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Mobilization of macro and micronutrients by promising microbial isolates in soil pot culture experiment

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Abstract
A short-term pot culture experiment was conducted to estimate effect of PGPR on mobilization of soil macro and micronutrients in soil using guava as test crop. Newly grafted nearly equal height seedling was planted in pot contains equal amount of soil. Hoagland nutrient solution was only used as a nutrient source. The laboratory stock cultures (*Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium, Bacillus thuringiensis, Pseudomonas fluorescens, Pseudomonas striata, Trichoderma viride. Trichoderma harzianum, Azotobacter chroococcum* and *Azospirillum lipoferum*) selected on the basis of their nutrient solubilizing potential and the experiment is design in completely randomized design with eleven treatments (Ten microbial isolates and one uninoculated control) and three replications. Obtained result indicated that inoculation of strain *Bacillus megaterium* in pot culture experiment was found better microbial strain in increasing microbial activity, availability phosphorous, zinc and copper of soil, followed by strain *Pseudomonas striata*. Whereas, strain *Trichoderma viride* was superior in availability of potassium and sulphur in soil of pot experiment followed by *Bacillus megaterium* and *Pseudomonas striata*. Nitrogen availability in soil was found higher with inoculation of *Azotobacter chroococcum* and maximum availability in soil and content of Fe in leaves was found with inoculation of strain *Pseudomonas fluorescens*.

Keywords: Pot experiment, PGPR and Mobilization of macro and micronutrients

Introduction
Plant growth-promoting microbial isolates are heterogenous population of microorganism found on root surface, in near the root surface (rhizosphere) and as well as in cooperation with plant roots which can directly and indirectly improve the extent or quality of plant growth. In last past couple of decades, a large array of bacteria including the species *Burkholderia*, *Azospirillum, Pseudomonas, Enterobacter, Azotobacter, Klebsiella, Alcaligenes, Arthrobacter*, *Bacillus* and *Serratia* have reported to enhance plant growth by producing the substances that is synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the soil environment. The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effect of one or more phytopathogenic organism.

There has been significant rise in the human's population on Earth, particularly in the post-industrial era. With increased population, there has been the increase in basic need and demands. Resources on the earth are depleting at very rapid rates. It's expected to reach the mark of billion in the next 5 decades. Degrading environment, rising human population, increasing food demand, exhausting environmental resources, and demand some significant change and contribution in the field of agriculture to feed people. A technology that could lead towards sustainable development at the same time increasing yield (Ladeiro, 2012 and Glick, 2014) [15, 8]. Efforts have been made to increase crop production by improving the soil qualities. This approach, by heavy use of chemical fertilizers, was successful in improvement in crop productions. However, it also caused problem such as high input of energy, labour, money, as well as environmental pollution and resources depletion. Therefore, more recently the best approach is to improve the nutrients uptake and their use efficiency which can be possible via. using beneficial microorganism. As they are ecofriendly and cost effective and can enhance the crop yield and reduced the dependence on synthetic source of nutrients

Here, in the present studies our idea is to study effect of Plant Growth Promoting microbial agents on mobilization of macro and micronutrients. We will try to understand how PGPR increasing soil nutrient availability. Thus, keeping this, in the view present study was undertaken for "Effect of promising microbial growth promoting agents on mobilization of macro and micronutrients in pot culture experiment.

Material and Methods

To find out the effect of PGPR, a short-term experiment was carried out in pot culture using guava as test crop. Newly grafted nearly equal height seedling was planted in pot contains equal amount of soil. The plant in pot was treated with ten microbial inoculants and one is control and Hoagland nutrient solution was use as nutrient source which was compulsory to each pot (On the basis of field capacity of soil). Seedling in pot was kept for 90 days. Seedling were harvested after 90 days seedling are cut and separate from root. Soil and leaf sample were collected from each pot and analysed for physico-chemical and biological properties.

The pot culture experiment was carried out in *kharif* season during year 2019 on Typic haplustepts in front of Department of Soil Science and Agricultural Chemistry, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. The experiment was performed under Completely Randomized Design (CRD) comprising eleven (11) treatments replicated three (3) times. The initial soil pH was 8.11, Electrical Conductivity- 0.19 dSm^{-1}, OC- 4.20 g kg^{-1}, $CaCO_3$- 124.8 g kg^{-1} Available Nitrogen-0.0736 mg kg^{-1}, Phosphorous- 0.00498 mg kg^{-1}, Potassium– 0.2437 mg kg^{-1} and Sulphur-10.117 mg kg^{-1}. The initial micronutrient status DTPA Cu 1.721 mg kg^{-1}, DTPA Manganese 4.485 mg kg^{-1}, DTPA Zinc 0.561 mg kg^{-1}, DTPA Iron 4.373 mg kg^{-1} and Boron 0.491 mg kg^{-1}. Soil pH and EC were measured in a 1:2 soil: water suspension using pH and conductivity meters. Soil organic carbon determined by wet oxidation method and were analysing available nitrogen by Alkaline permanganate method (Subbiah and Asija), available phosphorous and available potassium by (neutral normal ammonium acetate method). Fe, Mn, Zn and Cu were determined by using Atomic Absorption Spectrophotometer as explained by Lindsay and Norvel (1978). Hot water-soluble boron was determined with method described by Berger and Troug, 1978 expressed in terms of mg kg^{-1}.

