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Full Length Article

Boron Tolerant Phosphorus Solubilizing *Bacillus* sp. Strain MN-54 Improved Canola Growth in Alkaline Calcareous Soils

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Abstract

Boron (B) and phosphorus (P) application is mandatory as most of the Pakistani soils, being calcareous in nature, fail to supply nutrients in sufficient quantity to plants. Few bacteria have been reported as B-tolerant but their features for enhancing nutrient supply to plants have not been studied yet. In this study, growth of six bacterial strains was assessed in tryptic soy broth medium at different levels of B (0, 60, 120 and 180 m*M*) where *Bacillus* sp. strain MN-54 was found most tolerant. The phosphate solubilizing ability of MN-54 was improved by increasing molasses concentration from control to 1%. Moreover, this bacterium was capable of producing auxin, siderophores and 1-aminocyclopropane-1-carboxylate (ACC) deaminase. In incubation study, four levels of P *i.e*., 25, 50, 75 and 100% of recommended dose each with 0.5, 1.0 and 1.5% (0.06, 0.12 and 0.18 mg kg^{-1}) of B, further inoculated with MN-54 were used. However, only soil and soil with MN-54 pots were maintained as control. Moreover, canola was grown in soil filled pots with same set of treatments. However, in control pots, 100% P without B and inoculation, and 100% P inoculated with MN-54 but without B application were used. Pure culture of MN-54 was applied at 5 mL pot^{-1} and 12.5 mg P kg^{-1} of soil was used as 100% recommended. The presence of MN-54 improved B and P availability by 37 and 30%, respectively, in native soil. Moreover, application of 100% P along with 1.5% B and MN-54 showed significant improvement in shoot length (46%), root length (49%), shoot dry weight (41%), root dry weight (45%) and chlorophyll content (16%) of canola compared to control (100% P only). In crux, Bacillus sp. strain MN-54 was capable of improving canola growth in alkaline calcareous soils due to P solubilization, auxin and ACC-deaminase production. © 2019 Friends Science Publishers

Keywords: *Bacillus* sp. strain MN-54; Boron deficiency; Calcareous soils; Canola; P solubilization

Introduction

Boron (B) is a non-metal element, which was first time described as a micronutrient by Warington in 1923. It plays an essential role for optimizing plant growth and yield (Bolanos *et al.*, 2004; Silva *et al.*, 2011). During the last 60 years, B deficiency has observed in over 132 crops and 80 countries including Pakistan (Shorrocks, 1997; Zia *et al.*, 2006; Saleem *et al.*, 2011). Under agro-ecological conditions, about 65% of the tested soils in Pakistan were found B deficient. (Rashid *et al.*, 2005). After zinc, B is second most deficient micronutrient severely affecting the growth of crops on global scale (Alloway *et al.*, 2008). The critical concentration of B in plant tissues is 20-25 mg kg^{-1} (usually 35 mg kg^{-1}) on a dry mass basis however, the critical level of B deficiency in soil is 0.15-0.5 mg kg^{-1} (*i.e*., hot water extractable B) (Ahmad *et al.*, 2012). Soils, which are calcareous in nature and those having loessial or alluvial deposits and highly leached soils are extremely deficient in B (Borkakati and Takkar, 2000). Various other factors, which affect the availability of B to plants, include sandy/coarse texture, drought, alkalinity, liming and intensive cultivation with more nutrient uptake and less fertilizer application (Ahmad *et al.*, 2012). B deficiency results in substantial yield losses in cereals, oilseeds, perennials and legumes/pulses (Niaz *et al.*, 2002, 2007; Rashid *et al.*, 2005; Johnson, 2006; Zia *et al.*, 2006). In these B deficient crops, B fertilization causes significant economic benefits on average (Rashid, 2006).

B is highly important for human (Nielson, 2008), animals (Devirian and Volpe, 2003) and unicellular eukaryotes at specific level which varies according to the nature of living organisms (Bonilla and Garcia-Gonzalez, 1990). However, B had not been reported as an essential nutrient for bacteria except for cyanobacteria until 2007 (Ahmed *et al.*, 2007a). In *Azotobacter*, nitrogen fixation was stimulated in the presence of B, yet it was not found vital for its growth. In an *Arthrobacter nicotinovorans* strain, phenyl boronic acid (PBA) catabolism was demonstrated where B was released as orthoboric acid [$B(OH)_3$] (Negrete-Raymond and Wackett, 2003). Nevertheless, none of the study described that the absence of B restricts bacterial growth until 2007 (Ahmed *et al.*, 2007a). In case of B tolerance, considerable variation was observed in plant species where *Lysinibacillus boronitolerans, Chimaereicella boritolerans*,

