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Novel potassium solubilizing bio-formulation improves nutrient availability, fruit yield and quality of pomegranate (*Punica granatum* L.) in semi-arid ecosystem

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ABSTRACT

A carrier based bio-formulation of potassium solubilizing fungi, *Penicillium pinophilum,* MCC0114 was evaluated for its effect on availability of K and P, plant nutrient status, fruit yield and quality of pomegranate under field condition. Soil inoculation with this bio-formulation was found to improve availability of K and P in soil and plant nutrient status that increased fruit yield by 35% with perceptible improvement in fruit quality. The impact of bio-formulation on fruit yield and quality was much higher, when supplemented with insoluble K bearing mineral. The highest yield (27% higher than that recorded from the application of potassic fertilizer at recommended dose) and cost-benefit ratio of 1:2.60 were recorded with the conjunctive use of bio-formulation and insoluble K bearing mineral at the rate of 20 g K_2O tree^{-1}. Higher dehydrogenase, alkaline and acid phosphatase activity, microbial biomass carbon content and population of *Penicillium* spp. in inoculated soil were due to rhizospheric colonization of *P. pinophilum* in the bio-formulation indicating its suitability for pomegranate.

1. Introduction

Potassium (K) is the third major important essential nutrient for plant growth. It plays an important role in enzyme activation, protein synthesis and photosynthesis. Total soil K reserves are generally large in the soil. However, the distribution of K forms *viz.,* water soluble, exchangeable and non-exchangeable or mineral follow a dynamic equilibrium with each other that vary from soil to soil as function of the dominant soil minerals present (Rao and Srinivas, 2017). Potassium from water soluble and exchangeable pools is directly available for plant uptake. The issue of sustainable potassium management in soil has largely been ignored in India during the last decades on thinking that Indian soils are rich in potassium. With each successive cropping, a considerable amount of potassium from the soil is lost with the harvest, runoff and erosion. Further, pomegranate growing areas are characterized by the presence of 2:1 type expanding clay minerals in the soil (Challa et al., 2008). This leads to fixation of potassium as and when applied to the soil and make it unavailable to the plant.

With the intensification of pomegranate cultivation in arid and semi-arid regions of India, the available soil K levels have dropped to a considerable extent (Bhattacharyya et al., 2015). For optimal nutrition of pomegranate crop, the replenishment of K-depleted soil solution takes place mainly by release of exchangeable K from clay minerals. Consequently, soil solution and exchangeable K are required to be replenished continuously through the release of non-exchangeable K by weathering of potassium reserves (i.e. micas and feldspars) (Basak and Biswas, 2009) or the addition of potassic fertilizers. Many soil microbes are capable of solubilizing 'non-available' forms of K bearing minerals *viz.,* micas, illite and orthoclases by excreting organic acids. These organic acids either directly dissolve rock K or chelate silicon ions to bring K into solution (Friedrich et al., 1991; Ullman et al., 1996; Bennett et al., 1998). Therefore, application of K-solubilizing microbes can be of economic importance in increasing K availability in the soil. The bacterial strains like *Bacillus edaphicus* NBT, Bacillus mucilaginosus are reported to improve potassium uptake by cotton, rape seed and sudan grass by solubilizing the mineral reserves (Sheng, 2005; Basak and Biswas, 2009) under controlled conditions. However, these K solubilizing bacteria are less adaptable to the pomegranate growing soil ecology characterized by low organic matter, high soil pH, high temperature and solar radiation, low and irregular distribution of rainfall, and water deficit during plant growth period (Panwar and Tarafdar, 2006). Hence, indigenous microbial strains which are much more competitive are effective under field condition as they are adapted to the particular conditions of the site (Paau, 1989). Very recently, we reported a novel fungal strain NFCCI 2498 later on known as MCC 0114 (*Penicillium pinophilum*) from pomegranate soil ecology that

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substantially increased K and P uptake by the pomegranate plant (Maity et al., 2014). The major obstacle in commercialization of promising potassium solubilizing microorganism is the development of a shelf-stable formulated product that retain efficacy similar to that of the fresh cells of the micro-organisms (Leggett et al., 2011). To be of practical use *P. pinophilum* MCC 0114 must be formulated as product capable of storage, distribution and application in the agricultural market. Indeed, performance under field condition is a scenario not frequently explored in bio-fertilizer research particularly with perennial crop like pomegranate.

Hence, a study was conducted to evaluate the effect of carrier based bio-formulation of *Penicillium pinophilum* (MCC 0114) on nutrient availability, fruit yield and quality and economic viability in pomegranate under field condition.