Microbial isolates

The laboratory stocks microorganisms (T_2- *Bacillus subtilis*, T_3- *Bacillus lecheniformis*, T_4- *Bacillus megaterium*, T_5- *Bacillus thuringiensis*, T_6- *Pseudomonas fluorescens*, T_7- *Pseudomonas striata*, T_8-*Trichoderma viride.* T_9-*Trichoderma harzianum*, T_{10}-*Azotobacter chroococcum* and T_{11} - *Azospirillum lipoferum*) were procured from All India Network Project on Soil Biodiversity-Biofertilizers, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani.

The obtained data was statistically analysed and interpreted appropriately as per the method given in "Statistical Methods for Agricultural Workers" by Panse and Sukhatme, 1954 [21].

Results and Discussion

Soil chemical properties

Slight change in chemical properties of soil was observed with inoculation of different promising microbial isolates. Initial pH of pot culture soil was 8.11 which slightly decrease after inoculation of microbial isolates. Maximum reduction in pH soil of pot experiment was observed with inoculation of strain *Pseudomonas striata* (7.87) followed by T_4 *Bacillus megaterium* (7.91), T_8 *Trichoderma viride* (7.90) and other microbial isolates. The maximum soil pH of pot culture eith guava crop was notice under uninoculated control. There was non-significant effect of microbial inoculants on soil EC. Soil calcium carbonate of pot culture soil was also non-significantly influence with inoculation microbial agents The organic carbon content was found significantly high in pot treated with *Bacillus megaterium* (0.58%), which was followed by *Pseudomonas striata* (0.56), *Trichoderma viride* (0.53) and *Azotobacter chroococcum* (0.53) which was followed by strain *Bacillus megaterium*. Whereas lowest organic carbon was found in uninoculated pots.

Reduction of soil pH is might be due to acidification of rhizosphere could be through liberation of organic acids by proton extrusion mechanism and decomposing of organic matter. Slight reduction of pH and significant increase in organic carbon by inoculation of strain *Trichoderma viride*, *Bacillus megaterium* and *Pseudomonas striata* which might be due to organic acids secretion by Pawar (2016) [22]. Jadhav (2021) [12] also reported that maximum reduction in pH of soil inoculated with of *Pseudomonas striata, Trichoderma viride* and *Bacillus megaterium* might be due to secretion of organic acid. Found significant increased organic carbon and reduction of pH and $CaCO_3$ with inoculation of strain Pseudomonas *striata* in pot experiment.

Table 1: Effect of promising microbial growth promoting agent on chemical properties of soil in pot culture experiment.

Treatments	Soil chemical properties			
	pH (1:2.5)	EC (dSm^{-1})	$CaCO_3$ (%)	OC (%)
T_1 - Uninoculated control	8.06	0.21	12.40	0.39
T_2 - *Bacillus subtilis*	8.00	0.22	11.34	0.45
T_3 - *Bacillus licheniformis*	7.96	0.22	11.58	0.47
T_4 -*Bacillus megaterium*	7.91	0.25	11.18	0.58
T_5 - *Bacillus thuringiensis*	7.96	0.23	11.91	0.43
T_6 - *Pseudomonas fluorescens*	7.92	0.23	11.69	0.50
T_7 - *Pseudomonas striata*	7.87	0.26	11.30	0.56
T_8 - *Trichoderma viride*	7.90	0.25	11.22	0.53
T_9 -*Trichoderma harzianum*	8.03	0.22	12.11	0.41
T_{10} -*Azotobacter chroococcum*	7.94	0.24	11.36	0.53
T_{11} - *Azospirillum lipoferum*	7.95	0.23	11.83	0.39
S.Em.±	0.04	0.01	0.48	0.02
CD @5%	NS	NS	NS	0.07
Initial	0.86	7.04	7.10	8.85

Soil macronutrients availability

Available nitrogen

Slight increase in available nitrogen in pot soil was found with inoculation of different microbial isolates. The significantly higher nitrogen was found in pot treated with strain *Azotobacter chrococum* (0.0873 kg^{-1}) followed by strain *Azospirillum lipoferum* (0.0821g kg^{-1}) and *Bacillus megaterium* (0.0798 g kg^{-1}).

