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Efficiency of Nutrient Mobilisation of Green Gram (Vigna radiata var. radiata) Plant With and Without Applying Fertiliser

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Abstract

Nutrient mobilisation is the transformation of a nutrient from a less soluble form to an easily soluble one, thereby facilitates its availability to a plant. The process is helped by the activity of soil microbes. Fertiliser is added into soil to increase the soil capacity to support the plant growth. It can be of any substance, such as manure, a mixture of nitrates, phosphorus or potassium compounds added to soil or water to increase its productivity. For proper plant growth there are atleast 17 essential elements are required: carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, manganese, zinc, copper, boron, molybdenum, chlorine, and nickel. Plants receive carbon, hydrogen, and oxygen via air and water. The rest of the elements are derived from the soil. Fertiliser supplementation becomes necessary when the soil cannot meet the needs for proper plant growth. The study is trying to analyse the addition of fertiliser will make any progressive change in the nutrient mobilisation. The work mainly centres on the isolation and screening of rhizospheric bacteria from the rhizosphere microflora of a leguminous plant supplemented with and without fertiliser with an objective of comparing the activities shown by the two. Microbial activity in the soil was measured by soil respiration method. R: S ratio was also assessed in order to get an idea about the rhizosphere effect. Our present study reveals that without the addition of fertiliser the plant rhizosphere shows less activity. It will not help for the adequate plant growth. Hence after soil testing the addition of fertiliser is necessary according to the need.

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Introduction

Many ecosystem services on which one depends are indeed influenced by soils (Dominati *et al*., 2010). For performing vital functions in the soil, soil organisms are responsible, to a varying degree. They make up the diversity of life in the soil. Soil organisms are actually representing a large fraction of global terrestrial biodiversity. These organisms performing a range of processes in soils of both natural ecosystems and agricultural systems and this is important for soil health and fertility. During the construction process many urban soils are disturbed. Such as top soil is often scraped off and removed and, as a result, nutrient and organic matter levels are often lower in these disturbed sites than in native soils. Here lies the importance of adding fertiliser by adding organic matter as well as fertiliser may be necessary to enhance the growth of plants on these particular areas. For this accurate fertiliser recommendations are important, because problems may be arised due to either inadequate or excessive fertilisation. If the fertiliser is added too little it may lead to poor plant growth, but too much fertiliser addition can also reduce growth and quality of the plant. In addition to that excessive applications of fertiliser can also be harmful to the environment.

Based on the plant types, soil types and the results of soil tests fertilisers are recommended. Soil testing is done prior to the fertiliser application in order to obtain the information regarding the availability of nutrients in the soil and this is required for accurate lime and fertiliser recommendations. Soil pH is an important chemical property, which is checked during soil testing because it affects the availability of nutrients to plants and the activity of microorganisms in the soil. A pH measurement is therefore an important part of a soil testing program. The effect of pH on microbial activity and nutrient availability in mineral soils is shown in Figure 1. (Rosen *et al*., 2008). Cultivated soil has relatively much more microbes than the fallow land. Soil fauna like fungi and bacteria are the highest portion of microorganisms inhabiting in the rhizosphere (Morgan *et al*., 2005). The rhizosphere is the soil zone surrounding the plant roots and which helps for physical, chemical and biological properties of soil (Kennedy, 2005). The root exudates in the rhizosphere region provide amino acids and growth factors required by soil bacteria. The root colonizing bacteria which inhabiting the rhizosphere and form symbiotic relationships with many plants is popularly known as rhizobacteria. Root free soil outside the rhizosphere that is not penetrated by plant roots is commonly known as bulk soil (Andrade *et al*., 1997). Here microbial populations are low in number compared to rhizospheric soil.

Based on the studies about rhizosphere and the effect of root exudates to the microbes (particularly bacteria), the term rhizospheric effect/phenomenon was come into existence for the first time. Then later Katznelson in 1946 introduced the term R/S ratio (Rhizosphere: soil ratio) to express the rhizospheric effect. The ratio of microbial population per unit weight

of rhizosphere soil (R), to the microbial population per unit weight of the adjacent non-rhizosphere soil (S) is the R: S ratio. Type and moisture content of the soil, temperature, age of the plant, etc. are the factors, which influence the rhizospheric effect. R: S ratio is decreasing with increase in soil depth (Vassileva, 1998).

