

IMPACT OF ECO-FRIENDLY SOIL MICROBE ON *MACROTYLOMA UNIFLORUM* (LAM.) VERDC: A MEDICINAL PULSE.

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ABSTRACT

The present study was done with the chief objective of enhancing the cultivation and growth of *Macrotyloma uniflorum*, (Lam.) Verdc. a medicinal pulse belongs to fabaceae and one of the chief source important proteins. The whole experiment was done in the light of macro and micronutrients analysis of for pot experiment. Potassium (K) was found in greater amount whereas phosphorous (P) was found as a limiting factor than their recommended value. So, *Paenibacillus polymyxa* was selected to dissolve the nutrients into their organic form. The results were observed in 0, 10 and 25 days, reflected in comparison to control, treatments with used plant growth promoting bacterium (PGPB) ultimately increases root, shoot, total lengths and no. of leaves in plant. Moreover, such improved results have been found in the measurement taken after 10 days and these more enhancement after 25 days respectively. Furthermore, it can be concluded that use of *P. Polymyxa* can enhance growth and development of *M. uniflorum* to meet the current demands.

Keywords: *Macrotyloma*, PGPB, macro and micro nutrients, medicinal, pulse etc.

INTRODUCTION

Pulses can serve as a good source of dietary protein constituents for human consumption; it is rich source of protein in our diet. Pulses intake fulfill the protein requirement specially those people who does not depend on animal or fish sources. Various types of pulses are used in our diet. Horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.] is one of the important early domesticated and enriched nutritious crops cultivated mainly in tropical countries in a post-rainy season¹, Horse gram (*M. uniflorum*) vern.

Kulthi belongs to Fabaceae. It is an herbaceous plant with annual branches, sub-erect or twining, leaflets of 2.5 to 5 cm. Its seed is 6 to 8 mm long and 3 to 4 mm broad. The seeds are trapezoidal oblong or somewhat rounded in shape and pale to dark reddish brown or orange brown or all black in colour. Moreover, this might play a handful role in developing countries such as in India and fulfill nutritional security in rural and tribal areas²

Classification/taxonomical position

Kingdom : Plantae
Class : Dicotyledons
Sub class : Polypetalae

Order : Rosales
Family : Fabaceae
Genus : *Macrotyloma*
Species : *uniflorum*

Figure 1
Impact of P. polymyxa on M. uniflorum (Different aspects)



The cultivation of aforesaid pulse is cultivated mostly in Tamilnadu, Andhrapradesh and Chennai. Traditionally, it has been widely used in the treatment of kidney stones, inflamed joints, fever, musculoskeletal disorders, sinus wounds and localized abdominal tumors. This is one of the unutilized legumes due to difficulty in its cultivation³. The difficulty might be due to the odd climatic conditions, also comes under stressed one. These uneven things can be overcome with a group of beneficial microbes called as plant growth promoting rhizobacteria⁴. These bacteria enhance the plant growth either directly or indirectly⁵. In Direct mechanisms elicit growth promotion by microorganism determinants, together with production of plant hormones like IAA, GA and phosphate solubilization^{5, 6}, and in other way it indirectly suppresses harmful microorganisms and elicited general resistance (ISR)⁷. In the soil, heterogeneously distributed microbe communities together with dependent rhizobia and different plant growth promoting rhizobacteria perform a dynamic role in plant nutrition by remodeling nutrients in soils that square measure useful to plant growth through a method referred to as biogeochemical athletics, and directly transporting these nutrients to plants. Soil testing is one of the most important instruments for analysis of macro and micro nutrient. The odd availability of nutrients also makes it interesting. It is also cultivated as cover crop to maintain soil fertility in dry land⁸. Some important macro and