The increase in nitrogen availability as compared to control is might be due atmospheric N fixation by different promising microbial isolates. These results corroborate with finding of Chandra *et al.* (2015) [6] examined that Azotobacter, *Azospirillum. Bacillus spp.* and *Pseudomonas spp.* having greatability to fix atmospheric nitrogen. *Azotobacter* and *Azospirillum* can able to fix 30 to 40 kg N ha^{-}, whereas *Bacillus spp* and *Pseudomonas* spp are facultative N fixer Isawa *et al*. (2009) [11]. Similarly, Pawar (2016) [22] observed, significant increased nitrogen availability with inoculation of strain *Trichoderma viride, Bacillus megaterium* and *Pseudomonas striata* along Hoglond solution in pot experiment.

Available Phosphorus

Pot with guava seedling treated with strain *Bacillus*

megaterium show significantly high available phosphorus content in soil (0.00674 g kg^{-1}) which was followed by treatment T_7 i.e., inoculation of strain *Pseudomonas striata* (0.00651 g kg^{-1}) and T_8 i.e., *Trichoderma viride* (0.00625 g kg^{-1}). These treatments were at par with treatment *Bacillus megaterium* (T_4) (0.00674 g kg^{-1}).

Increased phosphorous availability with tested isolates might be due insoluble phosphate solubilization effect by the secretion of organic compound, as a result there is significant improvement in available phosphorous content of the soil. Similar finding also given by Osorno *et al.* (2018) [19] reported that, desorbtion of phosphorous from the surface of clay mineral and soil organic matter by indigenous soil microorganism were identified. PSB + Aspergillus awamori inoculation increase availability of Phosphorous in soil explain by Vidhyashree *et al.* (2017) [25]. Phosphorous availability was significantly increases with inoculation of *Pseudomonas fluorescens* followed by *Pseudomonas striata* and *Bacillus megaterium* along with RDF (Nelwade *et al.*, 2019) [17].

Available potassium

Inoculation of promising microbial isolates show significant increase available potassium as compared to T_1 i.e. control. However, significantly highest value of soil available potassium was observed in soil of pot having guava seedling treated with strain *Trichoderma viride* (0.276 g kg^{-1}), which was followed by pot soil treated with strain *Bacillus megaterium* (0.262 g kg^{-1}) and *Pseudomonas striata* (0.258 g kg^{-1}).

Increased potassium availability in soil of pot culture after inoculation listed is might due to microbial mobilization of metal by secretion of organic and inorganic acid (e.g. Malic acid and citric acid) by redox reaction and by exudation of complexing agents. Amount of organic acid release in the rhizosphere was accountable for increasing potassium mobilization. Our experimental results found similar with finding of Waghmare *et al.* (2019) [26] reported that maximum increase in phosphorus content in soil with inoculation of strain *Pseudomonas striata* followed by *Trichoderma viride* and *Bacillus megaterium* with recommended dose of fertilizer. Highest potassium availability in soil of pot experiment was found with inoculation of *Trichoderma viride* after that *Pseudomonas Striata* and *Bacillus megaterium* along with Hoagland nutrient solution reported by Pawar, 2016 [22].

Available sulphur

The available sulphur was ranged in pot culture soil from 9.976 mg kg^{-1} 11.611 mg kg^{-1}. Pot soil treated with strain *Trichoderma viride* was high in available sulphur (11.611) which was followed by *Bacillus megaterium* (11.448 mg kg^{-1}), *Pseudomonas striata* (11.260 mg kg^{-1}), *Bacillus licheniformis* (11.016 mg kg^{-1}) and so on.

Table 2: Effect of promising microbial growth promoting agents on available macronutrients in soil of pot culture.

Treatment	Available macronutrients (mg kg^{-1})			S (mg kg^{-1})	DTPA micronutrients (mg kg^{-1})		
	N	P	K		Fe	Zn	Cu
T_1 - Uninoculated Control	70.879	4.144	227.808	9.976	4.405	0.530	1.701
T_2 - *Bacillus subtilis*	73.469	4.568	246.996	10.622	4.467	0.552	1.744
T_3 - *Bacillus licheniformis*	75.958	5.675	251.262	11.016	4.477	0.605	1.811
T_4 -*Bacillus megaterium*	79.800	6.744	262.007	11.448	4.718	0.633	1.975
T_5 - *Bacillus thuringiensis*	72.854	5.147	250.265	10.517	4.444	0.560	1.804
T_6 - *Pseudomonas fluorescens*	72.701	4.960	240.976	10.265	4.843	0.610	1.776
T_7 - *Pseudomonas striata*	78.819	6.512	258.150	11.260	4.769	0.653	1.945
T_8 - *Trichoderma viride*	77.568	6.247	275.858	11.611	4.753	0.614	1.913
T_9 -*Trichoderma harzianum*	75.762	5.498	251.038	10.385	4.327	0.569	1.795
T_{10} -*Azotobacter chroococcum*	87.324	5.941	248.198	11.037	4.483	0.578	1.809
T_{11} - *Azospirillum lipoferum*	82.113	5.179	244.064	10.325	4.460	0.566	1.787
S.Em.±	2.29	0.274	11.30	0.375	0.112	0.022	0.045
CD @ 5%	6.72	0.804	7.99	NS	0.330	0.066	0.131
Initial	73.60	4.98	243.17	10.117	4.373	0.561	1.721