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Gracilibacillus boraciitolerans and *Bacillus boroniphilus* have been stated to tolerate more than 150, 300, 450 and 450 m*M* B, respectively (Ahmed *et al.,* 2007b). Similarly, substantial variation in terms of B tolerance has been described amongst microorganisms (Miwa *et al.,* 2009; Yoon *et al.,* 2010). Raja and Omine (2013) isolated B tolerant bacteria from a site where fly ash was being discarded. B tolerant bacteria were also resisting the high levels of NaCl (15%). Such bacterial isolates could tolerate higher B concentrations due to the mechanism of high B efflux and exclusions (Ahmed and Fujiwara, 2010). Microorganisms, tolerating high level of B, are of biological interest for their ability to work under extreme conditions. Such B tolerant bacterial strains can also be used as a PGPR when applied along with specific level of B in B deficient soils.

In addition, fertilizer use efficiency of phosphorus (P) is about 10-25% throughout the world while bioavailable P concentration in soil is around 1 parts per million (ppm) that is extremely low (Khan *et al.,* 2009). Huge amount of P applied through fertilizers moves to the immobile pools by precipitation reactions of orthophosphates with highly reactive Ca^{2+} in normal or calcareous soils (Gyaneshwar *et al.,* 2002). Nowadays, phosphate solubilizing bacteria (PSB) are being utilized by the agro-industry to enhance P availability in soils (Bagyaraj *et al.,* 2000; Trivedi and Sa, 2008; Hussain *et al*., 2016). The most auspicious PSB falls in several genera including *Bacillus* species (Bhattacharyya and Jha, 2012). Such soil microbes are playing important role in soil P dynamics, releasing several organic acids, dissolving P minerals and making it available to plants (Richardson, 2001; Sarker *et al.,* 2014). PSB help in solubilizing the fixed soil P along with applied phosphates for improving crop production and yields (Gull *et al.,* 2004). The PSB strains exhibit mineralizing abilities of organic P ranging between 8–18 μg mL^{-1} and P solubilizing abilities from inorganic sources between 25–42 μg P mL^{-1} (Tao *et al.,* 2008). The rock minerals containing phosphate are often too insoluble to fulfill P requirements for crops, however the application of phosphate solubilizing microbes (PSMs) can upsurge crop yields about 70% (Verma, 1993). The application of arbuscular mycorrhiza along with PSB enhance P uptake from both native soil P and from the phosphatic rock (Goenadi *et al.,* 2000; Cabello *et al.,* 2005). However, PSB were found most efficient in solubilizing the calcium bound P forms compared to Mn-, Fe- or Al-bound P (Fankem *et al.,* 2006). Thus, the use of PSB for solubilizing fixed soil P along with applied phosphates results in higher crop yields (Khan *et al.,* 2009; Namli *et al.,* 2017).

Canola (*Brassica napus* L.) was grown as a test crop as it requires high level of B and shows sensitivity to B deficiency (Abat *et al.,* 2014). Canola is ranked as one of the most significant oilseed crop round the globe. After soybean and palm oil, canola oil ranks 3rd largest by volume (Wittenberger, 2012). Canola oil is preferred because of low levels of glucosinolate and erucic acid (Lin *et al.,* 2013). Previously, B tolerant bacteria were studied for analyzing their behavior towards stress tolerance while PSBs were investigated separately for improving P availability to plants. As per our knowledge, this study is a novel approach to examine the behavior of B tolerant bacteria for enhancing P solubilization thereby improving canola growth.

Materials and Methods

Measurement of B Tolerance of Bacteria

The bacterial strains S-5, MN-34, MN-42, MN-17, MN-54 and MN-13 were collected from Soil and Environmental Microbiology Laboratory, Institute of Soil and Environmental Sciences (ISES), University of Agriculture Faisalabad (UAF), Pakistan. These strains had already been evaluated for enhancing growth and yield of crops (Naveed *et al*., 2014; Yang *et al*., 2016). The growth of six bacterial strains was assessed in tryptic soy broth (TSB) medium at different levels of B (0, 60, 120 and 180 m*M*). The bacterial inoculum adjusted to optical density (OD) of 0.6 to obtain uniform population of 10^8 to 10^9 CFU mL^{-1}, was applied at 1 mL per 100 mL medium and incubated for 72 h at 28±2ºC and 150 rpm. Bacterial growth was estimated by measuring OD at 600 nm using spectrophotometer (Thermo Electron Corporation, Evolution-300LC, England) (Ahmed *et al.,* 2007a).

