Phosphate-solubilizing *Bacillus* sp. enhances growth, phosphorus uptake and oil yield of *Mentha arvensis* L

Jai Prakash¹ · Naveen Kumar Arora²

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Abstract

In the present study, phosphate solubilizing rhizobacterial isolate STJP from the rhizosphere of *Stevia rebaudiana* was identified as a *Bacillus* sp. on the basis of phenotypic, biochemical, and 16S rRNA gene sequencing. In addition to phosphate solubilization ability, isolate *Bacillus* sp. STJP produced a significant quantity of siderophore (16.06 µg/ml) and indole 3-acetic acid (30.59 µg/ml). In the greenhouse experiment, treatment with STJP along with tricalcium phosphate (TCP²⁰⁰) showed significant increase in the plant growth parameters, oil yield and P uptake in *M. arvensis* as compared to the control plants. Amongst all the treatments, highest oil yield and menthol content were observed when treated with *Bacillus* sp. STJP + TCP²⁰⁰. Hence, an integrated approach of using *Bacillus* sp. STJP along with TCP can be used to increase the production of menthol and oil yield of *M. arvensis*. This approach of using fertilizer along with phosphate solubilizing *Bacillus* sp. worked very well and was more effective in comparison with individual treatment of fertilizer or plant growth promoting rhizobacteria. A combined use of efficient phosphate solubilising bacteria loaded with plant growth promoting characters along with TCP can thus be far effective way for enhancing the yield of crops in a sustainable manner.

Keywords *Bacillus* · *Mentha arvensis* · Menthol · Plant growth promoting rhizobacteria · Sustainable agriculture

Introduction

Medicinal and aromatic plants (MAPs) have proved to be the major sources of medicines in the world. They are extensively used for the treatment of several diseases and boost the economy as well as the living standard of farmers in several countries including India (WHO 2013). But nowadays, assurance of the efficacy, quality, and supply of MAPs and their products has become a major issue around the globe. Day by day, we are losing the natural reserves of MAPs mainly due to degradation of habitats (Stutte 2016). Hence, the need has arisen to cultivate the MAPs in an eco-friendly

manner so as to maintain their continuous supply to get the useful products. For the cultivation of MAPs, the use of biological methods, so as to enhance the productivity is better and this can reduce the load of chemicals in the agroecosystems (Singh and Arora 2016). The use of plant growth promoting rhizobacteria (PGPR) can thus be more useful and good for the environment.

Mentha arvensis (Japanese mint or menthol mint) of family Lamiaceae, is widely used for the treatment of several diseases such as abdominal pain, cough, diarrhoea, fever, headache, nausea, sunburn, skin irritation and vomiting (Alankar 2009). The leaves of *M. arvensis* contain high levels of flavonoids and antioxidants. In addition, *M. arvensis* also contains an important secondary metabolite known as menthol which has potential applications in several industries including pharmaceutical, flavoring, food, agrochemicals and cosmetics. The worldwide production of menthol is estimated as 20,000 metric tons every year (Srivastava et al. 2002). India is the chief menthol producer (more than 80% of the total produce in the world) and its leading exporter as well (Bakry et al. 2016). Approximately, 0.145 million hectares (ha) of agricultural land is being used for growing *M. arvensis* in northern India's Indo-Gangetic plains

✉ Naveen Kumar Arora
nkarora_net@rediffmail.com; nkarora.bbau@gmail.com

¹ Department of Environmental Microbiology (DEM), School for Environmental Sciences (SES), Babasaheb Bhimrao Ambedkar University (A Central University), Vidya Vihar, Raebareli Road, Lucknow, Uttar Pradesh 226 025, India

² Department of Environmental Science (DES), School for Environmental Sciences (SES), Babasaheb Bhimrao Ambedkar University (A Central University), Vidya Vihar, Raebareli Road, Lucknow, Uttar Pradesh 226 025, India

(Kumar et al. 2011). Day by day the demand for *M. arvensis* and its essential oil is increasing; hence it is important to increase the yields without affecting the quality and purity of agro-ecosystems.

