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ORIGINAL RESEARCH

Differential response of native *Bacillus* spp. isolates from agricultural and forest soils in growth promotion of *Amaranthus hypochondriacus*

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ABSTRACT
Bacillus spp. has emerged as agriculturally important bacteria with its productive gears in plant growth and health promotion. In the present study, different bacilli from agricultural and forest soils were characterized and identified as plant growth promoting bacteria. The isolates from both eco-habitants showed differential responses influenced with their mechanistic traits. Agricultural soil isolates were found best with maximum IAA production (9.5 µg ml^{-1}), phosphate solubilization (11.98 µg ml^{-1}) and siderophore production. However, isolates of forest soil were potent antagonist of *Fusarium oxysporum* and *Rhizoctonia solani*. Results of drop assay using root exudates of amaranth revealed voracious root colonization ability of agricultural soil isolates. It was interesting that BS-58, the isolate of agricultural soil has shown significant outcomes in blotter paper and pot assay to enhance growth promotion of amaranth. This study reveals comparative assessment on growth promotion behaviors of agricultural and forest isolates and reported the suitability of the isolates from different eco-habitants with divergent activity.

KEY WORDS: *Agricultural soil, Antagonism, Bacillus spp., Forest soil, PGPB*

INTRODUCTION

Plant growth promoting bacteria are ideal contender for crop productivity and bear mechanistic approach to increase crop production in sustainable manner. The plant growth promoting bacteria (PGPB) are soil-borne bacteria those exist either in free or symbiotic plant associations and thereby promote the plant growth (Chauhan *et al*., 2016). In the recent scenario, these bacteria are being challenged to bear miraculous ability of niche adaptation by competing or dominating on other microbes so could be colonized in the rhizosphere with least rhizospheric rejection (Dheeman *et al*., 2017).

PGPBs are now understood for their versatile plant growth behavior compatible with wide range of hosts (Shrivastava *et al*., 2015). The holistic plant growth promoting behavior in indigenous and non-indigenous PGPBs has earlier been studied by Aeron *et al*. (2010). Further, supported with many reports those propound the utilization of non-indigenous bacteria for crop productivity enhancement (Deepa *et al*., 2010; Gopinathan and Prakash 2014). However, a few have suggested host-specificity in particular and ability to be colonized in the rhizosphere of unrelated crop(s) in general (Dasgupta *et al*., 2015; Kumar *et al*., 2017). The plant growth

promoting bacteria act as 'cook' in the rhizosphere and provide ready prepared food to plant. The phosphorus, potassium and other essential minerals required for plant growth are made available in solubilized form by PGPBs. Due to production of other metabolic weapons in the category of diffusible and non-diffusible metabolites, PGPBs are called 'security' to protect plant from deleterious phytopathogens.

Bacillus spp. are endospore formers and are able to survive in diverse climatic conditions of Uttarakhand Himalayas and exist in significant diversity (Dheeman *et al*., 2017). Numerous researches have been explored the applications of indigenous and non-indigenous *Bacillus* as biocontrol agent for the inhibition of different phytopathogens (Zalila-Kolsi *et al*., 2016). Though, the scientific community has focused on the isolation of PGPBs from the agricultural soil but, micro-organisms are omnipresent. Hence, in the present study we focused to explore out the beneficial *Bacillus* spp. from forest soil as well as agricultural soil. Forest soil is enriched with the diverse microflora not previously been exposed to the pollutants, chemical fertilizers, pesticides etc. This could be possible avenue to explore the beneficial bacteria for agricultural causes. Some of the studies have also reported the availability of PGPBs in forest soils (Deepa *et al*., 2010; Sukweenadhi *et al*., 2014). However, there is less data available on comparative performance of PGPBs isolated from forest and agriculture soils. Therefore, this study was conducted to isolate PGPBs from both the soils and to compare their potential of plant growth promotion in amaranth.