Growth of B Tolerant Bacterium in the Presence of Different Carbon Sources

The growth of *Bacillus* sp. strain MN-54 was assessed in the presence of different carbon (C) sources (glucose, glycerol and molasses). The broth prepared by using TSB (1%) and each C source (0.25, 0.50, 0.75 and 1%) was autoclaved, cooled and inoculated with selected strain. The broth was incubated in a shaker at 150 rpm and 28°C while, OD was measured by spectrophotometer at 600 nm after 72 h (Ahmed *et al.,* 2007a).

Plant Growth Promoting Traits of B Tolerant Bacteria

Phosphate solubilization potential of bacterial strain MN-54 was analyzed qualitatively and quantitatively in National Botanical Research Institute's phosphate (NBRIP) growth medium (Mehta and Nautiyal, 2001), which was further amended with different levels of B (0, 60, 120 and 180 m*M*) and molasses (0, 0.25, 0.5, 0.75 and 1%). For quantitative estimation of P solubilizing activity, 50 mL NBRIP broth amended with different levels of B and molasses were taken in 100 mL Erlenmeyer flasks inoculated with selected bacteria at 1 mL per 100 mL. The flasks were incubated under shaking conditions at 28°C and 150 rpm for three days. The supernatant was obtained by centrifugation at 8000 rpm for 10 min. The available P contents of each sample were determined by measuring the absorbance at 420 nm by spectrophotometer (Olsen and Sommers, 1982).

In agar assay, aforementioned NBRIP media was supplemented with 1.5% agar and poured into autoclaved plates. The strain MN-54 was spot inoculated in the center of agar plates using sterile needle. The plates were incubated at 28°C. The colony and Halo zone diameters were measured and P solubilizing potential of bacteria was determined by calculating P solubilization index (PSI) (Edi-Premono *et al.*, 1996; Babana *et al.*, 2013). In addition, bacterial strain MN-54 was also characterized for IAA (Indole-3-acetic acid) (Sarwar *et al.*, 1992) and organic acid production (Butsat *et al.*, 2009) in the presence of different concentrations of B and molasses as mentioned above. In addition, siderophores production by qualitative method (Schwyn and Neilands, 1987) and ACC-deaminase activity (Penrose and Glick, 2003) of strain MN-54 were also assessed.

Soil Incubation and Plant Growth Experiments

A growth room study was conducted in ISES, UAF to measure temporal release of B and P in soil by strain MN-54. The soil used in pots was collected from a research field of ISES. The soil was sandy clay loam in nature with pH 7.5, $CaCO_3$ 5.6%, saturation percentage 30.3%, available P 5.1 mg kg^{-1} and B 0.29 mg kg^{-1}. Pots were filled with 500 g sieved and air dried soil. In incubation study, four levels of P *i.e.*, 25, 50, 75 and 100% of recommended dose each with 0.5, 1.0 and 1.5% (0.06, 0.12 and 0.18 mg kg^{-1} of soil) B, further inoculated with MN-54 were used. However, only soil and soil with MN-54 pots were maintained as control. Moreover canola was grown in above mentioned soil filed pots with same set of treatments. In control pots, 100% P without B and inoculation and 100% P inoculated with MN-54 but without B application were used. Pure culture of *Bacillus* sp. strain MN-54 was applied at 5 mL pot^{-1} and, 12.5 mg P kg^{-1} of soil was used as 100% recommended P. dose of P. Five seeds of canola per pot were sown which were thinned to one plant per pot. The recommended doses of N, P and K were applied at 45, 12.5 and 20 mg kg^{-1}, respectively. Pots were irrigated regularly to avoid moisture stress. The concentrations of B and P were determined with seven days interval using spectrophotometer (Bingham, 1982; Olsen and Sommers, 1982). After 3 weeks, crop was harvested and growth parameters including shoot length (cm), root length (cm), shoot fresh weight (g), shoot dry weight (g) and root dry weight (g) were recorded. Chlorophyll contents were measured by a light weight handheld SPAD meter.