2. Materials and methods

2.1. Fungal culture and bio-formulation

The fungal strain *Penicillium pinophilum* (NFCCI 2498 later on known as MCC 0114) which was isolated from the pomegranate rhizosphere in semi-arid ecosystem and previously well characterized at genus and species level based on morphology and molecular studies (Maity et al., 2014) was used in the present study. The said fungal strain was stored on potato dextrose agar (PDA) slants at 4 °C, and grown in darkness at 20–25 °C for 7 days to produce conidial inoculum for solid-state fermentation. A carrier based bio-formulation of *Penicillium pinophilum* (MCC 0114) was developed following solid state fermentation (De Cal et al., 2002). The bio-formulation had conidial count ranging from 4 to 5×10^{12} CFU g^{-1} formulation and this bio-formulation was used for field experiment to evaluate its effect on nutrient availability, fruit yield and quality of pomegranate.

2.2. Field experiment to evaluate the effect of bio-formulation in pomegranate

The study was conducted at Research Farm, ICAR-National Research Centre on Pomegranate (ICAR-NRCP), Solapur, Maharashtra, India located at 17°68′N latitude and 75°91′E longitude, at an altitude of 457 m above mean sea level. The climate of the study area is semi-arid, showing hot summer and moderate winter with a mean annual maximum and minimum temperature of 40.4 °C and 14.9 °C, respectively and average annual rainfall of 694 mm (approximately) occurring mostly during the months of July–September. The farm soil was loamy in texture with 48.8% sand, 14.8% silt and 26.4% clay. Taxonomically the soil is categorized as Entisol (Lithic Ustorthents). Some of the characteristics of the soil are: pH (1:1 w/v water) 7.82; organic carbon 7.8 g kg^{-1}soil; available N 134.40 mg kg^{-1}soil; available P 8.14 mg kg^{-1}soil; available K 150.20 mg kg^{-1}soil and cation exchange capacity 17.8 cmol (p^+) kg^{-1}soil. Pomegranate (*Punica granatum* L.) cv. Bhagwa of three years old orchard was selected for the field experiment. The trees in the orchard were planted in regular rows, with a 3 x 4.5 m frame (740 trees ha^{-1}) and irrigated by drip irrigation system.

2.3. Experimental design

The non-inoculated soil was considered as control (T_0). Rest of the treatments were: soil inoculated with bio-formulation of *P. pinophilum* (T_1), soil inoculated with bio-formulation of *P. pinophilum* with addition of insoluble K (Potassium feldspar powder, K_2O 10%) at the rate of 10 g K_2O $tree^{-1}$ (T_2), soil inoculated with bio-formulation of *P. pinophilum* with addition of insoluble K (Potassium feldspar powder, K_2O 10%) at the rate of 20 g K_2O $tree^{-1}$ (T_3), soil inoculated with bio-formulation of *P. pinophilum* with addition of insoluble K (Potassium feldspar powder, K_2O 10%) at the rate of 40 g K_2O $tree^{-1}$ (T_4) and non-inoculated soil with addition of soluble K as KCl at recommended dose (125 g K_2O $tree^{-1}$) (T_5). The experiment was laid out in a randomized block design (RBD) comprising of six treatments with four replications for the study. Twelve trees were used for each treatment.

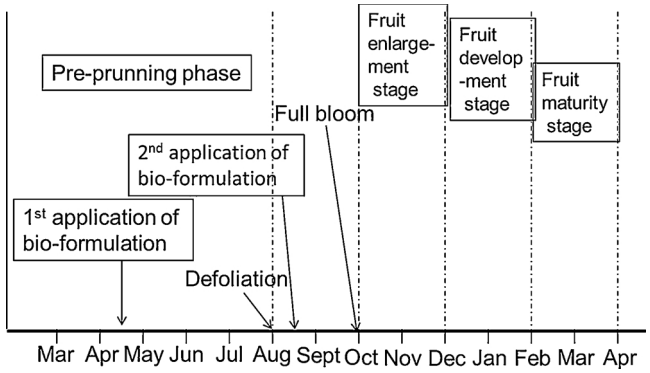

Fig. 1. Scheme showing different phonological stages of pomegranate crop.

The bio-formulation of *P. pinophilum* was applied at the rate of 10 g $tree^{-1}$ after inoculating and incubating with well decomposed farmyard manure in 1:25 ratio followed by addition of insoluble K (potassium feldspar powder) as per treatment combinations and mixing with the rhizospheric soil. A light irrigation was provided immediately after application of bio-formulation. The composition of farmyard manure was: organic material 14.5%, total N 0.50%, total P 0.25% and total K 0.45%. The bio-formulation was applied two times during the crop season viz. (i) 3–4 months before defoliation and (ii) at the time of release of stress (Fig. 1). A basal dose of nitrogen (225 g N $tree^{-1}$) and phosphorus (125 g P_2O_5 $tree^{-1}$) through urea and di-ammonium phosphate (DAP), respectively were applied to each tree and mixed thoroughly with the rhizospheric soil. The rest amount of nitrogen (275 g N $tree^{-1}$) was applied as top dressing in two splits (3:8 ratio) through urea. Adequate irrigation was provided as per the requirement of crop so as to raise the moisture content of soil to field capacity. The orchard was kept weed free. All the cultural practices viz. imposition of stress by withholding irrigation, pruning, defoliation and release of stress by applying irrigation and plant protection measures as and when required were followed as per the package of practices for pomegranate outlined in the technical bulletin NRCP/2014/1 (Sharma et al., 2014).