RESULTS AND DISCUSSION

In this study, a total of 140 isolates were isolated from different soil samples collected from forest and agricultural fields. Out of 140 isolates, 98 were isolated from agricultural soil samples whereas 42 were isolated from forest soils. The isolates were identified as *Bacillus* spp. and appeared as white, creamish-yellow with irregular margins, gram-positive and endospore former. Soil samples of both the eco-habitats were analyzed for their nitrogen and phosphorus content. Nitrogen and phosphorus contents were found higher in forest in comparison to agricultural soils. Higher nitrogen contents ranging from (388.00 kgha^{-1} to 467.26 kgha^{-1}) were found in different forest soil samples whereas, 235.60 kg ha^{-1} to 271.30 kg ha^{-1} nitrogen was found in the agricultural soil. Phosphorus contents were also high (ranged from 19.45 kg ha^{-1} to 44.80 kg ha^{-1}) in forest soils, however, 17.33 to 19.20 kg ha^{-1} phosphorus was available in agricultural soils. This is quite possible as forests generally have rich biodiversity that amends good amount of organic matter in soil. Additionally, presence of a number of diverse micro-flora in forest soil plays predominant role in enhanced nutrient availability by decomposition of forest liter and other organic matters. The fertility of soil is, therefore, also depends upon the microbial community and processes mediated by them (Das *et al*., 2013).

Screening for PGP traits revealed 11 phosphate solubilizers, 24 IAA producers, 6 HCN producers, and 15 siderophore producers from agricultural soils. On the other end, out of 42 isolates from forest, 4 phosphate solubilizers, 6 IAA producers, 4 HCN producers and 5 siderophore producers were observed. Among the isolates showing good PGP traits, three representative isolates from each of the two sources were selected for further study (Table 1). BS-58 showed maximum siderophore production (78.00%) among all three isolates of agricultural soil followed by BS-41 (70.00%). However, *Bacillus* spp. isolates of forest soil showed less siderophore production ranged from 35.29 to 56.86% (Fig. 1a, b). Higher IAA was produced by the isolates of agricultural soil that was ranged from 7.00-9.50 µg ml^{-1}. Among all three isolates of agricultural soil BS-58 showed highest IAA production (9.50 µg ml^{-1}) followed by BS-27 (7.90 µg ml^{-1}). However, *Bacillus* spp. isolates of forest soil showed IAA production ranged from 4.80 to 6.50 µg ml^{-1}. Among all these, maximum IAA production (6.50 µg ml^{-1}) was shown by BS-125 followed by BS-136 (6.30 µg ml^{-1}) (Fig.1c). Quantitative estimation for phosphate solubilization revealed highest phosphate solubilization (11.98 µg ml^{-1}) by the agricultural isolate BS-58 followed by BS-27 (11.60 µg ml^{-1}). While isolates from forest soil showed phosphate solubilization ranged from 3.32 µg ml^{-1} to 4.64 µg ml^{-1}. Among all the selected forest isolates maximum phosphate solubilization (4.64 µg ml^{-1}) was shown by BS-125, followed by BS-136 (4.52 µg ml^{-1}) (Fig.1d). All the selected agricultural and forest isolates were positive for HCN production as observed by yellow to moderate brown color of filter paper except BS-58 (Table 1). Agricultural isolates exhibited preeminent PGP traits in comparison to