Nutrient deficiency in soils is one of the major issue for agricultural production around the globe affecting the quantity and quality of crops (Tewari and Arora 2016). For the improvement of crop yields, farmers have commonly used chemical fertilizers for agricultural production causing an adverse effect on autochthonous organisms and deteriorating the quality of agro-ecosystems and aquatic resources (Arora et al. 2016). Most of the phosphate fertilizers remain unused and accumulate in the soil as precipitates. Apart from this, the accumulated phosphates (in soil) may also reach water bodies causing eutrophication. Therefore, the use of phosphate solubilising microbes along with the applied phosphate can provide a solution to the menace of accumulation of phosphates in the fields and water bodies. *Bacillus* sp. are well-known rhizobacteria which facilitate the plant growth either by solubilization of minerals like phosphorus or production of metabolites such as siderophores and phytohormones, and are excellent colonizers of the roots (Ahmad et al. 2018). In addition, *Bacillus* spp. have added advantage as their communities are found in the rhizosphere of wide varieties of crop plants and can survive stress condition very well (Abd-Allah et al. 2018). Use of *Bacillus* sp. has become a very important practice for eco-friendly and sustainable agriculture for enhancing crop production and control phytopathogens (Alori et al. 2017). An integrating approach of utilizing *Bacillus* spp. having multiple capabilities (including phosphate solubilization) along with phosphate fertilizers can enhance the growth of plants and maintain soil fitness even in presence of biotic and abiotic stresses (Mishra et al. 2016; Tewari and Arora 2018; Gouda et al. 2018).

Phosphate solubilizing bacteria (PSB) are emerging as important biofertilizers with the ability to utilize precipitated phosphorus (P) in the soil, leading to eco-friendly agriculture and maintaining the quality of agro-ecosystems (Pereira et al. 2015; Alori et al. 2017). PSB based biofertilizers are cheaper and can be of particular interest in nutrient (phosphorus) deficient soils (Shen et al. 2011). Although, in last few years, the role of PSB as attractive biofertilizers is on the rise, still, there are negligible reports on their utilization as biofertilizers with commercial use for enhancing the growth of *M. arvensis* and improving its oil content. With this aim, in the present study, PSB were isolated and checked for their plant growth promoting (PGP) characters followed by evaluation of the ability to enhance the growth, oil yield, and quantity of menthol in *M. arvensis*.

Materials and methods

Isolation and characterization of phosphate solubilizing rhizobacteria

PSB were isolated from the rhizosphere soil of *S. rebaudiana*, a medicinal plant growing in farmer's field at Lucknow (26.7679°N, 80.9263°E), Uttar Pradesh, India. For isolation, 1 g of sample was dissolved in 9 ml of sterile water (pH 7.1). From fifth dilution (10^{-5}), 0.1 ml of soil sample was spread on Pikovskaya's (PVK) agar (Pikovskaya 1948) containing 0.5% tri-calcium phosphate and incubated at 28 °C for 72–120 h for checking phosphate solubilization. Isolates were also grown at 30 °C for 120 h in National Botanical Research Institute Phosphate (NBRIP) broth medium to check the amount of phosphate solubilization. The soluble P content ($\mu\text{g/ml}$) was determined by spectrophotometer at 600 nm (Nautiyal 1999). Selected isolate STJP (on the basis of phosphate solubilization ability) was identified by phenotypic (flagellated or non-flagellated, shape and size) and biochemical features such as oxidase, urease, catalase, gelatinase, indole, H_2S , citrate, and nitrate production as per Bergey's Manual of Systematic Bacteriology (Garrity 2005). The cell shape of the bacterium was confirmed by scanning electron microscopy (SEM) performed according to the method of Golding et al. (2016). Isolate STJP was further identified by 16S rRNA sequencing and sequence analysis (Maidak et al. 2000). The evolutionary distance of isolate STJP and the closely related taxa was calculated using MEGA (version 6.00) software.

