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Induction of Plant Growth Promotion in *Camellia sinensis* by *Bacillus megaterium* and its Bioformulations

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Abstract: Plant growth promoting ability of *Bacillus megaterium* (TRS-4), isolated from rhizosphere of tea bushes, was tested on 5 varieties of tea- TV-18, TV-23, TV-25, TV-26 and T-17/1/154 in the experimental field and in potted condition. An increase in height, emergence of new leaves, branches and increase in leaf numbers as well as increase in leaf dry mass was observed following application of the bacterium. The experimental field was designed in randomized block design where three replicate plots with 10 plants /treatment for each variety was taken. In the field, the experimental plot of Tea Estate was arranged into 2-replicated randomized block design, with each treatment of variety T-17/1/154 having 100 bushes. Increase in number of leaves and number of branches were significant after 12 months of application in field but in potted plants, significant increase were obtained even after 2 months of application. Significant increase in accumulation of phenolics was also observed in tea plants. *B. megaterium* solubilized phosphate *in vitro* and *in vivo*. Following application of the bacterium, soil P content decreased, root and leaf phosphate increased and soil phosphatase activities were enhanced. Chlorophyll contents of leaves were enhanced by the application of *B. megaterium*. Bioformulations of the bacterium in saw dust, rice husk and tea waste were found to survive for more than 9 months *in vitro* with populations in the range of $1\times10^{6\text{-}7}$c.f.u/ml. The bioformulations were as effective as aqueous suspensions in plant growth promotion. There was no significant difference among the aqueous suspension or different bioformulations in increase in height and number of leaves after 2 months of application, indicating that the application of bioformulations could also effectively promote tea plant growth.

Key words: *Bacillus megaterium* · Growth promotion · Phosphate solubilization · Bioformulation

INTRODUCTION

Tea (*Camellia sinensis* (L.) O. Kuntze) is one of the most important plantation crops in Darjeeling and Dooars regions, which are the tea growing regions of West Bengal, India. Over the years, productivity of the plant has been decreasing and one of the reasons for this has been attributed to the continuous use of huge quantities of chemicals in tea plantations. Hence, there is a pressing need in tea industry for utilizing either biological products completely or reducing the use of chemicals by supplementing with biological products in integrated management practices.

The rhizosphere, a zone very rich in nutrients, supports large microbial populations, which exert beneficial, neutral or detrimental effects on plant growth. Plant growth promoting rhizobacteria, first defined by Kloepper and Schroth [1], include those bacteria that are able to aggressively colonize plant roots and stimulate plant growth when applied to roots, tubers and seeds. Excess use of chemical fertilizers and fungicides has resulted in adverse impact on soil environment which in turn leads to loss in productivity and hence plant growth promoting rhizobacteria (PGPRs) are now finding increasing applications as biofertilizers [2]. The potential benefit of plant growth promoting rhizobacteria is not fully realized because of limitation like inconsistence performance at different location and season.

The mechanisms by which PGPRs can influence plant growth may differ from species to species as well as from strain to strain. Growth promotion mechanism may be direct i.e. production of growth hormones, phosphate solubilization, nitrogen fixation or indirect viz., suppression of deleterious microorganisms by

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siderophore production or secretion of antifungal metabolites [3]. PGPRs have also been reported to protect plants from various pathogens by activating defense genes encoding - peroxidase, chitinase, phenylalanine-ammonia-lyase, β-1, 3- glucanase and others, involved in synthesis of phytoalexin [4, 5]. Isolates of *Bacillus megaterium* have also been reported to produce antibiotics against several fungal pathogens [6].