Increased sulphur availability in soil with inoculation of listed microbial isolates was might be due to greater root growth and increased sulphur oxidizing microorganism. Our study results found in collaboration with finding of Kumar *et al.* (2017) [17] examined that sulphur availability in soil was increased with application of *Trichoderma viride* followed by *Pseudomonas fluorescens* and *Pseudomonas striata* along with RDF. Similar results also given by Pagar *et al.* (2019) [20] observed that, significantly highest sulphur was found with application of RDF +*Pseudomonas striata*, followed by RDF + *Trichoderma viride* and RDF + *Bacillus megaterium.*

Soil micronutrients availability

DTPA extractable Iron

Iron content in soil of pot culture was significantly high in treatment inoculated with strain *Pseudomonas fluorescens* (4.843 mg kg^{-1}) which was followed by strain *Pseudomonas striata*, (4.769 mg kg^{-1}), *Trichoderma viride* (4.753) and *Bacillus megaterium* (4.718 mg kg^{-1}).

Our experimental result shows increased iron status of pot culture soil over control using listed promising microbial isolates, was might be due there siderophore producing capacity and solubilization of metal Fe by secretion of organic acid. Similar results was also given by Bagmare *et al.* (2019) [3] reported that *Pseudomonas fluorescens* inoculated soil show significantly higher Fe content followed by *Azospirillum lipoferum* and *Pseudomonas striata* because of their siderophore producing capacity. Adriana *et al.* (2010) [1] stated that the PGPR having an capacity to mobilized nutrients by formation of acids *viz*. organic or inorganic acids (e.g., citric acid, sulphuric acid) by redox reactions and by the exudation of complexing agents.

DTPA extractable Zinc

Availability of zinc in soil of pot culture experiment was also significantly improved with inoculation of promising microbial isolates. Significantly higher zinc availability was found with inoculation of strain *Pseudomonas striata* (0.653

mg kg^{-1}), which was followed by treatment *Bacillus megaterium* (T_4) (0.633 mg kg^{-1}), *Trichoderma viride* (0.614 mg kg^{-1}), *Pseudomonas fluoresces* (0.610 mg kg^{-1}) and *Bacillus licheniformis* (0. 606 mg kg^{-1}).

Mechanisms employed by different listed isolates as reported earlier in increasing zinc content by chelation or solubilization of mineral, with production of some organic acids such as gluconic, citric and /or fumaric acids in the rhizosphere which reduces the soil pH, leads to increase solubility of insoluble zinc compound and availability of zinc in soil, can increase plant metabolism leading to the enhancement of plant physiological activity and the development of root system. Our finding is corroborated with result given by Pawar (2016) [22] reported that drenching of *Trichoderma viride*, *Pseudomonas striata* and *Bacillus megaterium* in pot soil increased zinc availability due to their zinc solubilization efficiency. Similar results also shown by kumar *et al.* (2017) [17] observed that zinc availability in soil significantly increase due to application RDF+ *Trichoderma* viride followed by RDF+ *Bacillus megaterium* over uninoculated control treatment.

DTPA extractable copper

Soil of pot culture treated with strain *Bacillus megaterium* indicates significant higher value of DTPA copper (1.975 mg kg^{-1}), followed by treatment with strain *Pseudomonas striata* (1.945mg kg^{-1}) and *Trichoderma viride* (1.913mg kg^{-1}), these treatments are of par with each other. The DTPA copper in soil of pot culture was increase after inoculation of PGPR from initial (1.72 mg kg^{-1}) expect in T_1 i.e., uninoculated control having significantly lower value of DTPA Copper (1.701 mg kg^{-1}).