Statistical Analysis

The experiment was conducted in a completely randomized design (CRD) with three replications of each treatment. The data were subjected to analysis of variance (ANOVA) with Statistix ver. 8.1 software (Statistix, Tallahassee, FL, USA) and presented as mean of replications ± SE. Significance between treatments was checked by LSD, least significant difference test at $P \leq 0.05$ (Steel *et al.*, 1997).

Results

Screening of Bacterial Strain for B Tolerance

Among six bacterial strains, *Bacillus* sp. strain MN-54 showed maximum B tolerance in TSB medium containing 60, 120 and 180 m*M* B, however maximum growth was observed without B application. The bacterial strain, MN-13 was found most sensitive as it showed maximum growth reduction (-81%) at 180 m*M* B. The strains S5, MN-34, MN-42, MN-17 and MN-13 showing reduced growth of 67, 44, 56, 66 and 81%, respectively in the presence of 180 m*M* B. Thus, *Bacillus* sp. strain MN-54 was selected as it performed significantly better by showing minimum reduction (4.7%) at 180 m*M* compared to other strains (Table 1).

Growth of B Tolerant Bacteria in the Presence of Different C Sources

In Fig. 1, it was observed that increasing the level of C by the application of any source (glucose, glycerol and molasses) improved the growth of *Bacillus* sp. strain, MN-54. Maximum increase in bacterial growth (47.8%) was observed in the presence of 1% molasses while glucose and glycerol applied at same level also improved the growth of bacteria up to 12.5% and 14.3%, respectively.

Plant Growth Promoting Traits of B Tolerant Bacteria

Results of study revealed that the interaction of higher level of B and molasses improved the auxin production and P solubilization by the strain MN-54. The production of organic acids was also improved in the presence of 1% molasses (Table 2). Therefore, maximum P solubilization (PSI, 4.62 in agar assay; 295.43 mg L^{-1} in broth assay) and auxin production (39.13 μg mL^{-1}) was observed by MN-54 where 180 m*M* B was applied along with 1% molasses (Table 3 and Fig. 2). ACC-deaminase activity of selected bacterial strain was 0.689 μM α keto μg^{-1} protein hr^{-1} while MN-54 was also capable of producing siderophores (a qualitative test).

Soil Incubation and Plant Growth Experiment

In soil incubation study, *Bacillus* sp. strain MN-54 improved B and P release in native soil inoculated with MN-54 where 37 and 30% increase in B and P availability, respectively, was observed compared to un-inoculated soil on 28th day. Maximum B (0.765 mg kg^{-1}) and P (10.78 mg kg^{-1}) release was noted on 28th day after application of 1.5% B, 100% P and MN-54 (Fig. 3 and 4). In plant growth experiment, treatment containing 1.5% B, 100% P and MN-54 showed maximum plant growth which resulted in significant improvement in shoot and root length, fresh and dry shoot weight, fresh and dry root weight, and chlorophyll content (SPAD) as compared to inoculated and un-inoculated control treatments. In inoculated control, shoot length was increased 14% compared to un-inoculated control treatment.

Table 1: Effect of different boron concentrations in tryptic soy broth (TSB) medium on growth of six pre-isolated bacterial strains

Strains	Bacterial Growth (OD_{600})			
	0 m*M* B	60 m*M* B	120 m*M* B	180 m*M* B
MN-13	1.24 f	0.66 m	0.28 t	0.23 u
MN-54	1.69 b	1.73 a	1.65 c	1.61 d
MN-42	1.41 e	1.04 h	0.89 g	0.61 n
MN-34	0.98 i	0.73 l	0.62 n	0.55 p
S-5	1.19 g	0.87 k	0.58 o	0.30 s
MN-17	0.91 j	0.54 p	0.48 q	0.37 r

Means sharing different letters, within row or column, differ significantly from each other at $P<0.05$
Here OD: Optical density; B: Boron

Table 2: Bacterial metabolite profiles detected by HPLC by culturing *Bacillus* sp. strain MN-54 at different levels of molasses in TSB medium containing 180 m*M* of boron

Treatments	Concentrations of organic acids (μg mL^{-1})							
	Pyruvic acid	Tartaric acid	Citric acid	Oxalic acid	Malic acid	Malonic acid	Fumeric acid	Succinic acid
0.5% M	71.10 c	105.40 c	32.06 c	0.73 c	128.30 c	22.21 c	69.84 c	20.41 c
0.75% M	265.38 b	1457.24 b	263.91 b	21.13 b	1064.67 b	108.80 b	910.80 b	621.36 b
1% M	1262.70 a	1605.21 a	306.10 a	42.40 a	2036.30 a	293.02 a	1148.41 a	4653.62 a