Phosphorus is an essential nutrient for plants and an important component in cell metabolism. There are two components of P in soil, organic and inorganic phosphates. A large proportion is present in insoluble forms and therefore, not available for plant nutrition. Inorganic P occurs in soil, mostly in insoluble mineral complexes, some of them appearing after the application of chemical fertilizers. These precipitated forms can not be absorbed by plants. Organic matter, on the other hand, is an important reservoir of immobilized P that accounts for 20-80% of soil P [7]. Conversion of insoluble phosphates (both inorganic and organic) to a form accessible to the plants, like orthophosphate, is an important trait of a plant growth promoting bacterium (PGPB) for increasing plant yields. Phosphorus can be released from organic compounds in soil by three groups of enzymes including phosphatases. Acid phosphatases catalyse non- specific hydrolysis of inorganic phosphate (Pi) from phosphate monoesters in pH ranges from 4 to 6 and help in the supply of phosphate in plants [8-9]. Similarly alkaline phosphatases have a role in maintenance of cellular metabolism [10]. Use of plant growth promoting microbes as biocontrol agents and biofertilizers give eco-friendly and inexpensive alternatives to the use of chemicals. However, for easy handling of such bacteria, it is necessary to pack such bacteria in inert materials which can also be packaged and stored. Initially, it is essential to determine whether the bacteria can survive in the bioformulations for a reasonable period of time and whether they can induce similar effects to those observed by live bacterial cells. The efficacy of bacterial inoculants would largely depend on the type of formulation and delivery technology [11]. Experimental formulations of *Bacillus spp* that have effectively reduced plant diseases have included peat and chitin [12, 13], ca- alginate, alginate manucol [14].

The purpose of the present study was to evaluate the potential of *Bacillus megaterium*, which was originally isolated from tea rhizosphere [15] on tea growth in the nursery, in the experimental field as well as in tea plantation. Influence of the bacterium on phosphate solubilization in the soil and phosphate contents in the plants, as well as its influence on major biochemical components have also been studied. Further, efficacy of the bacterium as bioformulations for improving of plant health have also been determined.

MATERIALS AND METHODS

Isolation of Microorganisms: *Bacillus megaterium* TRS-4 was isolated from the rhizosphere soil of tea bushes from Hansqua Tea Estate, West Bengal, India and preliminarily identified on the basis of morphological, microscopic and biochemical tests [15]. The identity of the bacterium was confirmed from the Plant Diagnostic and Identification Services, UK. The bacterium was maintained on Nutrient Agar (NA) slants.

Selection of Plant Material and *In Vivo* Application of *B. megaterium*: Eighteen-month old seedlings of five varieties of tea- TV-18, TV-23, TV-25, TV-26 and T-17/1/154, were selected for experimental purposes. The selected tea seedlings were maintained in 12½ earthenware pots and also in the experimental field in randomized block design. Tea seedlings were watered regularly for proper maintenance. In the experimental plot of Hansqua Tea Garden, 8 yr old pruned plants of T-17/1/154 were selected for experimental purposes. The plot was also divided into a 2-replicated randomized block design for experiments. Aqueous suspension of *B. megaterium* (1×10^8 c.f.u/ml) was applied as a soil drench as well as foliar spray, @ 100 ml/plant to the rhizosphere of tea plants of the 5 varieties in experimental field, to the rhizosphere and leaves of 8 yrs old pruned tea plants of T-17/1/154 Hansqua Tea Estate field and also in potted plants. Application was done at an interval of one month and three applications were done in case of experimental field and pot. Application was also done at an interval of two months and two applications were done in case of Hansqua Tea Estate. Growth promotion was studied in terms of increase in height, number of leaves, number of branches and leaf dry mass in comparison to control, Observations were recorded at regular intervals.

Phosphate Solubilization: Primary phosphate solubilizing activity of *B. megaterium* was carried out by allowing the bacterium to grow in selective medium i.e., Pikovskaya's agar (PVK) (Himedia- M520; ingredients- yeast extract- 0.50 g/l, dextrose- 10.00 g/l, calcium phosphate- 5.00 g/l, ammonium sulphate- 0.50 g/l, potassium chloride- 0.20 g/l, magnesium sulphate- 0.10 g/l, manganese sulphate- 0.0001 g/l, ferrous sulphate- 0.0001 g/l and agar- 15.00 g/l) for 7 to

10 days at 37°C [16]. The appearance of transparent halo zone around the bacterial colony indicated the phosphate solubilizing activity of the bacterium. For phosphate determination, soil sample (1g) was air dried and suspended in 25 ml of the extracting solution (0.025N H_2SO_4, 0.05N HCl) to which of activated charcoal (0.01g) was also added, shaken well for 30 min on a rotary shaker and filtered through Whatman No. 2 filter paper [17]. In case of plant leaf and root samples, oven dried plant material was crushed with extracting solution. Quantitative estimation of phosphate was done following ammonium molybdate-ascorbic acid method [18].