Our experiment results show significant increase in Cu availability might be due to exudation of organic acids that both release the cation and reduces the acidity in the rhizosphere. This finding are also in the line of Altomare *et al.* (1999) [2] reported that reduction in soil pH is might be due to secretion of organic compound by promising microbial isolates. Our experimental finding collaborated with Waghmare *et al.* (2019) [26] observed that DTPA copper found significantly higher in treatment receiving RDF+ *Pseudomonas striata* after that RDF+ *Bacillus megaterium* and RDF+ *Trichoderma viride* as compared to uninoculated control

Soil biological properties

Dehydrogenase activity in soil

The perusal data on dehydrogenase activity in soil of pot culture, stated that the dehydrogenase activity was from 29.20 µg TPF g^{-1} of soil 24 hr^{-1} to 49.37 µg TPF $g^{-1}$of soil 24 hr^{-1}. It was found significant increased soil dehydrogenase activity in soil of pot culture with inoculation strain *Pseudomonas striata* (49.37 µg TPF g^{-1} of soil 24 hr^{-1}), followed by *Bacillus megaterium* (46.77 µg TPF g^{-1} of soil 24 hr^{-1}) and *Trichoderma viride* (44.63 µg TPF g^{-1} of soil 24 hr^{-1}) which was found at par with treatment *Pseudomonas striata*.

The increased production of dehydrogenase in inoculated rhizosphere soil can be due to the existence of high-quality organic compound/ substrate and thus to a boost in their microbial function (Madar *et al.*, 2010; Rana *et al.*,2012). Similar finding was also given by Pawar (2016) [22] reported that dehydrogenase activity was maximum in soil of pot culture experiment with inoculation of *Trichoderma viride* after that *Pseudomonas striata*.

Table 3: Effect of promising microbial growth promoting agents on enzymatic activity in soil pot culture experiment.

Treatments	Soil enzymatic activity			Soil microbial population		
	Dehydrogenase (µg TPF g^{-1} of soil 24 hr^{-1})	Alkaline phosphatase (µg PNP $g^{-1}$of soil hr^{-1})	Acid phosphatase (µg PNP $g^{-1}$of soil hr^{-1})	Bacteria (cfu x 10^{-7} g^{-1} of soil)	Actinomycetes (cfu x 10^{-5} g^{-1} of soil)	Fungi (cfu x 10^{-4} g^{-1} of soil)
T_1 - Uninoculated Control	29.20	82.00	49.33	44.00	18.67	4.33
T_2 - *Bacillus subtilis*	33.45	103.66	56.15	56.00	22.33	6.67
T_3 - *Bacillus licheniformis*	41.23	118.13	60.43	60.67	25.67	7.00
T_4 -*Bacillus megaterium*	46.77	130.00	66.89	72.67	30.33	8.67
T_5 - *Bacillus thuringiensis*	34.04	114.12	53.40	51.33	19.33	5.33
T_6 - *Pseudomonas fluorescens*	39.44	120.02	57.11	59.33	23.33	7.00
T_7 - *Pseudomonas striata*	49.37	128.68	64.07	68.00	27.00	8.00
T_8 - *Trichoderma viride*	44.63	125.44	62.69	53.33	26.67	10.33
T_9 -*Trichoderma harzianum*	35.94	119.18	56.79	43.67	20.00	9.00
T_{10} -*Azotobacter chroococcum*	42.83	124.20	58.17	66.00	25.33	7.33
T_{11} - *Azospirillum lipoferum*	32.83	116.48	54.14	52.67	20.67	6.67
S.Em.±	1.89	3.19	1.85	2.26	1.38	0.39
CD @ 5%	5.54	9.35	5.43	6.67	4.06	1.14
CV	8.38	4.74	5.52	6.86	10.19	9.17
Initial	17.28	43.51	28.43	29	19	3

Acid phosphatase activity in soil

Acid phosphate activity in soil of pot culture was ranged from 49.33 to 66.89 µg PNP g^{-1} of soil hr^{-1}. However, the pot with treatment *Bacillus megaterium* (66.89 µg PNP g^{-1} of soil hr^{-1}) show significantly high acid phosphatase activity which was at par with strain *Pseudomonas striata* (64.07 µg PNP g^{-1} of soil hr^{-1}) and *Trichoderma viride* (62.69 µg PNP g^{-1} of soil hr^{-1}). These treatments are superior over other treatment and at par with each other.

Alkaline phosphatase activity in soil: Acid phosphate activity was ranged from 82.00 to 130.00 µg PNP g^{-1} of soil hr^{-1}. There was increase in alkaline phosphate activity significantly with drenching of strain *Bacillus megaterium* (130.00 µg of PNP $g^{-1}$of $soil^{-1}$ which was at followed by treatment *Pseudomonas striata* (128.68 µg PNP $g^{-1}$of soil hr^{-1}), *Trichoderma viride* (125.44 µg PNP $g^{-1}$of soil hr^{-1}) and *Azotobacter chroococcum* (124.20 µg PNP $g^{-1}$of soil hr^{-1}).