2.4. Plant and soil analysis

Matured leaf samples were collected from non-bearing branches at the time of harvest of fruits. Leaf bio-mass were washed thoroughly, dried at 60 °C for 48 h and ground. Plant phosphorus (P) and potassium (K) status were measured after acid digestion of leaf samples according to methods of the Association of Official Analytical Chemists (1990). Rhizosphere soil samples collected at the time of harvest were assayed for dehydrogenase enzyme activity, alkaline and acid phosphatase enzyme activities based on the method of Tabatabai and Dick (2002) and microbial biomass carbon following the method of Vance et al. (1987). A sub-sample of soil (1.0 g) was removed from each sample and placed in 10 ml 0.01% Tween 80 (Acros Organics, Geel, Belgium) shaken for 4 min. using a vortex (Heidolph Instruments, Schwabach, Germany) and left to stand for 1 min. A dilution series in sterile distilled water were completed and the diluted suspensions were plated on Petri dishes containing the semi selective media. These Petri dishes were then incubated at 25 °C and final colony counts were made on plates after 5 days of incubation. There were three replicates for each soil sample. The identities of *Penicillium* spp. colonies were confirmed by characteristics greenish white velvety colony from front with reddish pigmentation on the back. Separately, rhizospheric soil samples were also collected thrice at 60, 120 and 180 days after full bloom in the whole crop season for available K and P determination. The available K (NH_4OAc-extractable K) and P in rhizosphere soil were determined

according to the method of Richards and Bates (1989).

2.5. Fruit physical properties and quality

Number of fruits harvested from each plant was recorded and fruits were weighed using a high precision weighing scale. After measuring the fruit size, the arils were separated manually from the fruits to estimate total arils and rind weight per fruit. Subsequently, a hundred arils were counted manually and weighed. The juice was extracted manually by squeezing a hundred arils in muslin cloth and the juice collected from these hundred arils was weighed. Finally, the aril, rind and juice per cent were calculated by dividing their corresponding weight by fruit weight and expressed in percentage.

Titratable acidity (TA) was determined by titration against 0.1 N NaOH solution and expressed in terms of g citric acid per 100 ml of juice (Ranganna, 2001). The total soluble solids (TSS) were determined using a digital refractometer (model SMART-1, ATAGO, Tokyo, Japan) and reported as °Brix at 21 °C.

Total phenolics was determined using the Folin-Ciocalteu colorimetric method (Makkar et al., 2007). Total phenolics was extracted from 10 g of fresh samples using 40 ml of 80% (by volume) aqueous ethanol. The mixture was extracted (in water bath at 80 °C), kept for 20 min. in inert atmosphere, and filtered through Whatman filter paper using a Buchner funnel. Extraction of the residue was repeated under the same conditions. The filtrates were combined and diluted to 100 ml in a volumetric flask with 80% aqueous ethanol and the obtained extract was used for determination of total phenolics and was expressed as mg of gallic acid equivalents (GAE) per 100 ml of fresh juices.

Total anthocyanins content in the extract from fruits was determined following method suggested by Wrolstad (1993). Fruit anthocyanins were extracted from 2 g of fresh samples using 2 ml of 0.1% HCl (by volume) in 96% ethanol and 40 ml 2% aqueous HCl (by volume). The mixture was centrifuged at 5500 rpm for 10 min. The obtained supernatant was used for the determination of total anthocyanins. The absorbance was measured at 520 nm. The molar absorbance value for cyaniding-3,5-diglucoside was used as a standard value. Results were expressed as mg of cyaniding-3,5-diglucoside equivalents per 100 ml fresh juice.

Ascorbic acid (AA) was determined by the 2,6-dichloroindophenol titrimetric method and total sugar, reducing sugar and non-reducing sugar was estimated by standard method described in AOAC (1995).

2.6. Economic analysis

In order to assess the effect of each treatment with the combination of insoluble source of potassium, the cost of cultivation was worked out. The average price of the produce was taken as Rs. 50.00 per kg fruits (based on prevailing market price). Cost benefit ratio of each treatment was determined by using the following formula.

Net return (Rs. per ha) = Gross value of the produce (Rs. ha^{-1}) – Cost of production (Rs. ha^{-1})

$$\text{Cost benefit ratio} = \frac{\text{N et return(Rs. per ha)}}{\text{Cost of cultivation(Rs. per ha)}}$$

2.7. Statistical analysis

The data obtained from the experiment were subjected to analysis of variance (ANOVA) appropriate to the experimental design. F-test was carried out to test the significance of the treatment differences and the least significant difference (LSD at $P < 0.05$) was computed and used for interpretation of results.