Acid and Alkaline Phosphatase Activities: About 2 g portions of each soil sample was used for enzyme extraction and assays. The activities of enzymes were expressed according to method of Tominaga and Takeshi [19] with modifications. For acid phosphatase assay, soil samples were extracted in 5 ml of 50 mM sodium acetate buffer (pH5.0) using a chilled mortar and pestle which was then transferred into a tube and solution was shaken well. 1 ml of 5 mM p- nitrophenyl phosphate solution was added to the tube. All the tubes along with control were allowed to incubate at 37°C for 1 hour. After incubation, 2 ml of solution was transferred into centrifuge tubes. Centrifugation was done at 10,000 r.p.m for 2 min at 4°C. Finally the supernatant was taken and 4.0 ml of 100 mM NaOH was added to it in a test tube and mixed well for termination of reaction. Three ml. of this mixture was taken in a cuvette (volume 4 ml.) and absorbance at 400 nm was noted for determination of the amount of p-nitrophenol liberated. Enzyme activity was expressed as one µmol p- nitrophenol liberated/sec/g of soil. The procedure for the assay of alkaline phosphatase was similar to acid phosphatase except that for enzyme extraction and incubation 100 mM sodium bicarbonate buffer (pH 10.0) was used.

Estimation of Phenolics and Total Chlorophyll Contents: Total phenols were extracted and estimated from tea leaves following the method of Mahadevan and Sridhar [20]. Quantification of total phenol was done using a standard of caffeic acid. Extraction and estimation of chlorophyll contents were performed by standard methods [21].

Development of Bioformulations: Three bioformulations were prepared- using saw dust, rice husk and tea waste. For the preparation of bioformulations, 2.5 g of carboxy methyl cellulose sodium salt (Himedia) was added to 250 g of saw dust or rice husk and pH was adjusted to 7 by adding calcium carbonate. They were sterilized by autoclaving for 30 min at 15 lbs. p.s.i. and 120°C twice. 100 ml of aqueous bacterial suspension (a concentration of 1×10^{10}c.f.u/ml) was added to the mixtures under sterile condition. The mixture was dried under shade to reduce the moisture to less than 20%. Formulations were packed in polythene bags, sealed and stored at room temperature. In preparation of tea waste bioformulation, the procedure was similar as mentioned above but tea waste was initially soaked overnight in distilled water to remove the phenolic components. Survivability of *B. megaterium* was checked at a regular interval of 1 month for a period of nine months using direct plating method in nutrient agar medium. The data was expressed in log form for analysis.

Results were analysed by ANOVA and Student's 't' test. Standard error was determined in each case.

RESULTS AND DISCUSSION

Application of *Bacillus megaterium* to the rhizosphere of five varieties of tea plants in the experimental field resulted in an increase in growth in terms of increase in leaf numbers and number of branches. Increase in number of leaves was significant after 12 months of application, whereas initial leaf number and increase in leaf number after 6 months of 1st application were insignificant. Similar trend was also observed in case of increase in branch numbers (Table 1). In a study conducted on groundnut, Kishore *et al.* [22] reported that *Bacillus firmis* GRS123, *B. megaterium* GPS 55 and *P. aeruginosa* GPS 21 promoted seedling emergence, root length, shoot length, dry weight and pod yield. In potted plants, application of *B. megaterium* revealed that increase in height and number of leaves after 2 months were significant but initial height and leaf numbers were insignificant (Tables 2 & 3). Growth promotion was also studied in terms of increase in number of leaves in T-17/1054 in comparison to control plot in the experimental field of Tea Estate (Table 4). Statistical analysis (ANOVA) revealed a significant increase in no. of leaves by application of the bacterium. Several species and strains of *Bacillus* have also been reported to induce plant growth promotion (seed germination, seedling emergence, increase in shoot and root biomass etc.) and disease resistance in crop plants. These include *B. pumilus* on cucumber, tomato [23, 24], tea [25] and *B. sphaericus* on cucumber [24]. The ability of *B.megaterium* to increase leaf number and biomass is especially important in tea plantations since tea is cultivated for its leaves.