From the data it was observed that acid phosphatase activity was lower than alkaline phosphatase activity. However, phosphatase activity was positively and significantly correlated with soil pH. In acid soil acid phosphatase activity

was maximum and in alkaline soil alkaline phosphatase activity. In general, acid phosphatase is predominantly due to plants and alkaline phosphatase is due to soil microbial activity. Higher alkaline phosphatase activity in our case followed by alkaline soil pH indicates high microbial biomass (Beura and Rakshit, 2011). Sable *et al.* (2016) [24] observed that, acid and alkaline phosphate activity was found significantly high with application of *Bacillus megaterium*, *Pseudomonas striata*, and *Trichoderma viride* along with *Rhizobium* and RDF.

Soil microbial activity

Bacterial population in soil

Bacterial population in pot culture soil was from 44.00 to 72.67 cfu x10 $^{-7}$ of soil. Significantly higher bacterial population was found in pot treated with strain *Bacillus megaterium* (72.67 cfu x10 $^{-7}$ of soil) which was on par with treatment *Pseudomonas striata* (68.00 cfu x10 $^{-7}$ of soil) and *Azotobacter chroococcum* (66.00 cfu x10 $^{-7}$ of soil).

Similar finding was also observed by Sable *et al.* (2017) concluded that higher number of bacterial populations was found with application of isolates *Pseudomonas striata* along with RDF and found at par with strain *Trichoderma harzianum* and *Burkholderia cepacia.*

Actinomycetes population in soil

Actinomycetes population in pot culture soil was ranged from 18.67 to 30.33 cfu x10^{-5} g^{-1} in pot soil. Significantly higher actinomycetes community was found in pot soil with strain *Bacillus megaterium* (30.33 cfu x10^{-5} g^{-1}of soil) followed by *Pseudomonas striata*, (27.00 cfu x10^{-5} g^{-1} of soil), *Trichoderma viride* (26.67 cfu x10^{-5} g^{-1} of soil) and *Azotobacter chroococcum* (25.33 cfu x10^{-5} g^{-1} of soil)

Highest actinomycetes population was due to chemical fertilizers and PSB inoculation stated by Bodkhe *et al.* (2014). Ramalakshmi *et al.* (2008) also noted that highest actinomycetes population was increased significantly in application of biofertilizers. Jadhav (2021) [12] also reported that significant increase in actinomycetes population due to inoculation of strain *Pseudomonas striata*, *Bacillus megaterium* and *Trichoderma viride* along with RDF.

Fungi population in soil

Fungi population in pot culture soil ranged from 4.33 to 10.33 cfu x 10^{-4} g^{-1} of soil. Significantly higher fungal population was found in pot treated with strain *Trichoderma viride* (10. cfu x 10^{-4} g^{-1} of soil). However significantly lowest fungal population was found in uninoculated control treatment (4.33 cfu x 10^{-4} g^{-1} of soil)

Microbial population of fungi were noted significantly highest in treatment RDF + *Rhizobium* + *Trichoderma viride* and was found at par with RDF + *Rhizobium* + *Trichoderma harzianum* (Kumar and Ismail, 2017) [17]. It was also suggested by studies of sale *et al.* (2016) that the population of fungi were noted significantly higher in RDF + Rhizobium + *Pseudomonas Striata.*

Relationship between soil organic carbon with enzymatic and microbial activity in soil of Pot culture experiment

Soil organic carbon estimated from soil of pot culture was positively and significantly correlated with root stock density of pot seedling, soil enzymatic activity like dehydrogenase activity, acid phosphatase activity, alkaline phosphatase activity and microbial population i.e. bacterial population, actinomycetes population and fungal population in soil of field experiment.

Table 4: Relationship between soil organic carbon with enzymatic and microbial activity in soil of pot culture experiment

Sr. No	Parameters	Correlation Coefficient
1	Root stock density	0.82**
2	Dehydrogenase activity	0.72**
3	Acid phosphatase activity	0.55**
4	Alkaline phosphatase activity	0.86**
5	Bacterial population in soil	0.92**
6	Actinomycetes population in soil	0.58**
7	Fungal population in soil	0.86**

Fig 1: General view of pot experiment significant at 5% (r = 0.349 *) significant at 1% (r = 0.449 **)