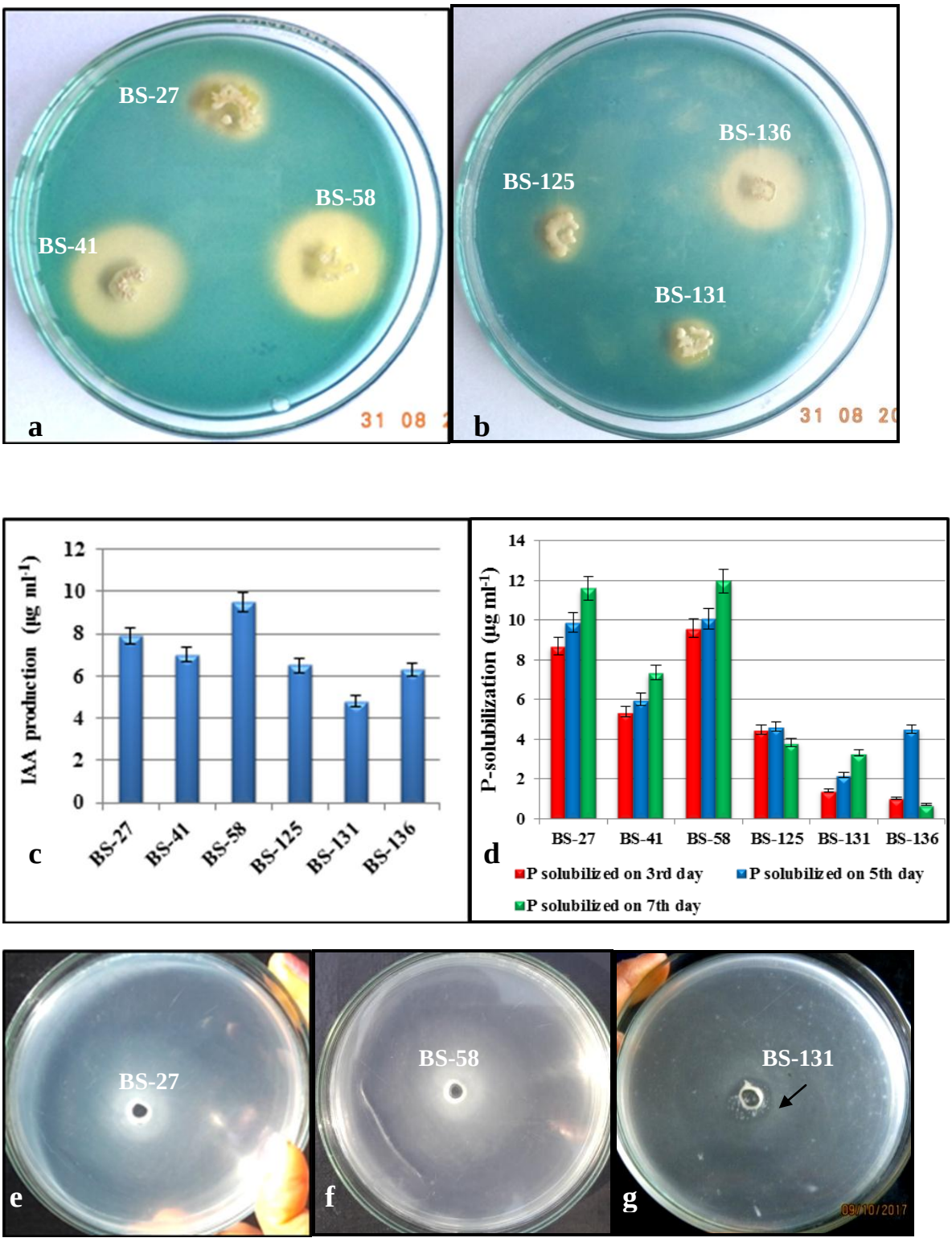

Figure 1: Plant growth promoting and chemotaxis activity of selected *Bacillus* spp. isolates. a) Siderophore production by the isolates isolated from agricultural soil; b) Isolates from forest soil; c) Quantitative estimation of IAA production by different *Bacillus* spp. isolates; d) Quantitative estimation of phosphate solubilized by different *Bacillus* isolates; e, f) Agricultural isolate BS-27 and BS-58 showing ring formation with high turbidity around the root exudates; g) Forest isolate BS-131 forming clear zone around the root exudates of amaranth (note arrow).

Table 1: Plant growth promoting traits of different *Bacillus* spp. Isolates

Isolates	IAA	P-solubilization efficiency (%)	Siderophore production (%)	Antagonistic activity		HCN production
				F. oxysporum (%)	*R. solani* (%)	
BS-27[a]	+	175.00	62.00	29.41	33.33	+
BS-41[a]	+	156.66	70.00	56.86	68.88	+
BS-58[a]	+	165.00	78.00	64.70	73.33	-
BS-125[b]	+	137.50	48.00	56.66	91.11	+
BS-131[b]	+	114.28	35.29	70.00	93.33	+
BS-136[b]	+	111.11	56.86	76.66	95.55	+

[a] Isolated from agricultural soils, [b] Isolated from forest soils, +: Positive, -: Negative. Values given in table are average of three replicates.

forest isolates. This might be due to the potential of these isolates to survive in every situation and could be correlated with nutrient scarcity in such soils that provoke them to develop effective strategies to produce/solubilize nutrients from soil. On the other end, forest isolates may take up the nutrients easily than that of other soil ecology.

