean [12] and in Lentil [13]. The possible reason of root and shoot elongation and increase in seedling length might be due to growth response attributed to IAA production and containing increases amount of phosphorous which uptake with the help of phosphorous solubilizing bacteria (PSB). They suggested that increase in fresh and dry weight might be due to production of phytohormones like auxins, cytokinin and gibberellins and also microbial inoculants provides more uptake of nutrient from soil.

Present study also indicated that primed seed had highest seed vigour over un-inoculated seed. Such types of finding were also reported by Dwivedi and Ram Gopal [12] in Soybean and Rawat and Prasad [13] in Lentil. They suggested that vigour of seed indicated the quality of seed and it might be increased by increasing the availability of soluble phosphorous, antagonistic property by phosphate solubilizing bacteria and increase more vigorous plant growth. It is reported that for germination percentage, primed seeds had lower Mean Emergence Time (MET) compared with non-primed seeds. These positive effects on germination might be due to the stimulatory effects of priming on the beginning times of germination process of germination process by mediation of cell division in germinating seeds [14]. It has been reported that primed seeds showed better germination pattern and higher vigor level than non- primed [15].

On the basis of laboratory experiment, PSB proved to be the best bio-agent for enhancing seed quality parameters than other tested bio-agents and in respect of selected varieties for priming purpose, Shilajeet was found most promising. The interaction between PSB and Shilajeet also had good combination for above mentioned characters. It can be concluded from the present investigation that low quality seeds need to be treated before sowing for quality enhancement. The present study thus reveals that seed bio-priming enhances seed quality in soybean. It is therefore suggested that before sowing, soybean seed need to be treated with appropriate bio-agents for better quality seed.

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