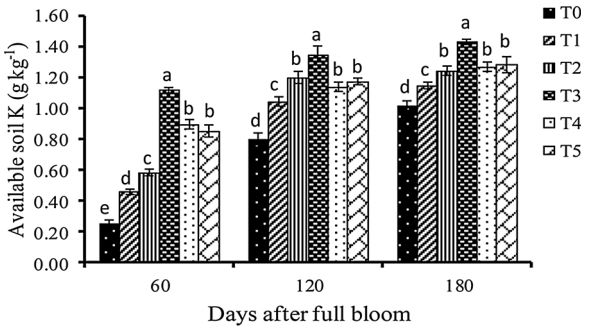

Fig. 2. Influence of potassium solubilizing bio-formulation (*P. pinophilum* MCC 0114) on soil available K content at different phonological stages (T0: Control; T1: bio-formulation; T2: bio-formulation + insoluble K @ 10 g K_2O $tree^{-1}$; T3: bio-formulation + insoluble K @ 20 g K_2O $tree^{-1}$; T4: bio-formulation + insoluble K @ 40 g K_2O $tree^{-1}$; T5: Soluble K at recommended dose. Vertical bars are standard errors of the means. Means sharing a common letter along the row are not significant at $P < 0.05$.

3. Results

3.1. Mobilization of potassium and phosphorus in trees

Soil available potassium (K) and phosphorus (P) content were measured at three phenological stages *viz.* end of fruit enlargement (60 days after full bloom, DAFB), fruit development (120 DAFB) and fruit maturity (180 DAFB). The results (Figs. 2 and 3) showed that application of *P. pinophilum* MCC 0114 based bio-formulation significantly increased available K as well as P during all the phenological stages. With the addition of insoluble source of K (K-bearing mineral, K-feldspar), there was further increase in available K and P content of soil. The highest improvement in available K and P content was recorded when this bio-formulation was supplemented with insoluble K at the rate of 20 g K_2O $tree^{-1}$. It resulted even significantly higher available K and P content in soil than those recorded from the application of soluble potassic fertilizer at recommended dose during all the phonological stages. The enhancement of available K content was much higher (88.47% increase) than that of available P content of soil (42.82% increase). It was also observed that available K content of soil continued to increase throughout the fruit growth and development period while, available P content of soil increased up to fruit development stage and then again declined during fruit maturity stage.

Further, acid and alkaline phosphatase enzymes play an important role in mineralization of soil organic phosphorus. Alkaline phosphatase activity is derived from microorganisms only while acid phosphatase is contributed by both plant roots and soil inhabiting microorganisms.

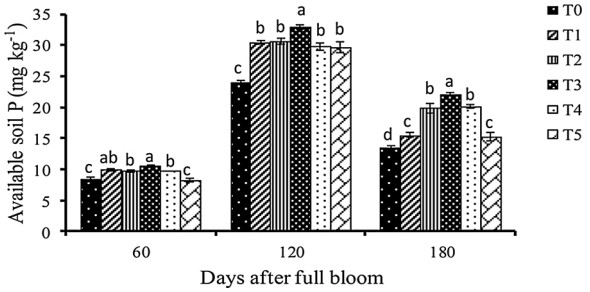

Fig. 3. Influence of potassium solubilizing bio-formulation (*P. pinophilum* MCC 0114) on soil available P content at different phonological stages (T0: Control; T1: bio-formulation; T2: bio-formulation + insoluble K @ 10 g K_2O $tree^{-1}$; T3: bio-formulation + insoluble K @ 20 g K_2O $tree^{-1}$; T4: bio-formulation + insoluble K @ 40 g K_2O $tree^{-1}$; T5: Soluble K at recommended dose. Vertical bars are standard errors of the means. Means sharing a common letter along the row are not significant at $P < 0.05$.