All the isolates from agricultural soil and forest soil showed migratory ring with higher turbidity around the wells loaded with root exudates of amaranth (Fig.1 e, f). Similar findings have been reported by Melnick *et al*., (2008) for *Bacillus cereus* using cacao plants. Since the root exudates are the primary source of nutrients for microorganisms, therefore, suitability of these exudates to microbes is necessary for effective root colonization. In addition, root exudates may be responsible for the enhancement of the auxin biosynthesis because they are natural source of L- tryptophan act as precursor of IAA production in bacteria (Martens and Frankenberger 1994; Tsavkelova *et al*., 2007). However, BS-131 from forest soil showed a clear zone around the root exudates of amaranth (Fig.1 g) which indicates its unsuitability towards the root exudates of amaranth.

Good antagonistic activity of the selected *Bacillus* isolates from both the locations was found against two pathogenic fungi. Forest soil isolates were found potential bio-control agent in order to inhibit *F. oxysporum* and *R. solani* up to 76.66% and 95.55% respectively. On the other hand, agricultural isolates could inhibit both the deleterious fungi at a lower extent of 64.70 % and 73.33% for *F. oxysporum* and *R. solani* respectively (Fig 2, Table 1). The reason behind the excellent antagonistic activity of forest microbes could be the presence of diverse population of micro-flora in the forest and the competition for space and survival therein. The microbes therefore secrete some antimicrobial metabolites to supersede over other microbial forms present in the habitat (Golinska and Dahm 2013; Sivasakthi *et al*., 2014; Zalila-Kolsi *et al*., 2016). Golinska and Dahm (2013) isolated different species of *Streptomyces* from forest soil and reported 40-50% inhibition of *Rhizoctonia solani* and *Fusarium culmorum* by *Streptomyces exfoliatus* (SR5).

All the selected isolates from the agricultural soil and forest soils were found effective to promote the growth of amaranth in comparison to control except BS-131. However, agricultural isolates (BS-27, BS-41 and BS-58) were found more potential than their counter parts to enhance planting value parameters of amaranth in blotter paper assay. The significant outcomes of pot assay also revealed astonishing growth promotion potential of agricultural isolates in comparison to forest isolates (Fig. 3; Table-2, Table 3). The increased seedling growth of amaranth in blotter and pot assays might be due to mechanistic PGP traits of agricultural isolates as we observed in our study. *Bacillus* spp. isolates with the ability to produce/solubilize significant IAA and phosphate have previously been reported as effective inoculants for the growth and yield enhancement of cotton in the field condition (Qureshi *et al*., 2012). Similarly, Sharma and Johri (2003) reported siderophore producing *Pseudomonas* spp. GRP3A and PRS9 able to increase root and shoot length of maize in pot assay.