Phosphatase enzyme assay

Phosphatase enzyme assay was performed as per the method of Tabatabai and Bremner (1969). Briefly, 100 μl bacterial culture was inoculated in NBRIP broth (50 ml) and incubated at 28 °C for 7 days. After incubation (24 h), the broth culture was centrifuged (speed: 10,000 rpm, time: 10 min, temp: 4 °C) and supernatant was collected. One ml of supernatant was mixed with modified universal buffer (4 ml; pH 6.8) and subsequently, disodium *p*-nitrophenol phosphate (1 ml; 0.025 mM) was added in supernatant for quick analysis of phosphate enzyme activity and incubated at 28 °C for 60 min. After incubation, 1 drop of toluene was added and further incubated (time: 1 h; temp: 37 °C) and finally 0.5 M CaCl_2 and 0.5 M NaOH (ratio 1:4) was mixed for inhibition of reaction. The obtained sample was filtered via the Whatman filter paper no 42 and the quantity of enzyme were examined in 1 μmol of *p*-nitrophenol/ml/min through spectrophotometer (420 nm). For standard, *p*-nitrophenol was used for the quantification of phosphatase.

Investigation of plant growth promoting characteristics

Bacterial isolate STJP was inoculated in tryptic soy broth (TSB) comprising (g/l); tryptone:15, NaCl: 8, soy peptone: 5, tryptophan: 1 (HiMedia, Mumbai), 1000 ml sterilized water; pH 7.0 in 50 ml Erlenmeyer flask (EF) and incubated at 30 °C for 72 h (120 rpm) for IAA production. After incubation, 5 ml culture broth was centrifuged (speed: 5000 rpm, time: 10 min, temp: 4 °C). The obtained supernatant was mixed with Salkowski reagent in the ratio of 3:2 and was left in the dark (20 min). Afterwards, sample was analyzed spectrophotometrically at 530 nm for quantitative estimation of IAA ($\mu\text{g/ml}$) (Brick et al. 1991). For quantifying of siderophore, isolate (STJP) was inoculated in nutrient broth (NB) and incubated for 72 h at 28 °C (130 rpm). After incubation, the culture was centrifuged to obtain supernatant (speed: 5000 rpm, time: 15 min, temp: 4 °C) and the collected sample was taken to examine the siderophore production (Reeves et al. 1983). The siderophore quantity was expressed in μmol benzoic acid/ml. The ability to fix nitrogen was checked as per Line and Loutit (1971) and production of hydrogen cyanide (HCN) was evaluated according to Millar and Higgins (1970).

Effect of isolate STJP on the growth of *M. arvensis*

The pot experiment was performed in University Greenhouse Horticulture Research Farm (HRF) for 90 days. Selected isolate STJP was grown in NB in a shaker (speed: 120 rpm, time: 48 h, temp: 28 °C). Afterwards, growth culture was centrifuged (speed: 5000 rpm, time: 10 min, temp: 4 °C) for collecting the pellet. Further, sterilized deionized water was mixed in the pellet to obtain population density of 10^8 CFU/ml (Maheshwari et al. 2012).

Sandy-loam soil [pH: 7.69, EC: 0.48 dS/m, N: 108 (kg/ha), K: 89 (kg/ha), and P: 35.26 (kg/ha)] was collected from HRF and 6 kg was filled in clay pots (20 cm $\times$ 20 cm $\times$ 20 cm). Rootlets of seedling of *M. arvensis* (var. CIMAP SARYU) were disinfected by ethanol (70%, time: 2 min) followed by sodium hypochlorite (2%, time: 5 min) and subsequently washed with deionized water for 10 min (Arora et al. 2001). The seedling was then dipped in inoculum suspension (10^8 CFU/ml; time: 20 min) and allowed to dry (time period: 30 min). Subsequently, the seedlings (five per pot) were sown as per following sets: T-1 control (*M. arvensis*); T-2 STJP; T-3 TCP¹⁰⁰ (100 mg/kg in soil); T-4 STJP + TCP¹⁰⁰; T-5 TCP²⁰⁰ (200 mg/kg in soil); T-6 STJP + TCP²⁰⁰ as per Mamta et al. (2010). The work was done with five replications for each set in a completely randomized design (CRD). Irrigation was done using tap water (in equal amount) for each pot as per requirement. The plants were uprooted 90 days after sowing (DAS) and

checked for the shoot and root length, number of leaves, fresh and dry weight.