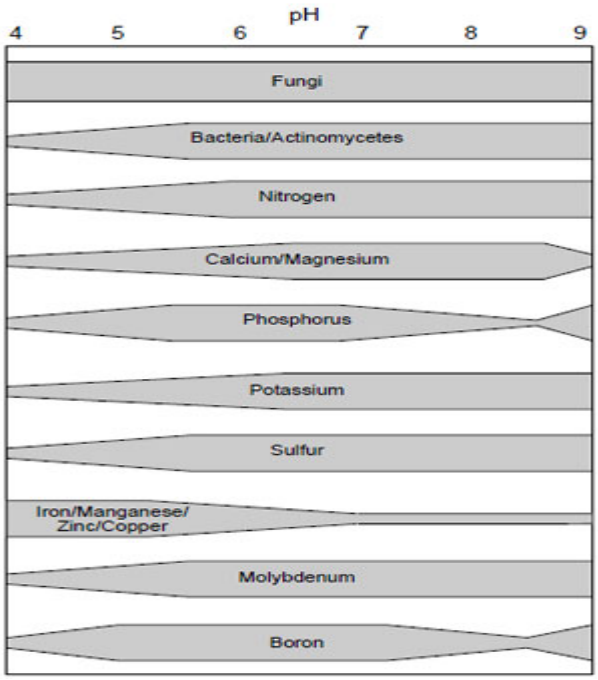

Figure 1. Nutrient availability and microbial activity as affected by soil pH; the wider the band, the greater the availability or activity (Adapted from Brady, N. The Nature and Properties of Soils, 10th ed. Macmillan Publ. Co., New York, 1990).

Many studies revealed that drought, cold, pests and diseases withstanding capacity of plants can be improvised with the help of Nitrogen (N), Phosphorus (P) and Potassium (K) (Lin *et al*., 2010; Tsai *et al*., 2007). The average soil contains about 0.05 % (w/w) phosphorus but the available phosphorus to plants is very low and it is only 0.1% of the total soil phosphorus because of poor solubility and its fixation in soil (Illmer and Schinner, 1995). Microorganisms have the capacity to solubilise organic and inorganic phosphorus into plant available form. These microbes are living in rhizosphere; root surface or it may be also seen in connection with roots. PSB secrete organic acids and phosphatases and the organic acids convert inorganic phosphates into monobasic and dibasic ions, which is absorbable by plants. This process is known as mineral phosphate solubilisation (Dave and Patel, 2003; Chung *et al*., 2005; Richardson and Simpson, 2011). The role of phosphatases is to improve mineralisation (hydrolysis) of organic phosphorus. So, PSB has an essential role in plant nutrition to increasing the uptake of phosphorus (Rodriguez, 2006). It is well known that CO_2 production, transport and emission in soil depend on environmental factors such as aeration condition, soil temperature, soil moisture,

supplies of organic carbon, fertilisation, pH etc. (Lin *et al*., 2010; Bowden *et al*., 2004). Therefore sound management of fertilisation is must to ensure the soil quality. In this paper, special focus was given separately to fertiliser added and non-fertiliser added leguminous plants. In order to satisfy crop nutritional requirements, Nitrogen, Phosphorus and Potassium are externally added. Fertilisers have long-term impacts on the environment in terms of eutrophication, soil fertility depletion and carbon footprint. Moreover, plants can use only a little amount of these fertilisers and 76-90% added fertilisers rapidly become fixed in soils by re-precipitation (Podile and Kishore, 2006). So fertilisers must be added in an adequate manner after soil testing like procedures.

Miranda and Jayadev, (2015) has studied and compare nutrient mobilisation efficiency, especially phosphorus and nitrogen mobilisation by the microflora of the rhizospheres of chemical fertliser and biofertilizer applied leguminous plant *Vigna radiata* and non-leguminous plant *Capsicum frutescens*. Both type of plants are treated separately using both type of fertilisers. The activity difference of microflora of rhizosphere of leguminous and non-leguminous plant was also analysed. The objective of the present study was to compare separately the efficiency between fertiliser added and non-fertiliser added rhizospheres of leguminous plant *Vigna radiata* var. *radiata* by analysing the nutrient mobilisation activities of the rhizospheres through ammonification, nitrification and phosphate solubilisation activities. Microbial activity in soil was also measured by soil respiration method. In addition to that to assess the rhizosphere effect R: S ratio was also estimated**.**