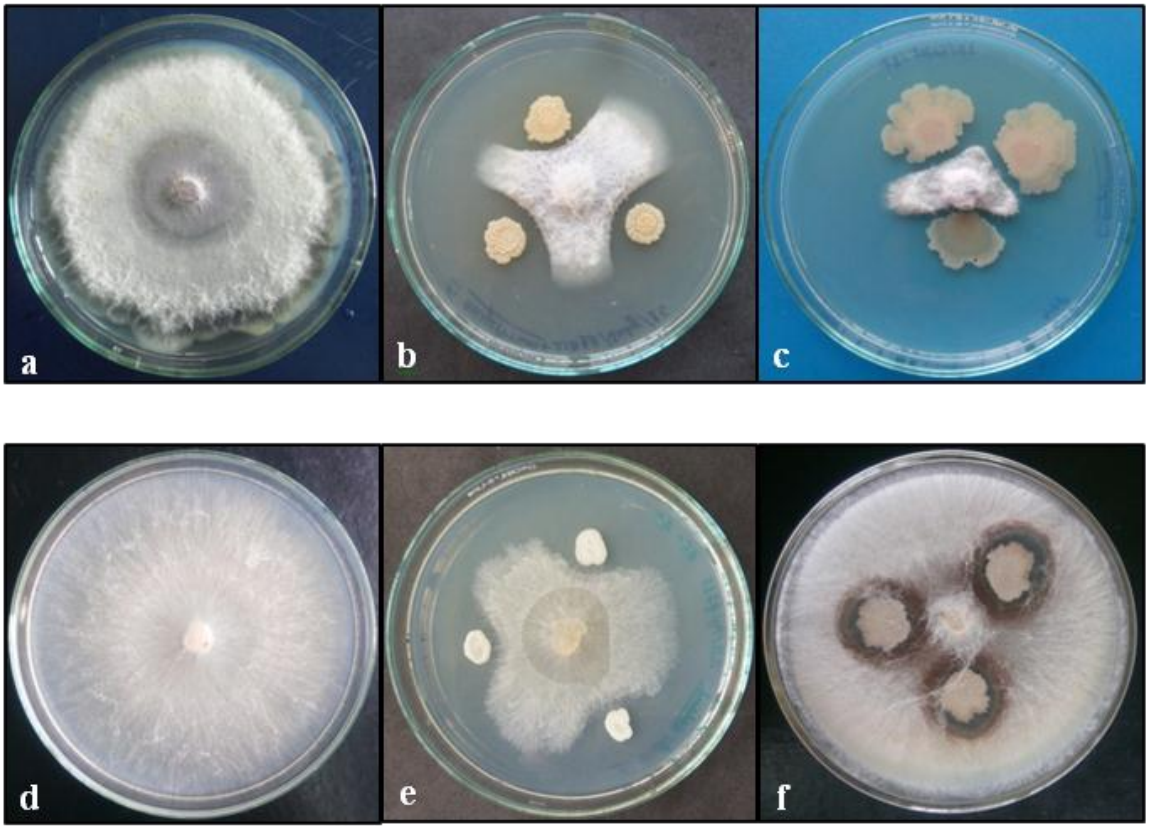

Figure 2: Antagonistic activity of selected *Bacillus* spp. isolates. a) *F. oxysporum* control plate; b) Antagonistic activity of agricultural soil isolate (BS-58) against *F. oxysporum;* c) Antagonistic activity of the forest isolate (BS-136) against *F. oxysporum*; d) *R. solani* control plate; e) Antagonistic activity of BS-58 against *R. solani*; f) Antagonistic activity of isolate BS-136 against *R. solani*.

Table 2: Effect of different *Bacillus* spp. isolates on seedling growth parameters (*in-vitro*) of *Amaranthus hypochondriacus*

Isolates	%Germination	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Fresh weight (gm)	Dry weight (gm)	Vigor Index-I	Vigor Index-II
Control	84.44	2.24	1.78	4.02	0.68	0.51	340.07	43.04
BS-27[a]	97.77*	3.00*	1.79ns	4.79*	0.96*	0.55*	468.92*	53.71*
BS-41[a]	97.77*	2.30ns	2.56*	4.86*	0.98*	0.52ns	475.86*	51.22*
BS-58[a]	97.77*	2.95*	2.79*	5.75*	0.98*	0.54ns	561.70*	50.97*
BS-125[b]	91.10*	2.42ns	1.88ns	4.31*	0.80*	0.52ns	392.84*	47.41*
BS-131[b]	88.88ns	2.40ns	1.82ns	4.22ns	0.78*	0.51ns	375.70ns	45.86ns
BS-136[b]	93.32*	2.45ns	1.90ns	4.36*	0.85*	0.52ns	407.22*	49.14*
SEM	2.05	0.07	0.04	0.09	0.01	0.01	13.44	1.44
CD at 5%	6.24	0.23	0.13	0.27	0.03	0.03	40.77	4.37

[a] Isolated from agricultural soils, [b] Isolated from forest soils. Values given in table are average of three replicates. *: Significant, ns: Non-significant.