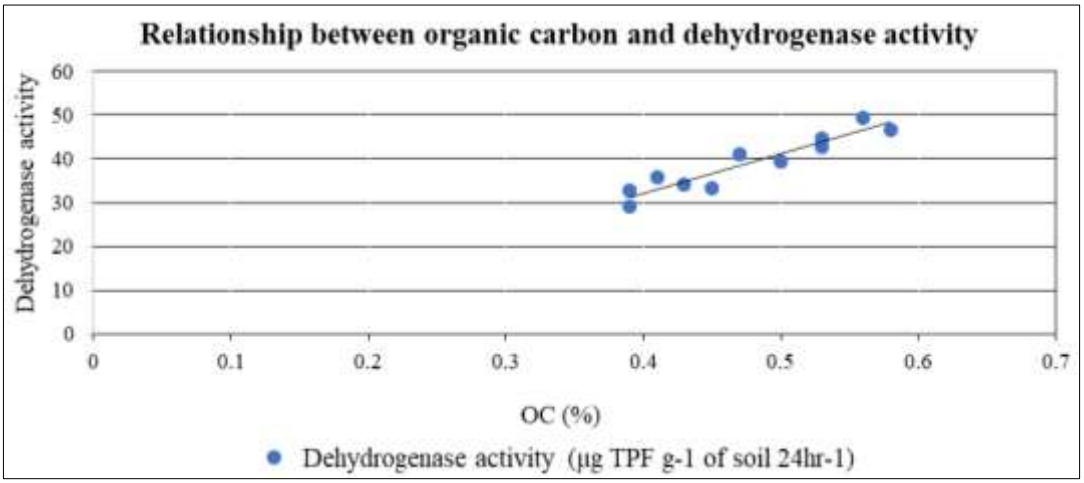

Fig 2: Relationship between organic carbon and dehydrogenase activity in soil of pot culture experiment

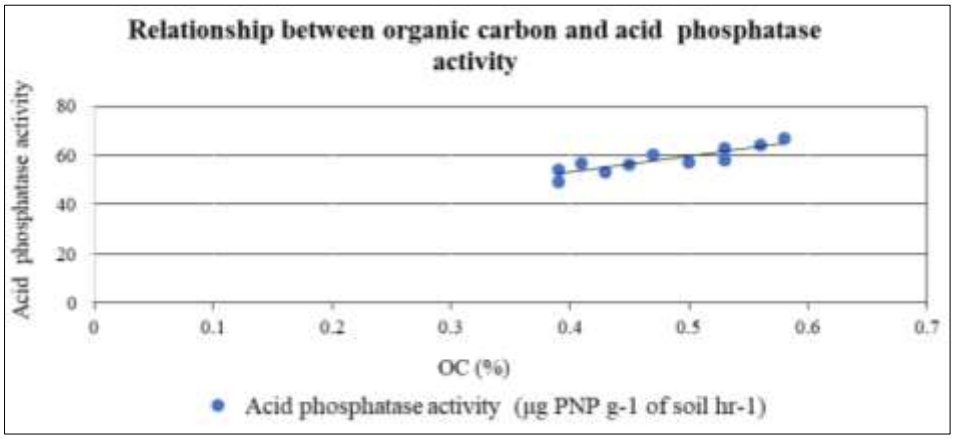

Fig 3: Relationship between organic carbon and acid phosphatase activity in soil of pot culture experiment

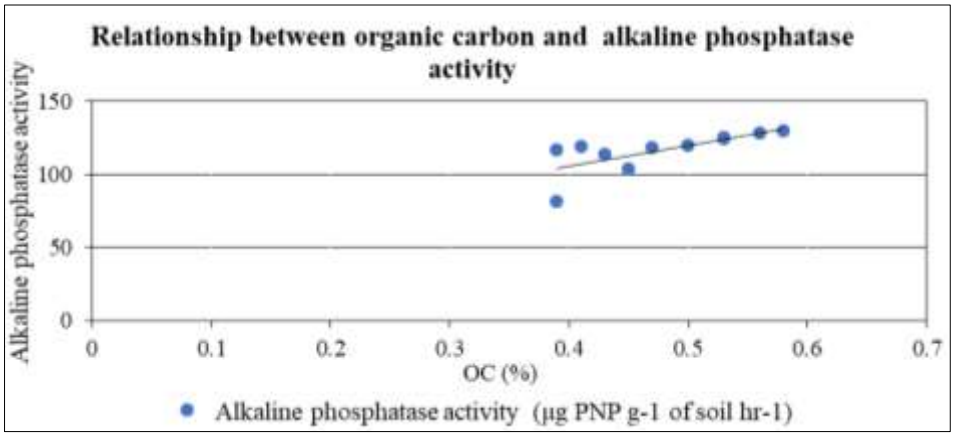

Fig 4: Relationship between organic carbon and alkaline phosphatase activity in soil of pot culture experiment