Materials and Methods

The plant used for the study was *Vigna radiata* var. *radiata* (*Vigna radiata* L.). It was earlier known as *Phaseolus aureus*. The plant is commonly known as green-gram or mung-bean. It is a leguminous plant. This work was done in order to study and compare nutrient mobilisation efficiency, especially phosphorus and nitrogen mobilisation by the microflora of the rhizosphere of fertiliser applied and non-fertiliser applied *Vigna radiata* plant. The activity difference of microflora of rhizosphere of fertilised and without fertilised leguminous plants is also analysed. The physico-chemical parameters of soil used (from All Saints' College Campus, Thiruvananthapuram) for growing the study plant *Vigna radiata* were tested in pathology section of Kerala Agricultural University, College of Agriculture, Vellayani just to get an idea about the type of the soil the plants are to be grown.

For microbial analysis work the following soil samples were used: (a) Rhizospheric soil from fertiliser added *Vigna radiate* and (b) Rhizospheric soil from non-fertiliser added *Vigna radiate*. Based on the colony morphology, a total of 7 strains of distinct bacterial colonies were isolated and selected for further studies. The pure cultures of bacterial strains obtained from different

samples were subjected to gram staining technique to differentiate them into gram positive and gram negative (Austrian, 1960).

Enrichment of microorganisms

The soil samples were enriched in nutrient agar plates using pour plate method after serial dilution. The plates were then incubated at 35°C– 40°C for 24 – 48 hours.

Isolation of pure culture

The morphologically distinct bacterial colonies observed on nutrient agar plates were subjected to streak plate method using sterilized inoculating wire loop on separate plates for isolation and identification of pure bacterial colonies from a mixed population. All plates were then incubated at same temperature and for time period as before indicated.

Morphological characterization of microorganisms

Purified microbial colonies were studied for 5 different morphological characters namely colour, margin of colony, surface form, surface texture and elevation (Cruickshank *et al*., 1975). The obtained microorganisms were subjected to Gram staining technique (Murray *et al*., 1994). The pure cultures were tested for the following abilities (Table 1).

Results and Discussion

The work was done to identify the capabilities of various microorganisms that can be isolated from a leguminous rhizosphere with and without applying fertiliser and to compare their efficiencies for the said activities and to find out the effect of addition of a fertiliser over a raw soil in which a plant was grown. The results obtained are presented below.

Analysis of physico-chemical parameters of soil

Chemical properties of soil are the most important among the factors that determine the nutrient supplying power of the soil to the plants and microbes. In the current work, analysis of physico-chemical parameters of the soil for the fertility status was analysed and the results are given in Table 2. Soil pH is the measure of soil acidity. A pH of 7 is neutral, a pH below 7 is acid, and a pH above 7 is alkaline. The pH of the study area's soil was 5.5. It was indicating that the soil acidity was approaching towards a highly acidic range. This can be due to different anthropogenic and natural activities including leaching of chemicals, acid rains, decomposition of organic materials etc. (Brady and Weil, 2002). Compared to corresponding native undisturbed soils the pH tends to be higher than in urban landscapes. A higher pH in urban soils is most likely due to

large amount of cement used during construction, scraping away of top soil and exposing the more calcareous subsoil, use of irrigation water high in bicarbonates (Rosen *et al*., 2008).

Table 1 Characters analysed and details of the tests carried out

Test	**Reagents/Procedure**	**Reference**
Identification of Ammonifying Bacteria	Peptone broth and Nessler's reagent	Cappuccino and Sherman, 1992
Test for nitrite production	Ammonium sulphate broth and Trommsdorf's reagent	Cappuccino and Sherman, 2008
Test for nitrate production	Nitrite Broth (Nitrate Forming Broth) for testing nitrate production using Diphenylamine reagent	Cappuccino and Sherman, 2008
Isolation of Phosphate Solubilising Microorganisms (PSM)	Pikovskaya's (PKV) agar medium supplemented with insoluble TCP	Pikovskaya, 1948
Measurement of microbial activity in soil	Soil respiration method	Anderson,J.P.E., and Domsch,K.H, 1978
Estimation of R: S ratio	Quantitative estimation is required to determine the R: S ratio and assess the rhizosphere effect. Divided the values of CFUs of rhizosphere microorganisms with non-rhizosphere to get the R: S ratio.	Katznelson, 1946; Timonin, 1966

Table 2 Physico-chemical characteristics of soil

Parameter	**Reading**	**Rating**
pH	5.5	Strongly acid
EC dSm^{-1}	0.04	Normal
Organic carbon (%)	2.2	High
Available phosphorus (kg/ha)	29	High
Available potash(kg/ha)	10	Very low

The very low content of potash may be due to the soil management activities or the parent materials. Wakene in 2001, clearly reported that the variation in the distribution of K depends on the mineral present, particles size distribution, degree of weathering, soil management practices, climatic conditions, degree of soil development, the intensity of cultivation and the parent material from which the soil is formed.