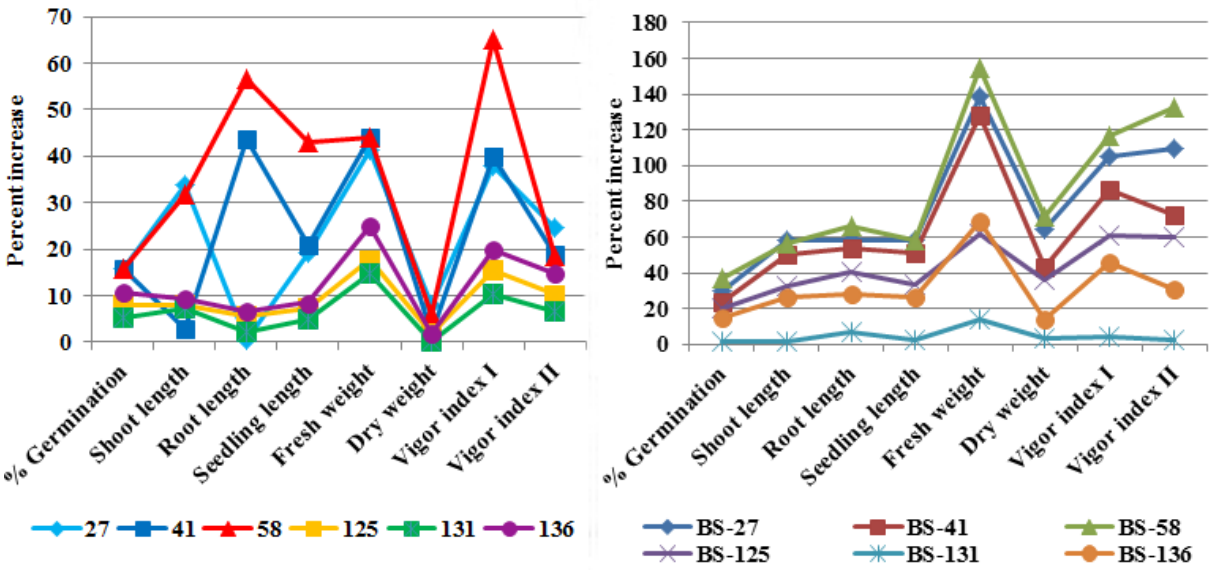

Figure 3: Effect of selected *Bacillus* spp. isolates on plant growth of amaranth. a) Percent increase in seedling growth parameters in blotter paper assay; b) Percent increase in different planting value parameters in pot assay

Table 3: Effect of different *Bacillus* spp. isolates on seedling growth parameters of *Amaranthus hypochondriacus* under pot assay

Isolates	% Germination	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Fresh weight (gm)	Dry weight (gm)	Vigor Index I	Vigor index II
Control	70.03	4.33	16.83	21.16	0.78	0.28	1483.61	20.08
BS-27[a]	91.06*	6.86*	26.60*	33.47*	1.86*	0.46*	3046.33*	42.15*
BS-41[a]	86.60*	6.66*	25.30*	31.96*	1.78*	0.40*	2768.73*	34.63*
BS-58[a]	96.03*	7.20*	26.26*	33.46*	1.99*	0.48*	3214.01*	46.72*
BS-125[b]	84.44*	6.07*	22.24*	28.31*	1.26*	0.38*	2391.27*	32.08*
BS-131[b]	71.15ns	4.62ns	17.08ns	21.70ns	0.89ns	0.29ns	1544.73ns	20.62ns
BS-136[b]	80.54*	5.54*	21.26*	26.81*	1.32*	0.32*	2159.66*	26.31*
SEM	0.49	0.34	0.57	0.67	0.053	0.009	63.40	0.77
CD at 5%	1.49	1.03	1.75	2.05	0.15	0.02	192.30	2.35

[a] Isolated from agricultural soils, [b] Isolated from forest soils. Values given in table are average of three replicates. *- Significant, ns- Non-significant.

Plant growth promoting attributes of *Bacillus* spp. isolates of agricultural soils were more effective than forest soil isolates. IAA production and phosphate solubilization as sole effect proved *Bacillus* of agricultural soil origin, as a suitable choice for the growth promotion of amaranth. However, being potential antagonistic bacteria, the forest isolates can used in the formation of biocontrol agents against the phytopathogenic fungi. Finally, it can be concluded that the PGPBs should be selected carefully and compatibility towards the host plant must be assessed. PGPBs may exhibit their best potential if are used in the same environment from which they were isolated.