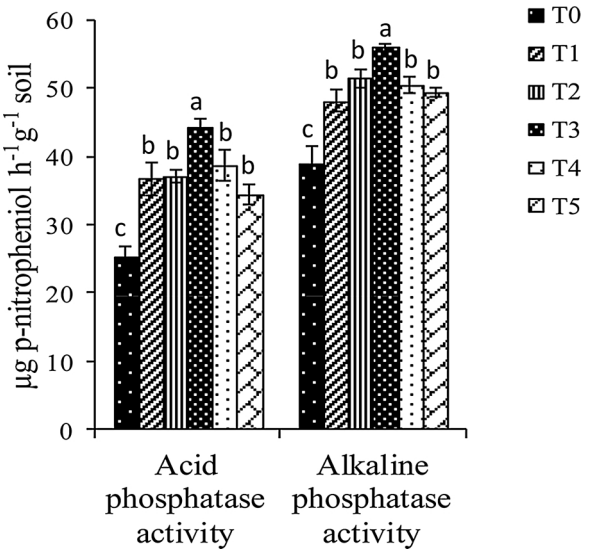

Fig. 4. Effect of potassium solubilizing bio-formulation (*P. pinophilum* MCC 0114) on soil phosphatase enzyme activities (T0: Control; T1: bio-formulation; T2: bio-formulation + insoluble K @ 10 g K_2O tree^{-1}; T3: bio-formulation + insoluble K @ 20 g K_2O tree^{-1}; T4: bio-formulation + insoluble K @ 40 g K_2O tree^{-1}; T5: Soluble K at recommended dose. Vertical bars are standard errors of the means. Means sharing a common letter along the row are not significant at $P < 0.05$.

Significant improvement in the activities of alkaline and acid phosphatase enzyme was observed upon application of bio-formulation (Fig. 4). The highest activity of these enzymes were recorded when bio-formulation was supplemented with insoluble K at the rate of 20 g K_2O tree^{-1} and the least activities of these enzymes were noticed in un-inoculated soil.

Significant increase in fruit yield, average fruit weight, hundred arils weight and juice content were recorded with the soil application of bio-formulation (Table 1). It increased fruit yield by 35.17% over un-inoculated trees. The highest fruit yield of 16.16 kg tree^{-1} was recorded in trees treated with bio-formulation plus insoluble K at the rate of 20 g K_2O tree^{-1} (i.e. 14.80 kg K_2O ha^{-1}). Fruit yield obtained from this treatment combination was even significantly higher (by 27.24%) than that recorded with application of soluble potassic fertilizer at recommended dose (Fig. 5). This treatment combination (i.e. application of bio-formulation amended with insoluble K at the rate of 20 g K_2O tree^{-1}) also recorded the highest average fruit weight (257.45 g) while the highest aril test weight (hundred arils weight) and juice content were recorded with the application of bio-formulation plus insoluble K at the rate of 10 g K_2O tree^{-1} (i.e. 7.40 kg K_2O ha^{-1}).

Application of this bio-formulation also had impact on the quality attributes of pomegranate fruit. Significant improvement in ascorbic acid, phenol and sugar content particularly reducing sugar was recorded upon application of bio-formulation (Table 2). Further the addition of insoluble K at the rate of 10 and 20 g K_2O tree^{-1} along with bio-formulation resulted the greatest improvement in phenol and reducing sugar content of fruit. It was interesting to note that significant decrease in non-reducing sugar content (which is not good for human health point of view) was recorded upon application of this bio-formulation.

Plant K and P status was also determined through leaf analysis and fruit nutrient content at the time of harvest of crop. The results indicated that the plant inoculated with bio-formulation had significantly higher content and concentration of K and P in fruits and leaves respectively at harvest (Table 3). Here also, the highest content and concentration of K and P in fruits and leaves respectively were recorded in trees inoculated with bio-formulation plus insoluble K at the rate of 20 g K_2O tree^{-1}.

3.2. Biochemical and microbial features of soil

The fungal strain *P. pinophilum* MCC 0114 in the bio-formulation was tested for its ability to colonize around pomegranate rhizosphere by soil respiratory enzyme viz. dehydrogenase activity assay, microbial biomass carbon analysis and population count of *Penicillium* spp. Dehydrogenase enzyme activity gives an indication of active microbial population in the soil. Significant increase in dehydrogenase enzyme activity in rhizospheric soil was noticed upon soil application of this bio-formulation (Table 4). The enzyme activity was much higher when the bio-formulation was inoculated with the addition of insoluble K and the highest dehydrogenase activity was recorded at the addition of 20 g K_2O (insoluble K) tree^{-1}.

Microbial biomass carbon analysis of soil after the harvest of crop also revealed that soil application of bio-formulation significantly influenced soil microbial community in the pomegranate rhizosphere and hence improved microbial biomass carbon content of soil. Like dehydrogenase enzyme activity, the highest microbial biomass carbon content was noted in soil treated with insoluble K at the rate of 20 g K_2O tree^{-1} and inoculated with bio-formulation.

Significant increase in the population of *Penicillium* spp. was observed in the rhizosphere soil inoculated with bio-formulation and amended with insoluble source of potassium. And highest *Penicillium* spp. population was recorded when the bio-formulation was supplemented with insoluble K at the rate of 20 g K_2O tree^{-1}. Twenty weeks after inoculation, it was still possible to recover *P. pinophilum* (identified on the basis of morphology and colony characteristics) from the rhizosphere soil supplemented with insoluble K source.

Table 1
Influence of potassium solubilizing formulation (*P. pinophilum* MCC0114) on fruit yield attributes of pomegranate. Data shown are mean of two seasons.