Microbial Analysis

Enrichment and isolation of bacterial isolates
From non-fertiliser added *Vigna radiata* rhizosphere = 3
From fertiliser added *Vigna radiata* rhizosphere = 4

Gram Staining

Four gram negative bacterial strains were obtained. The four were cocci. Three gram positive bacteria were also obtained and out of which one was bacilli and two were cocci.

Screening of bacterial strains for Nitrogen fixing activity

Identification of Ammonifying Bacteria

In the present study the isolated bacterial strains were tested for ammonification and nitrification. The results are shown in Table 3.

All the bacterial strains isolated from green-gram rhizosphere treated with fertiliser (FP1, FP2, FP3 and FP4) showed greater production of ammonia when compared to bacterial strains isolated from non-fertiliser treated rhizosphere (NP1, NP2 and NP3) (Table 3).

Table 3 The bacterial isolates showing ammonification and nitrification

Culture	Production of Ammonia	Nitrate production	Nitrite production
FP1	++	++	-
FP2	++	++	-
FP3	++	++	-
FP4	++	+	-
NP1	++	+	-
NP2	+	++	-
NP3	+	+	-

Note: '-' sign indicates no production, **'+'** sign indicates small amount of production and **'++'** sign indicates large amount of production.

Identifying bacteria involved in nitrification

Nitrite production

In the present study the selected strains were analysed for nitrite production and the results are shown in Table 3. None of the bacterial strains showed nitrite production. It may be due to the lack of oxidase enzymes in the selected bacterial isolates.

Nitrate production

Some bacteria are able to use nitrate (NO^{3-}) as an external terminal electron acceptor under anaerobic conditions. This kind of metabolism is analogous to the use of oxygen as a terminal electron acceptor by aerobic organisms and is called anaerobic respiration. Nitrate is an oxidised compound and there are several steps possible in its reduction. The initial step is the reduction of nitrate (NO^{3-}) to nitrite (NO^{2-}).

In the present study the isolated strains were screened for nitrate production and the results were showed in Table 3. It reveals that soil treated with chemical fertiliser contain no nitrate producing bacteria when compared to soil added with fertiliser, which contain five nitrate producing bacterial strains.

All the bacterial strains isolated from both green-gram rhizospheres showed nitrate production activity but that isolated from non-fertilizer treated rhizosphere showed a slight reduction in nitrate production. This may be due to the nature of the bacterial strains, which vary in their ability to perform these reactions.

The present study reveals that the presence of ammonifying and nitrifying bacteria seems to be decreased in non-fertiliser applied soils and plants compared to the fertiliser added ones. Fertiliser application also favours plant nutrient uptake and rhizosphere microbial activities. The spatial localisation of roots is important when nutrient is distributed heterogeneously (Ho *et al*., 2005).

Identification of Phosphate Solubilising Bacteria

Halo zones/ clear zones surrounding the bacterial isolates are considered as phosphate solubilisation zone and which is selected as positive (Gour, 1990). In the study period several attempts were made to isolate Phosphate Solubilising Bacteria (PSB). But only one bacterial isolate (FP4) with the said activity was obtained. Only FP4 was able to solubilise Tri Calcium Phosphate (TCP) in solid culture state. This result is also favourable to say that the addition of fertiliser is prompting the nutrient mobilisation activities of rhizospheric microbes. But it was not considered for further quantitative analysis since it showed less than 3

mm phosphate solubilisation zone. The isolates exhibiting 3 mm or more halo zone will be generally considered for further quantitative analysis.

At both low and high pH values, availability of phosphorus to the microbes will be low and which may be the reason for the above said observation (since in the present study, the soil sample having strongly acidic pH of 5.5). Acidic soil condition causes immobilisation of soil phosphorus which may cause unavailability of phosphorus to the microbes. The proper phosphate solubilisation pH for bacteria, which is suitable for solubilising TCP, is 7.2 (Malboobi *et al*., 2009).