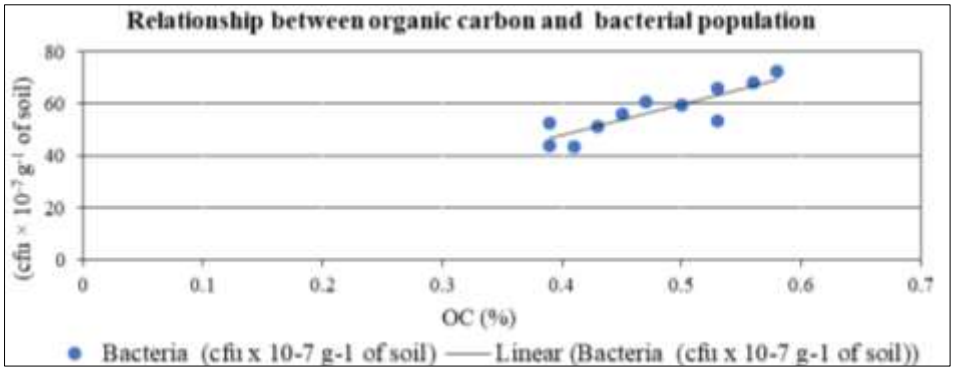

Fig 5: Relationship between organic carbon and soil bacterial population

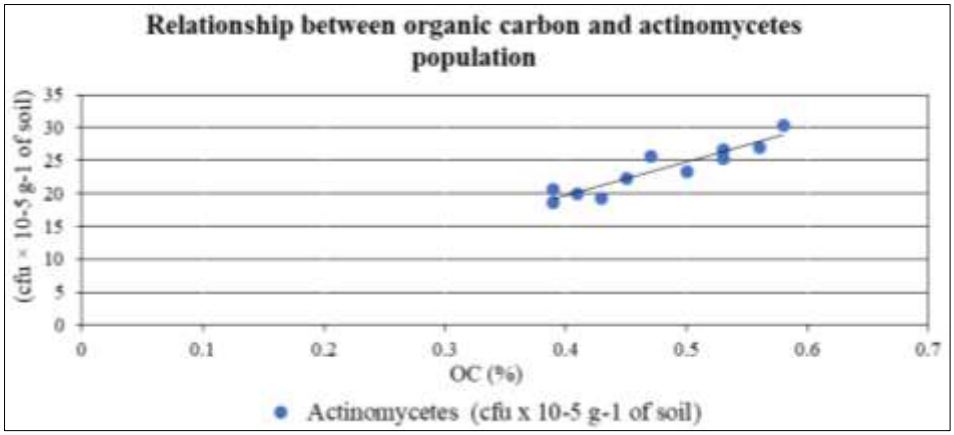

Fig 6: Relationship between organic carbon and soil actinomycetes population

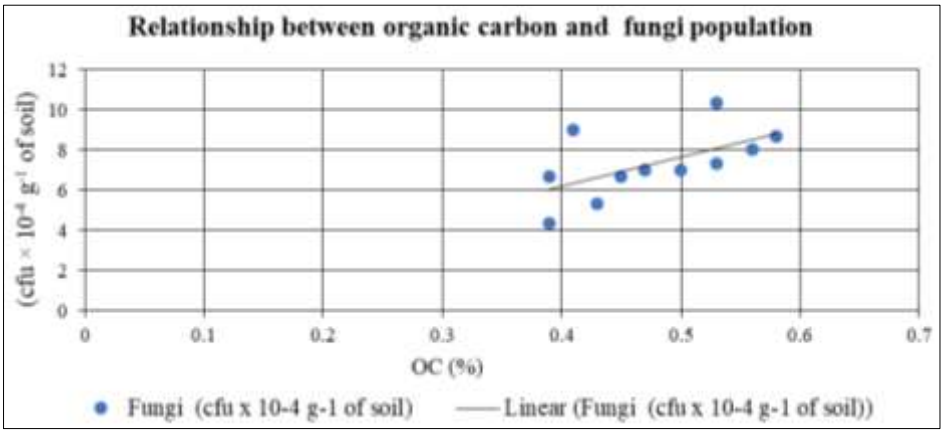

Fig 7: Relationship between organic carbon and soil fungi population

Conclusion

At a global scale, the effect of continuous fertilization can cause serious damage to the enviorment. Inoculation of microorganism is one of the most important sustainable practices in Indian agriculture, because microbial isolates established association with plants and promotes plant growth by means of several beneficial characteristics.

The combination of different fertilizers with these microbial isolates, such as identification of plant growth promoting traits, the identification of microbial isolates, as well as assay of seed inoculation in laboratory conditions and experiment in the field, are art of the search for new technologies for agriculture crop. Thus, when this search shows a potential of microbial inoculants it was observed that strain like *Bacillus megaterium*, *Pseudomonas striata* and *Trichoderma viride* not only increase nutrient content in soil but also found significant improvement in microbial activity as well as plant growth. The strategy of inoculation of PGPR individually, or in a mixture, represents a new biotechnological tool for mobilization of macro and micronutrients.

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Appendix A. Supplementary Data

Supplementary data of this article an be found online a http://krishikosh.egranth.ac.in/ handle/1/5810070599

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