Means sharing different letters, within column, differ significantly from each other at $P<0.05$
M: Molasses

Table 3: Phosphorus solubilizing potential of strain MN-54 in agar (index) and broth (μg mL^{-1}) medium at different levels of boron and molasses

Treatments	Colony diameter (mm)	Halo zone diameter (mm)	P solubilization index (PSI)	Soluble P concentration (μg mL^{-1})
Control	4.1 f	5.8 i	2.42 h	228 i
0.25% M + 60 m*M* B	4.5 ef	8.3 h	2.84 gh	236 hi
0.25% M + 120 m*M* B	4.8 ab	9.3 gh	2.95 fgh	238 h
0.25% M + 180 m*M* B	5.0 ab	10.3 g	3.07 e-h	244 fgh
0.50% M + 60 m*M* B	5.2 a	12.3 f	3.37 d-g	242 gh
0.50% M + 120 m*M* B	5.3 def	13.7 ef	3.58 c-f	247 fg
0.50% M + 180 m*M* B	5.5 c-f	14.7 de	3.68 cde	251 ef
0.75% M + 60 m*M* B	5.7 b-e	16.03 d	3.81 bcd	258 de
0.75% M + 120 m*M* B	6.0 b-e	18.27 c	4.05 abc	264 d
0.75% M + 180 m*M* B	6.1 a-d	19.43 c	4.19 abc	274 c
1.0% M + 60 m*M* B	6.3 a-d	21.27 b	4.38 ab	280 bc
1.0% M + 120 m*M* B	6.4 a-d	22.43 ab	4.50 a	287 b
1.0% M + 180 m*M* B	6.6 abc	23.87 a	4.62 a	295 a

Means sharing different letters, within column, differ significantly from each other at $P<0.05$
Here M: Molasses; B: Boron; P: Phosphorus; MN-54: *Bacillus* sp.; P Solubilization index = (Halo zone diameter + colony diameter)/colony diameter

Table 4: Effect of different levels of B and P on plant biomass of canola in the presence of B tolerant P solubilizing bacteria

Treatments	Shoot fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Shoot length (cm)	Root length (cm)
100% P	8.2 g	0.82 ef	0.083 d	15.5 fg	4.8 fg
100% P + MN-54	9.2 de	0.91 cd	0.090 bcd	17.8 de	5.5 cde
0.5% B + 25% P + MN-54	5.3 k	0.54 h	0.059 e	9.2 h	3.3 i
0.5% B + 50% P + MN-54	7.8 h	0.76 f	0.067 e	14.8 g	4.3 g
0.5% B + 75% P + MN-54	8.9 ef	0.89 de	0.092 bcd	16.9 ef	5.4 de
0.5% B + 100% P + MN-54	9.5 cd	0.94 cd	0.101 bc	18.2 de	5.8 cd
1% B + 25% P + MN-54	5.9 j	0.58 gh	0.062 e	10.1 h	3.7 h
1% B + 50% P + MN-54	8.6 fg	0.87 de	0.088 cd	16.5 efg	5.1 ef
1% B + 75% P + MN-54	9.7 c	0.97 c	0.097 bcd	18.8 cd	5.9 c
1% B + 100% P + MN-54	10.5 b	1.06 b	0.107 b	20.2 bc	6.5 b
1.5% B + 25% P + MN-54	6.3 i	0.64 g	0.062 e	10.8 h	3.9 h
1.5% B + 50% P + MN-54	9.3 cd	0.91 cd	0.092 bcd	18.1 de	5.7 cd
1.5% B + 75% P + MN-54	10.9 b	0.97 c	0.102 bc	20.8 b	6.4 b
1.5% B + 100% P + MN-54	11.5 a	1.16 a	0.121 a	22.8 a	7.1 a

Means sharing different letters, within column, differ significantly from each other at $P<0.05$
Here MN-54: *Bacillus* sp.; 100% P: 12.5 mg of P kg^{-1} of soil; 0.5, 1.0 and 1.5% B: 0.06, 0.12 and 0.18 mg of B kg^{-1} of soil

However, 46% increase in shoot length was observed by the combined application of B (1.5%), P (100%) and *Bacillus* sp. strain, MN-54 compared to un-inoculated control. The growth parameters including root length, fresh and dry biomass of roots and shoots also showed significant increase in the presence of strain MN-54, 1.5% B and 100% P (Table 4).