Table 1: Effect of application *B. megaterium* on branch and leaf numbers of tea plants in field

		No. of leaves			No. of branches		
Tea varieties	Treatment	Initial	After 6 months	After 12 months	Initial	After 6 months	After 12 months
TV-18	Control	11^{a}±0.8	47^{a}±1.4	51^{a}±1.6	3^{a}±0.5	4^{a}±0.5	08^{a}±1.2
	B.megaterium	12^{a}±0.9	51^{a}±1.5	61^{c}±1.5	4^{a}±1.0	9^{b}±1.7	12^{b}±1.1
TV-23	Control	13^{a}±1.0	21^{a}±1.4	30^{a}±1.0	2^{a}±0.4	3^{a}±0.9	08^{a}±0.9
	B.megaterium	12^{a}±1.2	33^{c}±2.2	35^{c}±0.9	3^{a}±0.5	5^{a}±1.0	12^{c}±0.8
TV-25	Control	12^{a}±0.7	29^{a}±1.7	31^{a}±0.8	2^{a}±0.6	3^{a}±0.6	07^{a}±1.0
	B.megaterium	09^{a}±0.5	79^{c}±1.7	80^{c}±0.7	3^{a}±0.7	8^{c}±0.5	13^{c}±1.1
TV-26	Control	14^{a}±1.0	68^{a}±2.3	69^{a}±2.9	5^{a}±1.0	8^{a}±1.1	10^{a}±1.0
	B.megaterium	17^{b}±0.4	90^{c}±2.2	92^{c}±2.8	4^{a}±0.8	8^{a}±1.4	14^{b}±1.2
T-17	Control	13^{a}±1.0	67^{a}±2.2	71^{a}±1.9	4^{a}±0.6	4^{a}±0.9	09^{a}±1.1
	B.megaterium	15^{a}±0.5	76^{b}±2.8	81^{c}±1.6	4^{a}±0.4	5^{a}±0.8	16^{a}±1.8
CD (P=0.05) (Treatments)		2.71	22.79	22.21	1.11	2.85	1.366
(Varieties)		4.30	36.03	35.11	1.75	7.35	1.527

Mean of 30 replicate plants/treatment; Differences between control and treatment in each variety insignificant when superscript same (^{a,a}), significant when superscript different (^{a,b} P=0.05; ^{a,c} P=0.01)

Table 2: Effect of application of *B.megaterium* on height of plants(± s.e.) and leaf numbers(± s.e.) of tea grown in potted condition

		Height (cm)		Leaf numbers	
Tea varieties	Treatment	Initial	After 2 months	Initial	After 2 months
TV-18	Control	08.0^{a}±1.1	10.0^{a}±1.2 (2)	26.0^{a}±1.9	29.0^{a}±1.6(3)
	B. megaterium	09.0^{a}±1.5	21.0^{c}±1.5(12)	29.0^{a}±1.7	38.0^{c}±1.4(9)
TV-23	Control	11.0^{a}±1.0	14.0^{a}±1.3(3)	29.0^{a}±1.9	34.0^{a}±1.8(5)
	B. megaterium	13.0^{a}±1.4	30.0^{c}±1.8(17)	32.0^{a}±2.4	47.0^{c}±2.3(15)
TV-25	Control	14.0^{a}±1.5	17.0^{a}±1.1(3)	26.0^{a}±1.8	32.0^{a}±2.7(6)
	B. megaterium	13.0^{a}±1.5	24.0^{c}±1.2(11)	29.0^{a}±2.7	41.0^{b}±2.9(12)
TV-26	Control	12.0^{a}±1.7	18.0^{a}±1.5(6)	22.0^{a}±1.5	29.0^{a}±2.4(7)
	B. megaterium	15.0^{a}±1.1	31.0^{c}±1.4(16)	22.0^{a}±1.1	39.0^{b}±3.0(17)
T-17	Control	10.2^{a}±1.1	16.2^{a}±1.5(6)	17.1^{a}±1.4	21.2^{a}±1.8(4.1)
	B. megaterium	12.0^{a}±1.4	22.5^{b}±1.7(10.5)	17.2^{a}±1.5	35.0^{a}±2.6(17.8)
CD (P=0.05) (Treatments)		1.862	5.063	2.007	2.834
(Varieties)		2.944	8.006	3.173	4.48

Mean of 10 replicate plants/treatment; Differences between control and treatment in each variety insignificant when superscript same (^{a,a}), significant when superscript different (^{a,b} P=0.05; ^{a,c} P=0.01); Figures in parenthesis indicate the increment over a period of 2 months

Table 3: Biomass of tea leaves of potted tea plants 2 months after application of *B.megaterium*