Examination of oil yield from *M. arvensis*

The dry *M. arvensis* plants were used for extraction of oil by Clevenger's apparatus in hexane solvent. Before extraction, the fresh shoots (leaves and stem) were harvested and separately dried (temp: 45 °C, time: 96 h). Completely dry shoots were crushed, samples were dissolved in *n*-hexane (50 ml) for 10 h and oil was extracted as per method described by Langenau (1948). The obtained oil was filtered via Whatman filter paper no. 42. The filtered oil was evaporated using rotatory evaporator (temp: 40 °C, speed: 50 rev/min, vacuum pressure: 80 mbar) to obtain pure oil. The oil yield was calculated (g/pot) as per the method of Singh et al. (2013).

Effect of isolate STJP on menthol content

To determine menthol content, 5 ml of the methanol and water in a ratio of 75:25 v/v were properly mixed with mentha oil obtained from plants. Further, the samples were filtered through 0.45 M Millipore filter membrane. The filtered sample was injected in the high-pressure liquid chromatography (HPLC) (Model No. WATER—2489) with C-18 column for determination of menthol content. Menthol: water (HPLC grade) (75:25) with 2×10^{-5} M *n*-heptyl-*p*-aminobenzoate was used as mobile phase and pressure and flow rate was set 800 psi, and 1.5 ml/min (Perkin 1984). The wavelength was monitored at 290 nm and compared with standard menthol (Sigma-Aldrich, USA).

Examination of P uptake in *M. arvensis*

For examination of P uptake in *M. arvensis*, roots, stem, and leaves were harvested (90 DAS) from each pot and dried separately. Further, dried sample was digested and analyzed using atomic absorption spectroscopy (Model: AA240FS Fast Sequential AAS, USA) for quantification of P as described by Koenig and Johnson (1942).

Statistical analysis

Pot experiments were organized in a complete randomized design (CRD) method. One-way analysis of variance (ANOVA) was chosen for data analysis. Duncan's multiple range test (DMRT) was used for comparison of means (Gomez and Gomez 1984) using the software of Statistical Package for Social Sciences (SPSS) version 15.

Results

Characterization of phosphate solubilizing rhizobacteria

Eleven PSB were isolated from rhizospheric soil of *S. rebaudiana*. Among them, six isolates showed greater than 6 mm zone on PKV agar plates (Fig. 1a). Isolates were further screened for the amount of phosphate solubilization in liquid NBRIP medium. All isolates were able to carry out phosphate solubilization ranging from 8 to 610.33 µg/ml (Table 1). Among them, isolate STJP showed maximum P-solubilizing activity (610.33 µg/ml) in NBRIP medium and hence was selected for further study. Isolate STJP was found to be flagellated bacterium with peritrichous arrangement and cell size of $1.0\text{--}1.28 \times 0.5\text{--}0.7$ µm (Fig. 1b). Isolate STJP was found positive for oxidase, urease, catalase, and gelatinase, but negative for indole, H₂S, citrate, and nitrate production. On the basis of phenotypic

and biochemical traits, isolate STJP was found to be a member of genus *Bacillus* sp. on the basis of Bergey's Manual of Systematic Bacteriology (Garrity 2005). The comparison of the whole 16S rRNA gene sequence of a neighbour isolate of STJP showed 99% similarities with *Bacillus safensis* strain NBRC 100820. The gene sequence data is submitted in NCBI database with Accession number KX372540 and can be said to belong to *B. safensis* clade. However, further studies are required to confirm the species of the strain.

Phosphatase assay

The production of phosphatase by isolate STJP was checked every 24 h up to 7 days in NBRIP medium. Isolate STJP produced maximum phosphatase (81.45 U/ml) after 96 h which reduced with further incubation (data not shown).