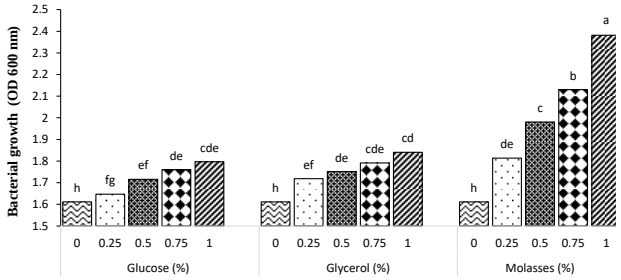

Fig. 1: Effect of different carbon sources on growth of strain MN-54. Means sharing different letters differ significantly from each other at $P<0.05$
Here OD: Optical density; MN-54: *Bacillus* sp.

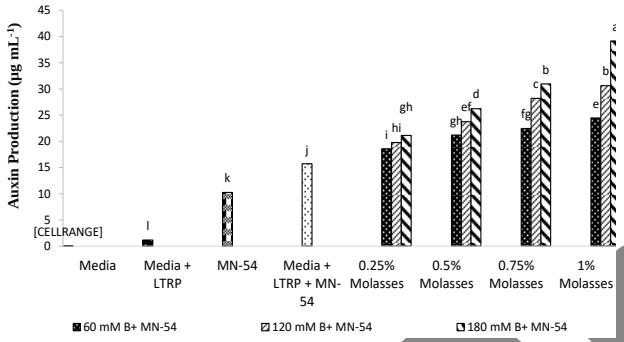

Fig. 2: Auxin production (μg mL^{-1}) by B tolerant strain at different levels of B and molasses. Means sharing different letters differ significantly from each other at $P<0.05$
Here LTRP: L-Tryptophan; B: Boron; MN-54: *Bacillus* sp.

In Fig. 5, the inoculated control improved the chlorophyll content (SPAD) up to 11% compared to un-inoculated control though, 16% increase in SPAD value was also observed by the application of MN-54, 1.5% B and full dose of P compared to inoculated control.

Discussion

Boron is highly important for human, animals and unicellular eukaryotes at specific level however, it had not been reported as an essential nutrient for bacteria until 2007 (Ahmed *et al.*, 2007a). In present study, *Bacillus* sp. strain MN-54 was found comparatively B tolerant as all others showed significantly reduced growth at higher levels of B application. The selected B tolerant strain MN-54 has already been reported as salinity tolerant (Yang *et al.*, 2016). Ahmed *et al.* (2007a) isolated a microbial sp. named *Bacillus boroniphilus* that requires B for optimum growth. However, intracellular B accumulation was also observed in *Variovorax boronicumulan* (Miwa *et al*., 2008). Several microbial species including *Bacillus boroniphilus and Gracilibacillus* have been known to tolerate above 450 m*M* of B (Ahmed *et al.*, 2007a, c), yet their significance as a PGPR was not studied.

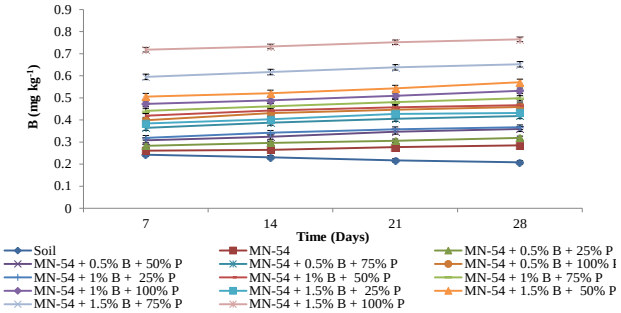

Fig. 3: Temporal release of B (mg kg^{-1}) in presence of B tolerant P solubilizing strain at different levels of B and P±SE
Here MN-54: *Bacillus* sp. MN-54: Bacillus sp.; 100% P: 12.5 mg of P kg^{-1} of soil; 0.5, 1.0 and 1.5% B: 0.06, 0.12 and 0.18 mg of B kg^{-1} of soil; SE: Standard error