Tea varieties	Treatment	Fresh weight (g)	Dry weight taken after 7 days (g)
TV-18	Control	04.5^{a}±1.5	02.4
	B. megaterium	15.0^{c}±1.4	07.4
TV-23	Control	09.0^{a}±1.0	04.0
	B. megaterium	25.0^{c}±1.4	10.5
TV-25	Control	10.0^{a}±1.0	06.5
	B. megaterium	18.0^{c}±1.2	08.8
TV-26	Control	02.0^{a}±0.5	01.3
	B. megaterium	15.0^{c}±1.0	04.3
T-17	Control	06.0^{a}±1.0	04.3
	B. megaterium	20.0^{c}±1.7	08.5
CD (P=0.05) (Treatments)		3.867	2.057
(Varieties)		6.114	3.253

Mean of 10 replicate plants/treatment; Differences between control and treatment in each variety insignificant when superscript same (^{a,a}), significant when superscript different (^{a,b} P=0.05; ^{a,c} P=0.01); Figures in parenthesis indicate the increment over a period of 2 months

Table 4: Effect of *B.megaterium* on leaf numbers of 8 yr old tea plants (T-17/1054)

		Mean leaf number		
Tea varieties	Treatments	After 2 months	After 4 months	Increment in leaf number
T-17/1054	Control	159±12.5	238±10.4	79
	T1 (*B.megaterium* treated)	184±13.2	314±12.9	130

Mean leaf no. of 40 replicates; Treatments were applied to 8 yr. old bushes soon after pruning; Differences between control and treatments significant at P=0.01, 4 months after 1st treatment

Table 5: Phosphate content of soil, roots and leaves of treated and control plants in field

		Phosphate content (µg/g)		
Tea varieties	Treatment	Soil	Root	Leaf
TV-18	Control	121.2±2.4	103.2±1.3	200.4±3.6
	B. megaterium	77.2±1.7	192.0±2.7	265.0±2.8
TV-23	Control	76.4±1.8	41.8±1.2	191.5±1.7
	B. megaterium	27.5±1.4	66.5±1.3	285.0±2.9
TV-25	Control	92.6±2.4	35.9±1.3	181.0±2.3
	B. megaterium	70.0±2.8	72.5±1.8	279.0±2.5
TV-26	Control	72.0±1.8	62.8±2.0	190.0±2.8
	B. megaterium	34.5±1.3	81.0±2.8	284.0±3.0
T-17	Control	134.6±1.4	149.6±2.9	193.0±1.9
	B. megaterium	110.2±1.4	235.0±4.2	284.5±2.7
CD (P=0.05) (Treatments)		21.72	42.06	16.76
(Varieties)		34.34	66.51	26.5

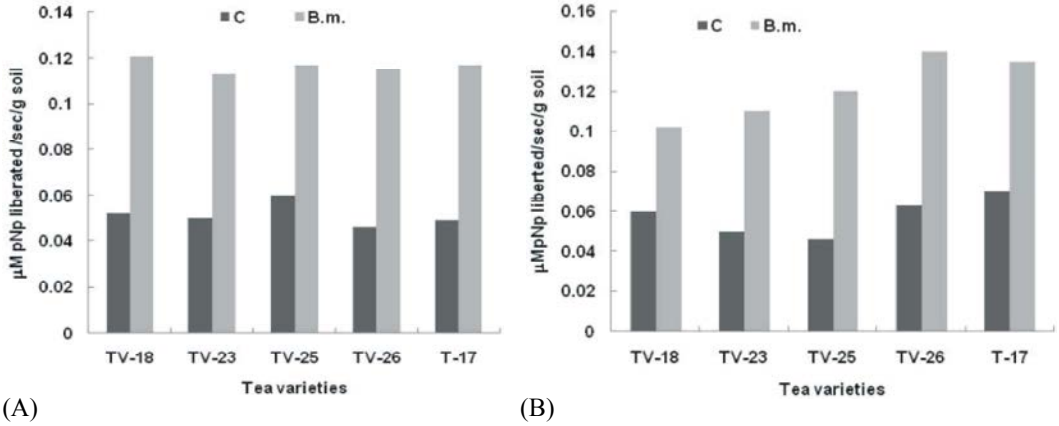
Average of 3 replicate sets

Table 6: Phosphate content of soil, root and leaves of treated and control potted plants