Fig. 1 **a** Phosphate solubilization by isolate STJP on Pikovskaya agar. **b** Cell shape of the bacterium STJP

Table 1 Quantitative examination of phosphate solubilization by different isolates (µg/ml) (1–5 days)

PSB isolates	1st day	2nd days	3rd day	4th day	5th day
B1TNP	48.33 ± 2.35	70.66 ± 2.49	122.33 ± 2.05	189.33 ± 2.49	188.33 ± 2.35
B2NPT	70.00 ± 4.08	80.66 ± 2.49	88.33 ± 2.35	234.33 ± 3.29	161.66 ± 2.35
B1PPoNP	10.00 ± 4.08	27.66 ± 2.05	68.33 ± 4.64	138.00 ± 1.63	134.66 ± 3.39
NPSP	89.33 ± 2.49	102.33 ± 6.12	31.00 ± 2.94	21.33 ± 1.88	8.00 ± 1.63
NPPsP2	83.33 ± 2.35	113.33 ± 4.71	159.33 ± 0.94	192.00 ± 1.63	185.66 ± 3.29
STJP	139.00 ± 2.94	188.33 ± 6.23	347.66 ± 2.05	610.33 ± 2.05	604.00 ± 4.10
STJP-12	052 ± 2.12	75.66 ± 3.29	101.66 ± 2.35	162.00 ± 1.63	235.00 ± 4.08
STJP-18	31.33 ± 1.88	53.33 ± 3.39	91.66 ± 1.24	161.33 ± 1.88	132.66 ± 1.88
STJP-20	89.66 ± 3.68	135.00 ± 4.08	237.66 ± 2.05	241.66 ± 2.35	208.33 ± 2.35
STJP-22	036 ± 561	092.00 ± 2.16	38.00 ± 1.63	132.66 ± 2.49	114.33 ± 3.29
STJP-30	37.66 ± 2.05	52.50 ± 1.87	88.33 ± 2.35	181.66 ± 2.35	132.33 ± 2.05

Bold values are for the selected isolate STJP which showed maximum phosphate solubilization on all the days of incubation

Data are mean of three replicates ± standard error of means

Plant growth promoting characteristics

The intensity of pink color production within 30 min is the first visible confirmation of IAA production. Isolate STJP showed intense pink color and recorded a significant amount of IAA (30.59 µg/ml). Further, isolate STJP showed a good amount of siderophore production (16.06 µg/ml) and observed a high affinity for iron chelation. The color change of the filter paper from yellow to brown is a key for detection of HCN. STJP did not show HCN production as confirmed by the absence of any color change of the filter paper laden with picric acid. Isolate STJP was unable to grow on nitrogen-free medium when tried repeatedly confirming its inability to fix nitrogen.

Effect of STJP on growth promotion of *M. arvensis*

Bacterial isolate STJP positively affected the plant growth parameters as observed after 90 days. STJP showed stimulatory effect, in term of growth parameters as compared to the control (Table 2). Among all treatments, STJP + TCP²⁰⁰ recorded maximum enhancement in vegetative parameters as compared to the control.

STJP treatment of *M. arvensis* enhanced fresh and dry weight by 46.27% and 49.57% respectively, in comparison with the control plant. The combined application of TCP¹⁰⁰ and STJP corresponded to 46.65% and 70.37% rise in fresh and dry weight respectively, in comparison with un-inoculated plants. Moreover, STJP + TCP²⁰⁰ treatment resulted in maximum fresh and dry weight enhancement (by 70.13% and 82.11%, respectively) as compared to the control plants (Table 2). The plant length of *M. arvensis* treated with STJP, STJP + TCP¹⁰⁰, STJP + TCP²⁰⁰ recorded an increase of 55.12%, 74.36%, and 86.57%, respectively, as compared with the control plants. However, treatment with 100 mg TCP and 200 mg TCP only, did not affect significantly in the length of the plant. Although, *M. arvensis*

treated with STJP showed significant increase in the leaf number (39.84%) as compared to the control plants but STJP + TCP²⁰⁰ application showed maximum increment in leaf number as compared to all other treatments. The number of leaves did not increase significantly after treatment with 100 mg and 200 mg TCP applications in comparison with the control plants.

