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INTEGRATED BIOINOCULANTS SIGNIFICANT FOR INCREASING FERTILITY LEVEL OF RED LATERITE SOIL

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Abstract
Soil microbes play an important role for increasing the soil minerals level under mineral deficient condition or stress condition. The dominant soil microbes are entophytic fungus and plant growth promoting bacteria .The interactions of soil microbes n most cases can positively affect the growth of host plant and their yield production and also increase the fertility level of soil.

Keeping the fact on mind the present study aims to evaluate the effect of Glomus aggregatum and Burkholderia fungorum on elevation of soil minerals of red laterite soil and we can use it as a biofertilizer in future. Due to this purpose here we analysis some soil parameters like soil ph, alkaline phosphatase, acid phosphatase, soil nitrogen, phosphorus, potassium, and iron also.

Key Words: Glomus aggregatum, Burkholderia fungorum Soil Phosphorus, alkaline phosphatase, acid phosphatase , soil nitrogen, phosphorus, potassium, and iron.

Introduction

In the late 19th and early 20th centuries inorganic compounds containing nitrogen, potassium and phosphorus (NPK) were used as fertilizers. Due to the growth in human populations fertilizers were used to amplify crop production and meet the increasing demands for food. Rising in the manufacturing cost, and the harmful nature of chemical fertilizers for the environment have led to a renaissance of interest in the use of biofertilizers for better environmental sustainability, lower production cost and good crop yields. Preparations of live microbes (like, bacteria, fungi etc.) utilized for improving plant growth and crop production are generally referred to as biofertilizers or microbial fertilizers (Subba Rao, 1998).

Researches in the field of biofertilizers have resulted in the development of different kinds of microbial inoculants or biofertilizers including nitrogen fixing bacteria, phosphate solubilizing microorganisms, vesicular – arbuscular mycorrhizae (VAM), plant growth promoting rhizobacteria (PGPR) and Plant growth promoting bacteria (PGPB).

Applications of bio fertilizers are one of the most acceptable approaches for high vigor with better quality and are also safe for human exploitation.

Associations of AM fungi play a vital role in mineral uptake, specially the slowly mobile ions such as phosphorous (Abbott and Robson, 1984). Minerals like N, P, K, Ca, S, Zn, Cu, are absorbed from soils by mycorrhizal fungi and are translocated to the host plant (Smith et.al.1994). As a result of increasing the soil fertility level ,AM fungi improve the growth of plants by increasing the absorptive surfaces of roots compared with root hairs, and thus help in the absorption of phosphorus, copper and zinc which are relatively immobile in the soil (Bagyaraj, 1992). AM infection in plant enhances plant growth and vigor and mineral levels of plants especially NPK becomes improved which lead to increase the yield and productivity.

Plant growth promoting rhizobacteria (PGPR) are the dominant group of rhizosphere bacteria which help in translocation, mobilization and solubilization of essential plant nutrients either in fixed or in organic forms and thus make them available to the growing plants. Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion. Inoculation of crop plants with certain strains of PGPR at an early stage of development improves biomass production through direct effects on root and shoots growth. Inoculation of ornamental plants, forest trees, vegetables, and agricultural crops with PGPR may result in multiple effects on early-season plant growth, as seen in the enhancement of seedling germination, plant health, plant vigor, plant height, and shoot weight, nutrient content of shoot tissues, early bloom, chlorophyll content, and increased nodulation in legumes (Chakroborty et al., 2007) PGPR are reported to influence the growth, yield, and nutrient uptake by an array of 6 mechanisms. They help in increasing nitrogen fixation in legumes, help in promoting free-living nitrogen-fixing bacteria, increase supply of other nutrients, such as phosphorus, sulphur, iron and copper, produce plant hormones, enhance other beneficial bacteria or fungi, control fungal and bacterial diseases and help in controlling insect pests. (Duchesne et al., 1989) Many plant growth promoting rhizobacteria have positive correlations with mycorrhizal fungi as they stimulate the growth of VAM fungi in the

soil (Ferguson, 1982; Lindeman and Paulitz, 1990; Jarstfer and Sylvia1992; Mukherjee and Rai, 2000; Bhowmik and Singh 2001, Hakoomat Ali et al., 2004).