		Phosphate content (µg/g)		
Tea varieties	Treatment	Soil	Root	Leaf
TV-18	Control	156.3±1.8	115.8±1.9	203.4±2.4
	B. megaterium	101.7±1.9	215.4±1.4	270.0±3.5
TV-23	Control	92.7±2.7	74.4±1.8	194.5±1.9
	B. megaterium	37.1±1.3	146.7±2.7	272.2±2.8
TV-25	Control	116.6±1.4	74.1±1.1	190.0±3.0
	B. megaterium	62.2±1.9	149.5±1.5	290.0±2.5
TV-26	Control	81.0±1.1	73.3±1.8	196.0±1.8
	B. megaterium	78.1±1.9	75.2±1.9	278.8±1.9
T-17	Control	135.8±2.4	151.8±2.4	191.5±1.8
	B. megaterium	107.5±2.9	258.0±2.7	268.2±2.9
CD (P=0.05) (Treatments)		28.21	55.79	15.25
(Varieties)		69.12	88.21	24.11

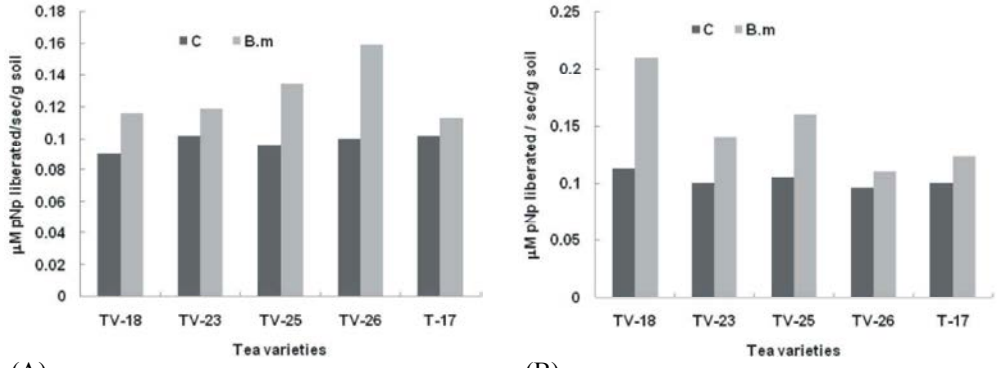
Average of 3 replicate sets

B. megaterium could solubilize phosphate *in vitro* as evidenced by the appearance of halo zone around the inocula in PVK medium. Estimation of phosphate contents in roots and leaves as well as rhizosphere soil revealed that while the soil P content decreased due to the activity of this phosphate solubilizing bacterium (PSB), root and leaf phosphate contents showed an increase both in treated tea plants in potted as well as field conditions in comparison to control (Tables 5 and 6). Maximum phosphate content was obtained in the leaf tissues. Increase in phosphate content was statistically significant. Both acid and alkaline phosphatase activities in rhizosphere soil of all five varieties were enhanced following application of the bacterium. Statistical analysis (ANOVA) of results showed that differences of control were significantly different from that of the treatment, but



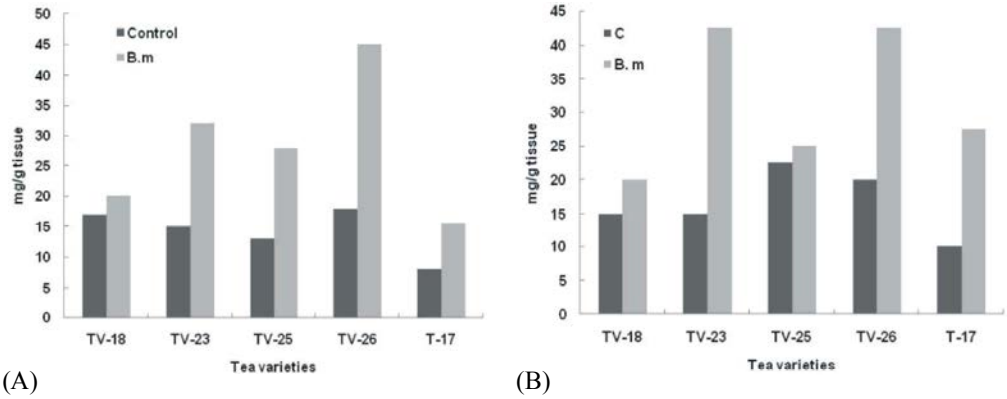
(A) (B)

Fig. 1: Acid phosphatase activities in rhizosphere soil of control and *B.megaterium* treated plants in pots (A) and field (B)



(A) (B)