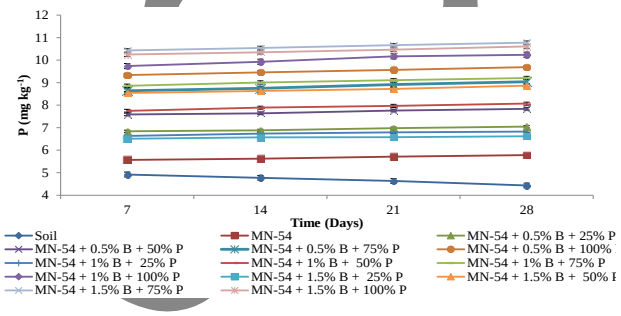

Fig. 4: Temporal release of P (mg kg^{-1}) in presence of B tolerant P solubilizing strain at different levels of B and P±SE
Here MN-54: *Bacillus* sp. MN-54: Bacillus sp.; 100% P: 12.5 mg of P kg^{-1} of soil; 0.5, 1.0 and 1.5% B: 0.06, 0.12 and 0.18 mg of B kg^{-1} of soil; SE: Standard error

The *Bacillus* sp. strain MN-54 showed maximum growth in the presence of molasses which is supported by several studies describing the significance of proper nutrition in microbial growth (Raizada and Singh, 1971; Coallier *et al.*, 1989; Miettinen *et al.*, 1997). In current study, molasses was found as an efficient carbon (C) source for *Bacillus* sp., strain MN-54 (Fig. 1). Recently, Gao *et al.* (2017) studied the effect of supplementation with C sources and microbial management for stimulating *Artemia* biomass production where molasses was found as a cheaper agricultural byproduct, which resulted in highest biomass production of *Artemia*, compared to glucose and sugar. Molasses also contains several inorganic and organic compounds such as biotin and glycine betain, thus considered as a better C source (Streit *et al.*, 1996).

The strain MN-54 was found capable of producing organic acid and solubilizing phosphates. It was also involved in the production of auxin, 1-aminocyclopropane-1-carboxylate (ACC) deaminase and siderophores. Plant growth promoting bacteria differ in their mode of action depending on host plant (Dey *et al.*, 2004) nevertheless, their general mode of action for plant growth aspects includes;

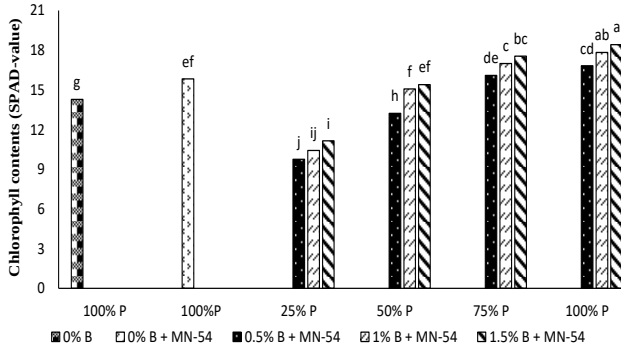

Fig. 5: Effect of B tolerant P solubilizing bacteria MN-54 on chlorophyll content (SPAD) of canola under varying levels of P and B. Mean sharing different letters differ significantly from each other at $P<0.05$

Here MN-54: *Bacillus* sp. MN-54: Bacillus sp.; 100% P: 12.5 mg of P kg^{-1} of soil; 0.5, 1.0 and 1.5% B: 0.06, 0.12 and 0.18 mg of B kg^{-1} of soil

enhancing abiotic stress tolerance in their hosts, nutrients fixation for plants uptake, release of several plant growth regulators, production of volatile organic compounds and siderophores, and plant disease prevention by producing different protective enzymes including glucanase, ACC-deaminase and chitinase (Choudhary *et al.,* 2011; Garcia-Fraile *et al.,* 2015). The bacterial strain MN-54 was also capable of solubilizing P in NBRIP medium (broth and agar) containing B and molasses. Similar consistent results of P solubilization by PSB were observed earlier in both agar and liquid broth assay (Nautiyal, 1999; Islam *et al.,* 2007). The solubilization of P is increased if sufficient energy is provided to organisms by the addition of a C source (Michael *et al.,* 1992). The bacterial strain MN-54 was found as an effective P solubilizer showing visible halo zone formation around its colony on agar plates incubated at 28±2°C (Table 3). Gupta *et al.* (1994) reported halo zone formation by bacterial isolates on PVK and MPVK agar plates which could be due to phosphatase activity of isolated strains. The results of our study were also consistent with Tripti *et al.* (2012) who isolated different microbial strains having variable P solubilizing potential in broth and agar medium. The maximum P solubilizing potential of 295 μg mL^{-1} was observed by MN-54. Park *et al.* (2011) showed that PSB isolates were able to solubilize 217–479 mg L^{-1} of P. Moreover Chatli *et al.* (2008) showed that different *Bacillus* species were involved in P solubilization. The organic acid production by MN-54 was also improved at higher levels of B and molasses that could be a reason of more P solubilization under these conditions. He *et al.* (2002) described that the inorganic P is dissolved by some heterotrophic microbes which are involved in secreting organic acids. These organic acids solubilize phosphatic minerals or may chelate cations associated with P ions (PO_4^{3-}) thereby directly leaving P into soil solution (Khan *et al.,* 2009).