Effect of isolate STJP on oil yield and menthol content in *M. arvensis*

M. arvensis grown in soil amended with TCP showed higher oil yield as compared to without TCP amended soil (Fig. 2). A significant rise in oil yield of *M. arvensis* was observed after inoculation of STJP (56.71%) as compared to the control (without any treatment). There was 107.68% and 79.85% enhancement in oil yield by the treatment with STJP + TCP²⁰⁰ and TCP¹⁰⁰ + STJP, respectively, in comparison with the control plant. Beside oil yield enhancement, the menthol content also increased significantly by the combined treatment of TCP and STJP (figure not shown). However, as in case of vegetative parameters, greatest enhancement in menthol content was shown when *M. arvensis* was treated with STJP + TCP²⁰⁰, as this treatment showed a maximum spike in peak (as compared to the control) when checked by HPLC.

Effect of STJP on P uptake in *M. arvensis*

Total P uptake in *M. arvensis* was studied after harvesting. The increase in P levels in roots, stem, and leaves were found to be 232%, 343.75%, and 327% respectively, by the STJP + TCP²⁰⁰ treatment which was maximum in comparison to any other treatment when compared with the control plant (Fig. 3).

Table 2 Effect of isolate STJP on growth promotion of *M. arvensis*

Treatments	Plant shoot length (cm)	Plant root length (cm)	Number of leaves	Total fresh weight (g)	Total dry weight (g)
Control	18.00 ± 3.20 ^c	09.03 ± 1.66 ^c	42.66 ± 1.88 ^c	28.83 ± 1.24 ^d	13.03 ± 1.23 ^d
STJP	27.20 ± 2.08 ^b	14.73 ± 0.55 ^{ab}	59.66 ± 1.69 ^b	42.17 ± 2.10 ^b	19.49 ± 1.52 ^{bc}
TCP ^{100A}	18.93 ± 1.23 ^c	09.63 ± 0.91 ^c	44.00 ± 1.63 ^c	35.61 ± 2.15 ^c	16.87 ± 1.22 ^c
STJP + TCP ¹⁰⁰	29.53 ± 0.80 ^{ab}	17.60 ± 1.62 ^a	61.66 ± 2.62 ^{ab}	42.28 ± 1.18 ^b	22.20 ± 0.98 ^{ab}
TCP ^{200B}	19.93 ± 0.66 ^c	10.13 ± 0.66 ^{bc}	44.33 ± 1.69 ^c	36.01 ± 2.12 ^c	17.22 ± 1.15 ^c
STJP + TCP ²⁰⁰	32.20 ± 2.01 ^a	18.23 ± 1.97 ^a	66.66 ± 3.39 ^a	49.05 ± 2.28 ^a	23.73 ± 1.81 ^a

Data are mean of three replicates ± standard error of means. Means, followed by the same letter in a column are not significantly different ($P=0.05$) by Duncan's multivariate test (DMRT)

^ATCP¹⁰⁰ (100 mg tricalcium phosphate in per kg of sterilized soil)

^BTCP²⁰⁰ (200 mg tricalcium phosphate in per kg of sterilized soil)

Fig. 2 Effect of isolate STJP on P uptake in *M. arvensis* [data are mean of three replicates $\pm$ standard error of means. Means, followed by the same letter in a column are not significantly different ($P=0.05$) by Duncan's multivariate test (DMRT) ($P \leq 0.05$)]

Fig. 3 Effect of isolate STJP on the yield of mentha oil in *M. arvensis* [data are mean of three replicates $\pm$ standard error of means. Means, followed by the same letter in a column are not significantly different ($P=0.05$) by Duncan's multivariate test (DMRT)]

Discussion

Menthol is one of the key plant metabolites with a wide range of applications in medicine and industries. According to an industrial report, the estimated production of menthol was 36,000 metric tonnes in 2017, which is much lower than the current global demand of approximately 45,000 metric tonnes (<http://www.aosproduct.com/MARKET-REPORT/Menthol-Crystals-and-Mentha-oil>). Hence, there is an urgent requirement to raise the menthol production which can be achieved in a better and sustainable manner if done through biological approaches. PGPR can thus play a key role in the enhancement of productivity of menthol producing plants. Among PGPR, *Bacillus* spp. are omnipresent in nature, and are widely used for enhancement of growth, development, and yield of plants

by producing bioactive metabolites and nutrients (Radhakrishnan et al. 2017). These bacteria have the ability to solubilize phosphate in the soil and also the ability to produce a number of metabolites such as IAA and siderophores which are directly involved in nutrients uptake and plant growth (Ribeiro et al. 2018).