Treatment	Fruit yield (kg per tree)	Average fruit weight (g)	Juice content (% v/w)	100 arils weight (g)	Total soluble solid (°Brix)
Un-inoculated soil (T_0)	7.45^{d}	208.70^{d}	71.83^{d}	34.82^{c}	14.25
Inoculated soil with bio-formulation (T_1)	10.07^{c}	220.19^{c}	73.50^{c}	36.34^{bc}	14.80
Inoculated soil with bio-formulation + insoluble K @ 10 g K_2O tree^{-1} (T_2)	11.22^{bc}	224.05^{c}	75.99^{a}	38.67^{a}	14.80
Inoculated soil with bio-formulation + insoluble K @ 20 g K_2O tree^{-1} (T_3)	16.16^{a}	257.45^{a}	74.33^{bc}	37.08^{ab}	14.63
Inoculated soil with bio-formulation + insoluble K @ 40 g K_2O tree^{-1} (T_4)	12.09^{b}	235.89^{b}	74.28^{bc}	37.71^{ab}	14.66
Un-inoculated soil + soluble K at recommended dose (T_5)	12.70^{b}	250.14^{a}	75.57^{ab}	36.62^{bc}	14.93
LSDα $_{0.05}$	1.56	10.81	1.42	2.03	NS

@- at the rate of.
Means sharing a common letter within the column are not significant at $P < 0.05$.

Fig. 5. Showing fruit bearing in trees (a) inoculated with bio-formulation of *P. Pinophilum* MCC 0114 plus potassium feldspar at the rate of 20 g K_2O $tree^{-1}$ (b) with potassic fertilizer at recommended dose (c) without potassic fertilizer application.

Table 2
Influence of potassium solubilizing bio-formulation (*P. pinophilum* MCC0114) on the fruit quality attributes of pomegranate. Data shown are mean of the two seasons.

Treatment	Acidity (%)	Ascorbic acid (mg /100 ml)	Phenol (mg l^{-1} GAE)	Sugar content (%)		
				Reducing	Non- reducing	Total
Un-inoculated soil (T_0)	0.35^{ab}	10.63^{b}	1328.72^{c}	11.57^{c}	1.92^{a}	13.50^{b}
Inoculated soil with bio-formulation (T_1)	0.36^{ab}	13.13^{a}	1386.70^{bc}	13.17^{b}	0.76^{bc}	13.93^{ab}
Inoculated soil with bio-formulation + insoluble K @ 10 g K_2O $tree^{-1}$(T_2)	0.33^{b}	14.38^{a}	1536.68^{a}	13.56^{ab}	0.43^{bc}	13.99^{ab}
Inoculated soil with bio-formulation + insoluble K @ 20 g K_2O $tree^{-1}$(T_3)	0.34^{b}	14.38^{a}	1521.93^{a}	14.10^{a}	0.09^{c}	14.19^{a}
Inoculated soil with bio-formulation + insoluble K @ 40 g K_2O $tree^{-1}$ (T_4)	0.38^{a}	13.75^{a}	1378.79^{bc}	13.33^{b}	0.80^{b}	14.13^{a}
Un-inoculated soil + soluble K at recommended dose (T_5)	0.35^{ab}	13.75^{a}	1464.62^{ab}	13.39^{b}	0.76^{bc}	14.15^{a}
LSDα $_{0.05}$	0.04	2.38	95.70	0.60	0.68	0.53

@- at the rate of.
Means sharing a common letter within the column are not significant at $P < 0.05$.

Table 3
Effect of potassium solubilizing bio-formulation (*P. pinophilum* MCC0114) on leaf K and P concentration and fruits K and P content at harvest. Data shown are mean of the two seasons.

Treatment	Nutrient concentration in leaves (g kg^{-1})		Nutrient content in fruits at harvest (kg ha^{-1})	
	K	P	K	P
Un-inoculated soil (T_0)	12.00^{d}	1.54^{d}	28.53^{e}	2.44^{e}
Inoculated soil with bio-formulation (T_1)	14.20^{b}	1.67^{b}	44.14^{cd}	3.53^{cd}
Inoculated soil with bio-formulation + insoluble K @ 10 g K_2O $tree^{-1}$(T_2)	14.70^{b}	1.71^{a}	38.07^{de}	2.98^{de}
Inoculated soil with bio-formulation + insoluble K @ 20 g K_2O $tree^{-1}$(T_3)	15.58^{a}	1.74^{a}	61.47^{a}	5.39^{a}
Inoculated soil with bio-formulation + insoluble K @ 40 g K_2O $tree^{-1}$(T_4)	14.63^{b}	1.62^{c}	49.82^{bc}	4.15^{bc}
Un-inoculated soil + soluble K at recommended dose (T_5)	13.00^{c}	1.54^{d}	54.22^{ab}	4.87^{ab}
LSDα $_{0.05}$	0.62	0.04	9.75	0.91

@- at the rate of.
Means sharing a common letter within the column are not significant at $P < 0.05$.