Due of these such advantages AM fungi Glomus aggregatum and Burkholderia fungorum can be consider as a potent bio inoculants and nutrient remedifier and it is also less expensive, it is therefore more important to use Glomus aggregatum arbuscular mycorrhizal (AM) fungi and Burkholderia fungorum (PGPR) as biofertilizer.

Material and Methods

The isolated AM fungus, (G.aggregatum and PGPR strain, Burkholderia fungorum were used either alone or in combination. The spores as starter inoculum of selected AM fungus, Glomus aggregatum were raised by Funnel Technique of Menge and Timer, (1982) and further mass inoculum was produced using Hordeum vulgare as the host plant. The bacterial isolate was designated as M3 and the species level identification was done (Sen, Dutta 2017) following 16s r RNA sequencing and was further used for mass culturing.

Analysis of Soil Nutrient Parameters during Crop Growth

Rhizosphere soil was collected from different pots at different growth phases of Sorghum bicolor plant. The soil was air dried, properly sieved and the following experiments were performed with a view to determine sol nutritional status.

1. **Determination of Soil pH:** It was done following the method of Jackson, (1973).
2. **Estimation of Organic Carbon present in the Soil:** It was done following the method of Walkley (1934).
3. **Estimation of Total Nitrogen:** It was done following the method of (Vogel, 1961).
4. **Estimation of Available Phosphorus:** present in the Soil (Jackson, 1973).
5. **Estimation of Total Phosphorus:** Total Phosphorus present in the Soil was estimated following the method of Jackson, (1973).
6. **Estimation of potassium:** Dipak Sarkar and Abhijit Halder 2010.
7. **Estimation of Soil Iron:** By Spectrophotometric methods (M. Jamaluddin Ahmed et al., 2009).
8. **Measurement of the activity of Phosphatase enzymes:** Sadasivam and Manickam, 1996.

Results and Discussion

Effect of (Vam) Glomus Aggregatum and Pgpr (Burkholderia Fungorum) On Soil Fertility

It is apparent from the results (Table -1) that inoculation of the selected soil with Glomus aggregatum and Burkholderia fungorum significantly increased the soil fertility of the red laterite soil.Co-inoculation of the seedlings of Sorghum bicolor with Glomus aggregatum and Burkholderia fungorum significantly augmented the percent colonization of the VAM fungus in the roots as well as in the rhizosphere soil of the plant more efficiently as compared to the plants which were inoculated by them separately. And as a consequence, Soil pH, soil phosphorus, carbon, potassium activity was measured and it exhibited a remarkable enhancement of these.

Table- 1: Interactive Effects of Glomus Aggregatum And Burkholderia Fungorum On Available Phosphorus (Ppm)*, Total Phosphorus (Ppm*), Total Nitrogen (%), Soil Carbon (%), Potassium (Kg Ha-1), Iron (Ppm), Ph Content, Phosphatase Activity of the Laterite Soil

	Treatment				
	Days of Inoculation	**Control**	**Soil Inoculated With Glomus Aggregatum**	**Soil Inoculated With Burkholderia Fungorum**	**Soil Inoculated With Burkholderia Fungorum + Glomus Aggregatum**
Available Phosphorus (Ppm)*	20	16.6±0.12	18.6±0.11	17.5±1.25	24.4±1.01
	40	18.2±0.13	19.8±0.90	18.8±0.88	26.2±1.00
	60	19.2±0.15	23.7±1.00	21.6±2.54	29.4±2.76
	90	21.3±0.17	28.8±2.76	23.5±2.76	31.4±1.05
Total Phosphorus (Ppm*)	20	52.5±1.31	61.2±1.87	60.3±2.02	83.4±1.20
	40	52.9±1.09	72.4±1.54	69.4±1.56	89.5±3.65
	60	54.1±1.21	87.6±1.55	79.5±1.09	96.7±1.56
	90	54.5±1.20	91.8±3.05	84.6±1.55	110.2±3.98