Fig. 2: Alkaline phosphatase activities in rhizosphere soil of control and *B.megaterium* treated plants in pots (A) and field (B)



(A) (B)

Fig. 3: Total phenol contents of leaves of tea plants grown in pots (A) or in field conditions (B) after application of *B. megaterium*

no significant differences were evident among the treatments (Figures 1 and 2). Results, therefore clearly revealed *B. megaterium* to have the ability of phosphate solubilization in the soil, which would be one of the mechanisms of observed plant growth promotion by *B.megaterium*. PGPRs convert insoluble phosphorus (P) to an accessible form to the plants. Phosphate is often the limiting nutrient for microbial and plant growth in soil, phosphatases remove the phosphate from organic compounds and convert it in soluble form to the plants. Some PGPR biofertilizers also influence the availability of phosphate by secreting phosphatases for mineralization

Table 7: Chlorophyll content of leaves of tea varieties following treatment with *B.megaterium*

		Total chlorophyll (mg/gm tissue)	
Tea varieties	Treatment	Field grown plants	Potted plants
TV-18	Control	1.601	2.348
	B.megaterium	1.979	2.495
TV-23	Control	1.050	0.539
	B.megaterium	1.320	2.540
TV-25	Control	1.040	1.330
	B.megaterium	1.320	2.360
TV-26	Control	1.340	1.090
	B.megaterium	1.970	2.520
T-17	Control	1.000	2.157
	B.megaterium	1.940	2.437
CD (P=0.05) (Treatments) (Varieties)		0.355	0.968
		0.561	1.531

Mean of 3 replicates

Table 8: Effect of application of *B.megaterium* as bioformulations on growth of tea plants in potted conditions

		2 months after treatment			
Tea varieties	Treatment	Initial height	Final Height	Initial leaf numbers	Final leaf numbers
TV-18	Control	19.0^{a}±1.3	26.0^{a}±1.5(7)	19^{a}±1.8	24.5^{a}±1.2(5.2)
	B. megaterium	25.0^{b}±1.5	30.0^{a}±2.1 (5)	19^{a}±1.3	26^{a}±2.0(7.0)
	B.m+saw dust	20.0^{a}±1.9	27.0^{a}±1.2(7)	17^{a}±0.9	24^{a}±1.8(7.0)
	B.m+rice husk	19.5^{a}±1.5	32.0^{c}±1.1(12.5)	18.1^{a}±1.0	28^{a}± 2.1(9.9)
	B.m+tea waste	21.0^{a}±1.8	29.0^{a}±1.8 (8.0)	22^{a}±1.2	27^{a}±2.4(5.0)
TV-23	Control	22.4^{a}±0.9	25.0^{a}±1.7(2.6)	17.1^{a}±1.2	24^{a}±1.0(6.9)
	B. megaterium	24.5^{a}±1.5	33.0^{c}±2.1(8.5)	19^{a}±1.1	28^{a}±1.9 (9.0)
	B.m+saw dust	24.0^{a}±1.4	38.0^{c}±2.5(14)	21^{a}±1.7	32^{c}± 2.3(11)
	B.m+rice husk	23.0^{a}±2.1	39.0^{c}±2.7(16)	18^{a}±1.0	31^{b}± 2.4(13)
	B.m+tea waste	22.0^{a}±2.2	36.0^{c}±1.9(14)	19^{a}±2.0	30^{c}±1.4(10.7)
TV-25	Control	21.4^{a}±2.0	24.5^{a}±1.9 (3.1)	19^{a}±1.0	25^{a}±1.8 (5.7)
	B.megaterium	29.0^{b}±1.9	32.0^{a}±2.1 (3.0)	34^{c}±2.0	45^{c}±1.1(10.8)
	B.m+saw dust	27.0^{a}±2.1	29.0^{a}±1.3(2.0)	34^{c}±2.1	45^{c}± 2.1(11.2)
	B.m+rice husk	26.1^{a}±2.0	28.1^{a}±1.1(2.0)	31^{c}±1.9	42^{c}±1.8 (11.0)
	B.m+tea waste	28.0^{b}±2.2	32.0^{a}±2.1(4.0)	30^{c}±1.3	41^{c}±11.9(10.8)
TV-26	Control	22.4^{a}±1.8	25.5^{a}±2.3 (3.1)	17^{a}±2.0	21^{a}± 1.5(3.5)
	B. megaterium	25.0^{a}±1.7	33.0^{b}±1.7(8.0)	19^{a}±1.5	32^{c}±2.2 (13)
	B.m+saw dust	22.0^{a}±1.1	29.2^{a}±1.1(7.2)	17^{a}±1.0	30^{c}±2.4(13)
	B.m+rice husk	21.0^{a}±1.7	31.0^{a}±1.8(10)	16^{a}±1.2	27^{b}±1.9(11)
	B.m+tea waste	21.2^{a}± 1.9	30.2^{a}±1.2(9.0)	18^{a}±1.1	26^{b}±1.7(8.2)
T-17	Control	24.0^{a}±2.4	27.2^{a}±1.2(3.2)	18^{a}±1.5	20^{a}±1.8(1.9)
	B. megaterium	25.0^{a}±1.5	34.0^{b}±2.1(9.0)	18^{a}±1.6	26^{a}±1.5(8.0)
	B.m+saw dust	22.0^{a}±1.4	33.0^{b}±2.3(11)	21^{a}±2.0	26.5^{b}±1.7(5.5)
	B.m+rice husk	25.2^{a}±1.3	32.5^{b}±2.2 (7.3)	19^{a}±1.6	27^{b}±1.8(8.0)
	B.m+tea waste	27.0^{a}±2.0	31.0^{a}±1.9 (4.0)	20^{a}±1.5	28^{c}±1.1(8.3)
CD (P=0.05)		2.58	2.98	3.84	2.85