Many studies support the significant increase in auxin production by the addition of C source in growth media. It was noted that the C source has significant role in improving bacterial growth (Akbari *et al.,* 2007; Tsavkelova *et al.,* 2007; Ali *et al.,* 2014; Aziz *et al.,* 2015). Makkar and Cameotra (1997) described the utilization of molasses by *Bacillus subtilis* strains for their growth. In this study, similar results were analyzed as the addition of molasses was helpful in improving bacterial growth. The strain MN-54 also showed siderophores production and ACC-deaminase activity. ACC-deaminase activity assists plants to endure various biotic and abiotic stresses by reducing the level of ethylene (Arshad *et al.,* 2007; Prisch *et al.,* 2012; Hassan *et al.,* 2016). The production of siderophores, auxins, ACC-deaminase and organic acids are main characteristics of plant growth promoting PGPRs (Hassan *et al.,* 2015, 2016). Such bacteria are also involved in solubilization of mineral nutrients like P (Pérez-Montano *et al.,* 2014). Therefore, *Bacillus* sp. strain MN-54 having all these characteristics was found as an effective PGPR.

After evaluating the PGPR for promising features under axenic conditions, they are further used for analyzing its effectiveness for plant growth by conducting pot and field trials under natural conditions (Pérez-Montano *et al.,* 2014). In present soil incubation study, bacterial strain MN-54 improved the release of B and P in soil compared to control. The release of P from insoluble and adsorbed forms into soil solution by PSB is an imperative feature for P availability as soil bacteria play a significant role in transmuting soil organic P to plant available forms (Halder and Chakrabartty, 1993; Tripti *et al.,* 2012), which could be a reason of improved P availability. The release of B might also be improved by the activity of MN-54 as PGPR are also involved in solubilizing the mineral nutrients (Pérez-Montano *et al.,* 2014).

The data of pot experiment showed improved growth of canola in the presence of 100% P, 1.5% B and MN-54 compared to lower levels of P and B (Table 4). Similar results were obtained as dry matter yield was increased at higher levels of applied P. Aziz and Aly (2012) observed that increasing the level of B application from 0 to 2 ppm results in improved dry matter production of plants. It was observed that B application show significant improvement in plant growth, seed yield, P and sulphur uptake, benefit: cost ratio and net returns as compared to control where no B was applied (Mallick and Raj, 2015). Bao-Luo *et al.* (2015) observed marked improvement in plant growth and yield of canola by combined fertilization of nitrogen, sulfur and boron. The aforesaid literature supports our findings by describing the significance of P and B, which play significant role in plant growth and development. Moreover, PSB significantly improved the root and shoot length, root and shoot dry weight and nutrient contents in plants compared to un-inoculated control (Sarker *et al.,* 2014; Zafar-ul-Hye *et al.,* 2015), supporting our PGPR strain MN-54 in improving plant growth by the production of growth regulators.

The data also showed that the application of 1% B, 50% P and MN-54 showed non-significant results as compared to control where 100% P was applied without B and MN-54. The combined use of PGPR and PSB could minimize P fertilization by 50% without significant drop in crop yield (Jilani *et al.*, 2007; Yazdani *et al.*, 2009). It was noted that PSB inoculants have great significance in sustainable crop production with optimum use of P fertilizers (Ahmad *et al.*, 2009). Henceforth, the interaction of B, P and B tolerant P solubilizing *Bacillus* sp. strain MN-54 was found highly efficient in improving nutrient availability and plant growth.

Conclusion

Considerable increase in canola growth was observed with optimum use of B and P, while the presence of *Bacillus* sp. strain MN-54 as B tolerant phosphate solubilizing strain was highly significant in improving nutrients availability in soil thus resulting in improved canola growth.

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