Total Nitrogen (%)	20	0.016±0.0008	0.032±0.0011	0.036±0.0018	0.042±0.0021
	40	0.019±0.00095	0.042±0.0021	0.049±0.0031	0.054±0.00245
	60	0.021±0.001	0.051±0.0053	0.054±0.0027	0.064±0.0032
	90	0.031±0.0015	0.058±0.0095	0.065±0.0032	0.084±0.0042
Soil Carbon (%)	20	0.86±0.04	1.38±0.05	1.18±0.04	2.21±0.06
	40	0.94±0.05	1.64±0.09	1.34±0.08	2.64±0.05
	60	1.03±0.07	1.98±0.09	1.46±0.07	2.95±0.05
	90	1.11±0.09	2.29±0.09	1.68±0.05	3.05±0.03
Potassium (Kg Ha-1)	20	9.97±0.64	11.38±0.75	10.12±0.80	16.86±0.62
	40	10.11±0.80	14.54±0.34	12.24±0.89	19.43±0.50
	60	12.12±1.01	16.30±0.89	13.67±0.76	21.52±0.51
	90	12.88±0.23	16.88±0.81	15.92±0.81	23.86±0.34
Iron (Ppm)	20	53.21±0.97	23.45±1.21	31.61±1.24	21.14±1.01
	40	49.54±3.11	20.34±1.21	27.56±3.05	19.09±2.56
	60	41.12±5.11	17.45±1.56	21.34±2.02	15.62±2.25
	90	39.35±1.49	12.67±2.02	19.98±3.87	10.19±1.10
Ph	20	2.63±0.13	4.7±0.23	4.9±0.245	5.3±0.265
	40	2.66±0.133	5.1±0.25	5.2±0.26	6.45±0.322
	60	3.69±0.18	5.2±0.26	6.1±0.3	6.81±0.34
	90	3.73±0.186	5.48±0.274	6.5±0.325	6.80±0.345
(**Alkaline Phosphatase) (M/Ml* Of Extract)**	20	0.63±0.011	2.77 ±0.044	1.22±0.021	4.01±0.078
	40	0.72±0.012	3.62±0.056	2.12±0.043	4.98±0.079
	60	0.76±0.013	3.89±0.049	2.71±0.045	5.16±0.082
	90	0.88±0.015	4.44±0.053	2.81±0.046	6.74±0.079
(Acid Phosphatase) (M/Ml* of Extract)	20	1.17±1.04	7.12±0.65	6.78±0.88	10.56±0.98
	40	1.90±0.90	8.54±0.76	7.44±0.65	11.76±0.98
	60	2.20±0.98	9.21±0.98	9.67±0.65	13.09±0.21
	90	2.26±0.86	10.54±0.99	9.98±0.98	14.54±0.69

*data are mean values of 5 replicates.

Nutrient in soil

It is evident from the results (Table-1) that combined effect of G. aggregatum and Burkholderia fungorum influenced on the soil fertility. Through the release of organic or inorganic compounds from the roots into the soil lead to the root-soil activities which adjust the soil physio-chemical properties. This is known to as rhizodeposition or root exudation which is influenced by plant and soil biotic and abiotic factors (Jones et al., 2004). Arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria (PGPR) are typical beneficial microorganisms which are able to influence the changes in rhizosphere functioning (Azcón-Aguilar and Barea, 1992; Barea et al., 2002b; Suresh and Bagyaraj, 2002;). As AM fungi supply an essential link between plants and the soil environments, they are very important to any rhizosphere studies (Timonen and Marschner, 2005).