Mean of 10 replicate plants/treatment; Differences between control and treatment in each variety insignificant when superscript same (^{a,a}), significant when superscript different (^{a,b} P=0.05; ^{a,c} P=0.01); Figures in parenthesis indicate the increment over a period of 2 months

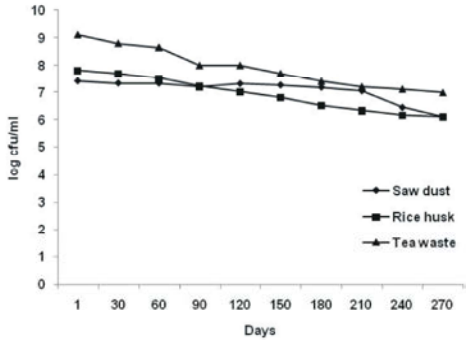


Fig. 4: Growth of *B.megaterium* in bioformulations at different periods of incubation

of organic phosphates [26]. Shankaraiah *et al*. [27] reported that *B. megaterium* alone, or in combination with *Agrobacterium awamori* could enhance phosphate solubilization and increase yield in sugarcane. They suggested that nutrient mobilization was one of the mechanisms of increasing yield by the microorganisms.

Application of *B. megaterium* in the soil increased accumulation of phenolics in tea leaves (Figure 3). In field grown plants, total phenol content in control leaves ranged from 10-25 mg/g tissue, while it increased upto 43 mg/g in treated plants. In case of potted plants, in control plants the phenol content ranged from 8-18 mg/ g tissue, whereas in case of treated plants it increased upto 45 mg/ g. Results revealed increase in total chlorophyll content of leaves from plants grown in control and treated plots and pots in the treatment by *B. megaterium* (Table 7). Ability of *B.megaterium* to promote growth in *Lactuca sativa* alone, or in combination with arbuscular mycorrhiza was reported previously, who also reported increased accumulation of chlorophylls and carotenoids [28]. Accumulation of higher levels of phenolics in plants resistant to various stresses have been reported by several authors [29].

The viability of the bacterium in formulations was tested during the storage period of 9 months at one-month interval. Results revealed that the isolate was viable till 9 months of storage; *B. megaterium* could survive in the range of 1×10^6c.f.u/ml in bioformulations of saw dust, rice husk and 1×10^7c.f.u/ml in tea waste respectively (Figure 4). Application of *B. megaterium* in bioformulations of saw dust, rice husk and tea waste led to significant increase in growth of tea seedlings. The growth was measured in terms of increase in height and number of leaves of seedlings two months after application. Increase in growth was more or less similar to that observed by application of aqueous suspension of the bacterium. Statistical analysis revealed initial height and leaf numbers were insignificant among the treatments but in TV-25 & T-17, significant results were observed. In case of increase in height and number of leaves after 2 months of application, there was no significant difference among the aqueous suspension or different bioformulations though all of them were significantly higher than control (Table 8).

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