MATERIALS AND METHODS

Isolation of agricultural and forest soil isolates

Non-Rhizospheric soil samples were collected from agricultural soils and forest soils (Deodara forest, Oak forest and mix forest) of

different locations (Salamkhet, Dandachali, Tehri etc.) of higher altitude in Trans-Himalaya lies with District Tehri Garhwal (78.15° E 30.27° N to 78.25° E 30.17° N), Uttarakhand, India. Soil samples collected from different forest regions and agricultural land from a depth of 6" from the surface and were analyzed for nitrogen and phosphorus contents in the particular soil by following the standard methods (Anderson and Ingram 1989). Isolation of *Bacillus* spp. was carried out by standard microbiological method as earlier stated by Agarwal *et al*., (2017). The cultures were a-prior identified on phenotypic traits following the Bergey's manual of determinative bacteriology (Holt *et al*., 1994).

Bioprospecting of PGP attributes

Plant growth promoting traits such as phosphate solubilization, and IAA production were carried out by following Kumar *et al*., (2012). Phosphate solubilization was done by spot inoculation of all the isolates from both the soil types on Pikovskaya's medium plate and clear zone around the bacterial colonies was considered as positive result. IAA production was determined by adding salkowski's reagent into the culture supernatant and positive reaction was observed by the appearance of pink color. Quantitative estimation of phosphate solubilization in Pikovskaya's broth was carried out following Chauhan *et al*., (2017). All the isolates were analyzed for IAA production quantitatively by following the salkowski's method as described by Rahman *et al*., (2010). Siderophore production was determined on Chrome-azurol S (CAS) medium by following the method described by Schwyn and Neilands (1987). Yellow to orange coloured halo zone around the bacterial colonies indicated siderophore production. HCN production by all the isolates was determined by modified method of Bakker and Schippers (1987) using exponentially grown cultures and change in the color of filter paper from yellow to moderate brown considered as positive.

Antagonistic activity of agricultural and forest isolates

Antagonistic activities of bacterial isolates were tested against two phytopathogens (*Fusarium oxysporum* and *Rhizoctonia solani*) on PDA plates using the dual culture technique as described by Negi et al., (2011). The chemotactic behavior of *Bacillus* isolates towards the root exudates was assessed to study their survivability in the rhizosphere of *Amaranthus hypochondriacus* using drop assay method (Grimm and Harwood 1997).

Evaluation of planting value parameters by blotter paper test

On the basis of PGP traits and biocontrol potential six isolates (BS-27, BS-41, BS-58, BS-125, BS-131 and BS-136, three from each source habitat) were selected to assess their effect on plant growth promotion of amaranth by blotter paper and pot trial. The Blotter paper experiment was conducted using PRA-1 variety of amaranth, procured from Department of Crop Improvement, College of Forestry, Ranichauri, Uttarakhand, India. Seeds were treated with the talc formulation @ 8g.kg^{-1}.Twenty seeds per treatment in triplicates were placed in each set and incubated at 28±1°C for 15days. The germination was recorded every day after the first seed germinated and continued until full germination was achieved in any of the experimental set. After 15 days, seedling growth parameters including shoot length, root length, seedling length, fresh weight, dry weight and vigor indices were recorded.

Evaluation of planting value parameters under pot assay

Pot experiment was conducted by using amaranth seeds which were bacterized individually with talc formulation of six different *Bacillus* spp. isolates isolated from agricultural and forest soil respectively and were sown in pots (12" diameter) containing pre-sterilized soil. Un-treated seeds were used as control to compare the respective performance of all treatments. Twenty seeds per pot were sown in triplicates. After 30 days, all the parameters were recorded as carried out for blotter paper experiment. The experiment was performed twice under controlled conditions in poly-house.

Statistical analysis

One way analysis of variance (ANOVA) to calculate the significance by magnitude of the F value (p= 0.05) was carried out (Gomez and Gomez 1984).

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