4. Discussion

Most of the potassium in soil exists in the form of silicate minerals. The potassium becomes available to the plant when the minerals are slowly weathered or solubilized. This process of weathering /solubilization of silicate minerals get accelerated with the intervention of microorganisms. The bio-formulation used in the study contain fungal strain *Penicillium pinophilum* MCC 0114 which is capable of solubilizing insoluble potassium as well as phosphorus (Maity et al., 2014). Our results showed that soil application of potassium

Table 4
Effect of potassium solubilizing bio-formulation (*P. pinophilum* MCC0114) on biological activity, microbial biomass carbon content and population of *Penicillium* spp. in rhizosphere soil.

Treatment	Dehydrogenase activity (μg TPF $h^{-1} g^{-1}$ soil)	Microbial biomass carbon (μg g^{-1} soil)	*Penicillium* spp. CFU g^{-1} dry soil*
Un-inoculated soil (T_0)	4.02[d]	220.50[d]	2.39[d]
Inoculated soil with bio-formulation (T_1)	6.03[c]	322.83[c]	2.71[cd]
Inoculated soil with bio-formulation + insoluble K @ 10 g K_2O $tree^{-1}$(T_2)	7.40[b]	372.62[b]	4.21[b]
Inoculated soil with bio-formulation + insoluble K @ 20 g K_2O $tree^{-1}$(T_3)	8.76[a]	456.58[a]	6.22[a]
Inoculated soil with bio-formulation + insoluble K @ 40 g K_2O $tree^{-1}$(T_4)	8.25[a]	325.05[c]	3.05[c]
Un-inoculated soil + soluble K at recommended dose (T_5)	6.50[c]	246.67[d]	2.54[cd]
LSDα $_{0.05}$	0.69	28.17	0.62

@- at the rate of.
CFU, colony forming unit.
*Data are mean values of 10 replicates transformed by log(X + 1) and represent concentration of CFU.
Means sharing a common letter within the column are not significant at $P < 0.05$.

solubilizing bio-formulation greatly increased available K and P content of soil throughout the crop season. The improvement in available K and P content of soil might have arisen from solubilization of unavailable K and P in their respective bearing minerals through production and excretion of organic acids like citric, oxalic and tartaric acid by fungal strain in the bio-formulation (Franz et al., 1991; Maity et al., 2014; Khan and Gupta, 2015). Organic acid produced can facilitate the solubilization of minerals by directly dissolving K and P from rocks or through the formation of metal-organic complexes or by forming chelate with silicon ion to bring the K and P into solution (Bennett et al., 1998; Grigis et al., 2008; Sheng and He, 2006).

In addition, it was also observed that alkaline and acid phosphatase enzyme activities in soil increased upon the application of bio-formulation. These phosphatase enzymes play important role in solubilizing organic P in the soil and making them available to the plant. Our observations are in conformity with earlier workers (Yadav and Tarafdar, 2011) who reported that *P. purpurogenus* releases phosphatase and phytase, resulting in solubilization/hydrolysis of soil unavailable P into plant available form. When this bio-formulation was inoculated in conjunction with the addition of insoluble rock/mineral materials, there was even a higher available K and P content in soil. Increasing the availability of K and P in the soil to a higher extent with the integrated use of K-bearing minerals and potassium solubilizing microorganisms has been reported by many researchers (Han and Lee, 2005; Badr, 2006; Basak and Biswas, 2009; Youssef et al., 2010). Bennett et al. (2001) reported that microorganisms preferentially colonize and dissolve feldspar that contain phosphorus and iron as minor components through producing organic ligands. In the present study addition of insoluble K bearing minerals (potassium feldspar) provided the site for colonization by *P. pinophilum* in the bio-formulation and it might have triggered expression of certain genes and activation of metabolite pathway leading to production of organic ligands thereby solubilizing insoluble minerals in the soil and enhancing the availability of K and P. The triggering effect of insoluble K bearing minerals (potassium feldspar) on microorganism was reported by Wang et al. (2015) in their study to explore the mechanism behind enhanced weathering of potassium feldspar by *Aspergillus niger*. The highest availability of K and P was recorded in soil amended with insoluble K bearing minerals at the rate of 20 g K_2O $tree^{-1}$ and inoculated with bio-formulation. This may be due to the optimization of micro-environment i.e. the production of organic ligands apparently sufficient near attached fungi to destroy the silicate framework while releasing the nutrient of interest (Rogers and Bennett, 2004). In addition, enhanced colonization by the inoculated fungi was evident from the increasing population of *Penicillium* spp., higher dehydrogenase enzyme activity and microbial biomass carbon content in rhizosphere soil. Our study also demonstrated that addition of higher dose of insoluble K bearing minerals (i.e. at the rate of 40 g K_2O $tree^{-1}$) along with bio-formulation did not increase available K and P content of soil any further, rather there was decrease in available K and P content in soil. This might be due to feedback inhibition mechanism in the fungi regulating the metabolic pathway and leading to lower production of organic ligands for solubilizing insoluble K in soil (Sanchez and Demain, 2008).