Soil PH

Soil PH was noticed to be influenced by VAM fungi. Here we have selected red laterite soil for AM inoculums production. This has a soil pH value more or less acidic. From the results (Table-1) it is evidenced that inoculation of Glomus aggregatum in the pot culture soil enhanced the pH value first and then it becomes maintained to the pH range of 5.1 to 5.48. It has been appeared to be increased in the soil inoculated with VAM fungus, G. aggregatum. The same trend was maintained in the soil inoculated with PGPR bacteria, B. fungorum. When soil inoculated by both the microorganisms i.e. VAM and PGPR, the soil pH value was increased steeply and it reached upto 6.84 from 4.9 at the 90 days of incubation. The results corroborate with the findings of (Chakroborti et al., 2007).

Nitrogen and Phosphorus

Total Nitrogen and phosphorus content in the red laterite soil inoculated with microbial inoculants, Glomus aggregatum and B .fungorum individually enhanced significantly after 20 days of inoculation and reached highest at 90 days. N2 – Content in the roots was more or less higher in Burkholderia fungorum inoculated plants rather than in the roots inoculated with Glomus

aggregatum. Application of VAM also enhanced the soil phosphorus content in the rhizosphere soil than the uninoculated plants. The soil inoculated with dual inoculation accumulated large amount of Phosphorus and nitrogen in the rhizosphere soil, and the increase was more pronounced under dual colonization at 90 days. The results corroborate with the findings of (Lakshmipathy et al., 2002, Chakroborti et al., 2007, Wani et al., 2007, Khare and Rodrigues (2009) ; Selvaraj et al., 2008, Choudhary et al.,2011).

Potassium

Synergistic effect of VAM fungi (Glomus aggregatum) + PGPR (Burkholderia fungorum caused a sharp increase activity upto 23.86 kg ha-1 soil after 90 days of inoculation as contrast to the control set (12.88kg ha-1). While the plants inoculated with Burkholderia and VAM alone exhibited 16.88±0.81 kg ha- 1.15.92±0.81 kg ha-1 similar results was suggested by Lakshmipathy et al., 2002; Chakroborti et al., 2007; Selvaraj et al., 2008).

Carbon

The host plant serve carbon to the mycorrhiza, as a result mycorrhizal inoculated soil showed higher amount of carbon than the soil inoculated with Burkholderia fungorum and control. It is clearly evidenced from the result that dual colonization of both VAM and Burkholderia fungorum together accumulated higher amount of carbon in Soil. This result was supported by Subramanian et al., 2009.

Iron

As we have selected the red laterite soil for present study which contains greater percentage of iron, we have measured the changes occurred in soil iron content as a result of mycorrhizal as well as PGPR colonization. It has been recorded that soil iron content has remarkably been reduced as a consequence of mycorrhizal inoculation of the host plants. It reduced into 12.67 ±2.02ppm from 39.35±1.49 as in control set 39.35±1.49 after 90 days of inoculation. Inoculation of host plants with the PGPR bacteria, Burkholderia fungorum also reduced the amount of iron. However, the effect was more pronounced when dual inoculation with VAM and PGPR was done. This result does not corroborate with the findings of N. Hemashenpagam et al., 2011.

Phosphatases Content

Mycorrhizal colonization had been shown to influence root phosphatase activity (Khade and Rodrigues, 2009). Greater enhancement of enzymatic and acid phosphatase (ACP) and alkaline phosphatase (ALP) activity occurred with AM fungi treated plant roots compared to non-mycorrhizal plant roots (Chakroborti et al., 2007, N. Hemashenpagam et al.,2011). Their view concomitantly support with our study.

The activity of Acid Phosphatase (ACP) and Alkaline Phosphatase (ALP) in the plants inoculated with dual inoculum of VAM and PGPR simultaneously were recorded to produce more enzyme (14.54µ/ml and 6.74 µ/ml of cell free extract) at 90 days of inoculation as compared to the plants under control set and the set inoculated with single inoculum.

Conclusion

The present experimental findings confirm the positive synergistic interaction between vesicular arbuscular mycorrhizal fungi (Glomus agggregatum) and a PGPR bacterium (Burkholderia fungorum) in the red laterite soil for changing the physical, chemical and biological properties and gives a positive impact on soil fertility of red laterite soil.

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