P is the second most important macronutrient which is also a limiting factor for plant growth and development apart from nitrogen. Most of the P in soil is found in an insoluble form such as aluminium phosphate (AlPO_4) or ferric phosphate (FePO_4) which cannot be taken up by crops (Chen et al. 2017). The ability of PSB to solubilize insoluble P and make it available to the plants by one of the several mechanisms such as producing phosphatase enzyme, releasing organic acids (gluconic, oxalic and succinic acids), ion exchange and chelation (Wei et al. 2018) are well known. *Bacillus* spp. and in particular, *B. subtilis* are widely reported as PSB (Radhakrishnan et al. 2017; Ahmad et al. 2017). In the current study, phosphate solubilizing isolate STJP from the rhizosphere of a medicinal plant and identified to be *Bacillus* sp. was tested as a potent PGPR for *M. arvensis*. Mukhtar et al. (2017) reported *B. safensis* and *Bacillus megaterium* as phosphate solubilizers, however in their study, *Bacillus megaterium* was found to be better phosphate solubilizing agent as compared to *B. safensis*. In the present study *B. safensis* isolate STJP was found to be effective phosphate solubilizer. The ability to solubilise phosphate by STJP was found to be better than reported earlier for *B. safensis* (Mukhtar et al. 2017).

Mechanisms of phosphate solubilization by PSB are very well known (Sharma et al. 2013; Behera et al. 2017). Phosphatase enzyme has been commonly reported for solubilization of inorganic phosphate (Wei et al. 2018). In our study, isolate STJP produced 81.25 U/ml phosphatase enzyme (after 96 h). There are many reports available on the

production of phosphatase by *Bacillus* spp. (Parhamfar et al. 2016; Behera et al. 2017). The phosphatase enzyme cleaves the insoluble phosphate into phosphate ions and thus helps in uptake of ions by the plants. Maximum growth enhancement of *M. arvensis* was observed in the presence of STJP in combination with TCP²⁰⁰ treatment. This might be associated with the presence of a phosphatase enzyme produced by *Bacillus* sp. STJP. Isolate STJP produced 8.64 U/ml phosphatase after 24 h which increased with incubation period up to 96 h (81.45 U/ml). Similarly, Swain et al. (2012) reported that *B. subtilis* produced 30.2 U/ml phosphatase at 12 h incubation period but further increase in incubation period resulted in higher production of the enzyme.

P content was also increased to the maximum level in *M. arvensis* when treated with STJP + TCP²⁰⁰ as compared to the addition of TCP only. Treatment of STJP + TCP²⁰⁰ also resulted in maximum enhancement of plant growth parameters. Pereira et al. (2015) examined that *Arthrobacter nicotinovorans* EAPAA inoculated in TCP amended soil enhanced biomass and uptake of P in *Zea mays* plants.

In addition to phosphate solubilization, isolate STJP also showed a significant amount of IAA and siderophore production. Phytohormone IAA stimulates the lateral root formation, cell elongation, and also helps in cell division and differentiation in plant cells (Goswami et al. 2016). Siderophore plays a significant role in chelation of micro-nutrients such as iron even under limiting conditions (Arora and Verma 2017). Earlier studies have reported *Bacillus* spp. to be good producers of IAA and siderophores (Walpoli and Yoon 2013). Researchers have also reported *B. safensis* as effective producer of IAA (Mukhtar et al. 2017) and siderophore (Chakraborty et al. 2013). Production of siderophore in the rhizosphere also helps in phosphate solubilisation and availability of phosphate ions to the plant through chelation of iron from precipitated forms (FePO₄) (Sharma et al. 2013). *B. safensis* is a well-reported PGPR which has the ability to enhance the growth and productivity under biotic and abiotic conditions (Lateef et al. 2015). However, in the present work *Bacillus* sp. STJP capable of production of IAA, siderophore and phosphate solubilisation, significantly enhanced the growth of *M. arvensis*.