Soil inoculation of this bio-formulation improved fruit yield attributes and also quality attributes (average fruit weight, aril weight, juice content, ascorbic acid, phenol and sugar content). The impact of this bio-formulation was much higher when it was used in conjunction with insoluble K mineral at the rate of 20 g K_2O $tree^{-1}$ and the impact was even higher than that obtained from use of soluble potassic fertilizer at recommended dose. So our study demonstrated that the synergistic use of potassium solubilizing bio-formulation and insoluble K bearing minerals/rocks could totally replace the potassic fertilizer requirement in pomegranate. Our results were in conformity with earlier report by Supanjani et al. (2006) who suggested that direct application of K and P rocks and solubilizing bacteria would be promising sustainable alternative to the use of classical fertilizers in hot pepper. From the present study, it may be suggested that the enhancement in fruit yield and quality attributes were related to increased solubilization of K and P and release of growth promoting substances at the root interface which stimulated root development. All these resulted in better absorption of water and nutrients from the soil (Sheng, 2005). The evidence of greater nutrient uptake, consequently higher leaf area index and photosynthetic activity resulting from the integrated use of *P. pinophilum* and insoluble K source was reported in our earlier publication (Maity et al., 2014). This was further supported by the fact that K and P status in inoculated plant (concentration in leaf tissues and content in fruits) were much higher than un-inoculated plants at harvest. All these growth promoting effects and nutritional physiology collectively resulted perceptible improvement in fruit yield and quality attributes of pomegranate. Among the quality attributes, improvement in ascorbic acid, phenol and sugar concentration might have resulted from the better K and P nutritional fulfillment and their involvement in fruit physiology of pomegranate plant.

As successful plant growth promoting inoculants, fungal strain in the bio-formulation must be able to rapidly colonize the root system during the crop season (Defreitas and Germida, 1992). Dehydrogenase enzyme activity and microbial biomass carbon give measurement of active microbial population in the soil. Our results showed that application of bio-formulation led to enhancement in dehydrogenase enzyme activity, microbial biomass carbon content and population of *Penicillium*

Table 5
Estimated economic return from the application of potassium solubilizing bio-formulation (*P. pinophilum* MCC0114) on 1 ha pomegranate orchard.

Treatment	Cost of cultivation (Rs.)	Income from the produce (Rs.)	Net profit (Rs.)	Cost-benefit ratio
Un-inoculated soil (T_0)	155049	275650	120601	1:0.78
Inoculated soil with bio-formulation (T_1)	164049	372590	208541	1:1.27
Inoculated soil with bio-formulation + insoluble K @ 10 g K_2O $tree^{-1}$(T_2)	165159	415140	249981	1:1.51
Inoculated soil with bio-formulation + insoluble K @ 20 g K_2O $tree^{-1}$(T_3)	166269	597920	431651	1:2.60
Inoculated soil with bio-formulation + insoluble K @ 40 g K_2O $tree^{-1}$(T_4)	168489	447330	278841	1:1.65
Un-inoculated soil + soluble K at recommended dose (T_5)	169294	469900	300606	1:1.78

@- at the rate of.

spp. in the rhizosphere soil even after the crop season. This implied that application of bio-formulation promoted microbial activity in the rhizosphere of pomegranate plant and also gives indirect evidence of rhizospheric colonization by the fungal strain. The fungal strain, *P. pinophilum* MCC 0114, used in bio-formulation was isolated from soil of Maharashtra. And this soil has been commercially cultivated with pomegranate. According to Paau (1989), microbial strians which are isolated from the pool of indigenous soil flora, are more competitive and effective for deriving maximum benefits, as they are adapted to the particular conditions of the site.

Further from the economic analysis (Table 5), it was inferred that use of this novel bio-formulation of *P. pinophilum* MCC 0114 could substantially improve farm income and also the benefit - cost ratio. However, the maximum economic benefit could be realized from the conjunctive use of this novel bio-formulation with insoluble K minerals at the of 20 g K_2O $tree^{-1}$.

The present study represents the positive response of bio-formulation in pomegranate under harsh field condition of semi-arid regions of India. Soil application of this novel bio-formulation exhibited significant impact on nutrient (K and P) availability, status in plant, fruit yield and quality attributes. Integrated use of this bio-formulation in conjunction with insoluble mineral source at the rate of 20 g K_2O $tree^{-1}$ (as against recommended dose of 125 g K_2O $tree^{-1}$) can completely fulfill the potassium requirement of pomegranate plant and hence could be a promising alternative to the use of imported potassic fertilizer for sustainable pomegranate production in the semi-arid Agro-Ecosystem.

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