In 2002, Fasim *et al*. suggested that the bacterial isolates solubilise phosphates only if the medium contains glucose. In the present study, the medium provided for isolating PSB, contained glucose but due to the acidic pH of the soil (5.5) the organic acid production may be blocked. There are several factors related to phosphate solubilisation and those may be nutritional, physiological, or it may be the culture growth conditions (Reyes, 1999).

Generally bacteria are famous for their mineral solubilisation properties. The bacteria such as *Bacillus megaterium*, *Bacillus circulans*, *Bacillus subtilis*, *Bacillus polymyxa*, *Bacillus sircalmous*, *Pseudomonas striata*, and *Enterobacter* could be referred as the most important phosphate solubilising strains (Subbarao, 1988). From the group of bacterial communities in the soil, Igual et al. (2001) had described ectorhizospheric strains from Pseudomonas and Bacilli, and endosymbiotic rhizobia as effective phosphate solubilisers. There are many bacterial (*Pseudomonas and Bacilli*) and fungal strains (*Aspergilla* and *Penicillium*) had been identified as Phosphate Solubilising Microorganism (PSM), by the study conducted by Seema *et al*. in 2013. But in the present study regarding the case of phosphate solubilisation, their performance was poorly established. But based on the type of organisms involved, the degree of phosphate solubilisation can also change.

Soil respiration

The microbial activity in soil was measured by soil respiration method in raw soil and fertiliser added soil for two weeks and the results are shown in Table 4. From the table it is clear that the amount of CO_2 after one week was high and after that it was decreased. It reveals that during the second week period, the growth of the microbes was approached to stationary phase and thus the microbial activity was also reduced. Hence the first week time was considered as optimum growth period for the growth of the microbes (Murray *et al.*, 2000; Bernhardt *et al*., 2006). The addition of fertiliser will enrich the nutrient quality of soil by transforming organic matter into nutrients and which the plants for healthy and productive growth use. A healthy plant usually having a healthy rhizosphere and should be dominated by beneficial microbes.

Table 4 Soil respirations in raw soil and fertilizer added soil

Soil samples	**Amount of carbon mineralised (mg)**	
	I week	**II week**
Raw soil	33.54	27.78
Fertiliser added soil	46.20	22.86

R: S ratio

It is evident that the rhizosphere microflora predominates as compared to non-rhizosphere ones. It was revealed from the table of R: S ratio (Table 5). From the table itself it was obvious that the microbial count was high in fertiliser added rhizosphere compared to that of non-fertiliser added one. The quantitative difference in the microbial population of the rhizosphere from that of non-rhizosphere/general soil is mainly due to root exudates in the rhizosphere region which supports more bacterial growth. R: S ratio gives a clear picture about the rate of microbial interaction with the plant roots. Egamberdiyeva in 2007, reported that the bacterial inoculation has a much better stimulatory effect on plant growth in nutrient deficient soil than in nutrient rich soil. Hence the result is supportive for the present work.

Table 5 The R: S ratio in rhizosphere microflora and non-rhizosphere

Soil Regions	**CFUs ($\times 10^{4/}$g)**	**R: S ratio**
Non–rhizosphere of green-gram plant without fertiliser	38	1.5
Rhizosphere of green-gram plant without fertiliser	58	
Non–rhizosphere of fertiliser added green-gram plant	42	2.7
Rhizosphere of fertiliser added green-gram plant	112	

Conclusion

Soil is not a static matter and it is enriched with the activity of soil microbes and this will essentially support the rhizosphere for nutrient mobilisation and thereby promoting the plant growth. If the soil is rich in

organic matter such as litter and also the soil is having sufficient nutrients including nitrogen, phosphorus and potassium there will be no need of adding any extra fertiliser. But in this study context the soil sample is from an urban area and hence there should be a need of adding additional fertiliser externally. This conclusion is finalised by analysing the physico-chemical characteristics of soil and this is proven by test results such as ammonification, nitrification, etc. The addition of fertiliser increased the microbial activity of rhizosphere as well as nearby soil. Therefore it is evident that fertiliser improved the efficiency of nutrient mobilisation of *Vigna radiata* plant. Fertliser helps to achieve increased development of roots, sufficient water and mineral uptake, proper growth of vegetation and even helps in nitrogen fixation. On the other hand, increased application of these fertilisers will lead to deficiency in micro-nutrients and high rate of water consumption. On that account prior knowledge about the fertiliser application is necessary. In order to achieve a sustainable agricultural system, try to nourish the soil in a natural way.

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