To the best of our knowledge, this is the first report on a PSB strain (*B. safensis* STJP) which has been employed successfully to significantly enhance the menthol content of *M. arvensis* simultaneously with plant growth and P content. Treatment with STJP + TCP²⁰⁰ (in pot trials) resulted in a maximum increment of oil content and it was approximately 37% higher than treatment with TCP²⁰⁰ alone. It has been informed earlier that the application of IAA increased the yield of menthol in *Mentha piperita* (Khan et al. 2015). Similarly, Hazzoumi et al. (2014) studied that essential oil content of Basil increased on the application of IAA. Isolate STJP is also a prolific IAA producer and this study

also suggests the role of this growth hormone in enhancing the oil content of the plants might be associated with better growth of roots and shoots. P plays an important role in the enhancement of total biomass, and yields of bioactive components in the medicinal plants (Sharma et al. 2013). The deficiency of P in soils/plants causes a reduction in the net photosynthesis rate (PN) resulting in a decrease of biomass and oil content in crops (Xu et al. 2007). P also induces the synthesis of secondary metabolites and highly modulates the oil synthesis in medicinal plants via a nutrient-plant growth enhancement mechanism (Malusà et al. 2006; Nell et al. 2009) and menthol is a type of metabolite which is produced by *M. arvensis*. Also, IAA production results in improved root hair formation. Simultaneously release of IAA and phosphate solubilization activities by STJP most probably resulted in enhanced P uptake by the plant. This may justify the maximum increase in root weight in case of plants treated with PGPR and TCP²⁰⁰. It has been reported earlier that PSB having the capability to produce other metabolites such as IAA and siderophore are far more efficient in enhancing the growth and yields of crop plants (Koo and Cho 2009). In fact, Gupta et al. (2012) also reported the role of IAA and siderophore produced by PSB strains along with TCP in enhancing the growth and Aloin-A content of *Aloe barbadensis*. Hence, using *B. safensis* as a biofertilizer in an integrated approach with TCP could be an effective tactic for improvement of soil health, growth enhancement and oil yield in *M. arvensis*. When applied alone most of the TCP gets precipitated in the soil and causes stresses such as salinity and eutrophication as runoff to the water bodies (Tewari and Arora 2018). By the way of integrated approach of utilizing an efficient PGPR with multiple growth traits including efficient phosphate solubilizing abilities we can reduce the precipitation of phosphates in the soil and simultaneously diminish the eutrophication of water bodies like ponds and lakes which are integrated for water supply in agro-ecosystems (Arora et al. 2016; Gouda et al. 2018). PSB possessing PGP characters such as siderophore and IAA production can thus not only enhance the growth and metabolite content of medicinal plants but also protect from unnecessary pollution due to the addition of phosphate fertilizers.

Conclusion

The present study reports *B. safensis*, an efficient PGPR with phosphate solubilizing abilities, for growth promotion and increasing the oil content of *M. arvensis*. The experiments clearly show the efficiency of an integrated approach wherein fertilizer TCP and the PSB are co-inoculated to enhance the productivity of a very important medicinal and commercial plant. *Bacillus* sp. STJP is also an efficient producer of IAA and siderophore. The cocktail of PGP

characters helped in enhancing the yield and oil content of *M. arvensis*. This is the first report suggesting the use of phosphate solubilizing *Bacillus* in enhancing the menthol content of *M. arvensis*. Menthol is a key plant metabolite having wide applications in various industries. Enhancement of oil yields that too through eco-friendly biological approach can be a major breakthrough for menthol production and development of the country's economy.

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Compliance with ethical standards

Conflict of interest All authors declare that there is no conflict of interest in this original article.

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