micronutrients are P, K, Zn, Cu, Fe and sulphur etc. Phosphorous and Potassium are most important nutrients. In general, phosphorous will be in lower amount compared to potassium which is higher in crop plants. The plant growth promoting bacteria (PGPB) solubilizes the inorganic phosphorous which reduces the efficacy of potassium. However, phosphate solubilization activity by plant growth promoting rhizobacteria is more when compared to potash solubilizing activity. Herein, the present study was done with special reference to soil analysis. The presence of K in greater amount forced to select the genera *P. Polymyxa*, a good source of potassium solubilization. The experimented strain was also found to be excellent solubilizer of phosphorous, prominent macronutrient for plant growth. After inoculum preparation, according to CLSI recommendations, seeds of *M. uniflorum* were treated. Moreover, after excellent growth promotion, antioxidant activity was measured which was also found interesting. Further recommendations based on present study for sugar alternate in the form of *Macrotyloma* plant can be made.

MATERIALS AND METHODS

Soil sampling and site of Experiment conduction

Soil samples for these experiments were collected from the ROXBURGH GARDEN, Department of Botany, University of Allahabad by digging depth

about 5 cm. All experiments were conducted in the Biological Product Lab.

Soil analysis

Soil is best nutrient medium for plant growth. The collected soil was followed by cleaning and removing the unwanted debris such as stones, glass pieces, polythene etc. Then, it was air dried, sieved and kept for overnight desiccation. Some amount of the soil samples were sterilized by autoclaving at 121°C, 15 lbs pressure for 15 minutes. The collected soil was prepared and given at the Indian Farmer Fertilizer cooperative Ltd., (IFFCO) Phulpur, Allahabad for macro and micronutrient analysis.

Procurement of PGPB culture, plant seeds and inoculum preparation

The selected culture, *Paenibacillus polymyxa* was procured from Microbial type culture collection (MTCC-9489), Chandigarh, India and the plant *M. uniflorum* (Lam.) Verdc seed from Gullu Mal Girdhari lal, Gumat Bajar, Jammu, J&K, India. Seeds were surface sterilized with 0.02% (w/v) HgCl₂ and washed three to five times with sterile distilled water to remove traces of HgCl₂. Inoculum was prepared according to CLSI recommendations⁹.

Biochemical characterizations

Phosphate solubilisation Test

P solubilising activity was observed in Pikovskaya plates¹⁰. The components of aforesaid medium (in gms/ltr) with 0.5 yeast extract, 10.00 dextrose, 5.00 calcium phosphate, 0.5 ammonium sulphate, 0.2 potassium chloride, 0.1 magnesium sulphate, 0.0001 manganese sulphate, 0.0001 ferrous sulphate, 15.00 agar were taken in 1000 ml deionised water. Freshly prepared medium was autoclaved at 121°C and 15 lbs pressure for 15 minutes. Then, streaked plates were kept in BOD at temp of 35±2 for 24 hrs.

Indole acetic acid (IAA) Test

IAA was measured by inoculating the 24hrs old culture in pre-sterilized Peptone broth containing 1 % of trypton for 48 hrs at 37°C. Then after, 1 ml of Kovac's reagent to the culture tube of *P. polymyxa* and shake after 15 minute. The appearance of red ring at the top is confirmed as indication of IAA.

Potassium solubilising activity

The potassium solubilization activity was observed in modified Aleksandrov medium plates by the spot test method¹¹ was autoclaved at 121°C and 15 lbs pressure for 15 minutes.

Siderophore production

Siderophore production was checked on solid CAS universal blue agar plates¹². Actively growing cultures were spot inoculated on the CAS blue agar plate and incubated at 30°C for 48 h. Formation of yellow-orange halo zone around the colony indicated production and release of the siderophores on the agar plate.

Experimental setup

The seeds were washed with detergent and sown in pots which were already filled with autoclaved soil, according to the agronomic practices to determine the effect of *P. polymyxa* on *M. uniflorum*. The pods were filled with the autoclaved soil and were performed in triplicate. The dose of *P. polymyxa* was 50 ml per pods. The seeds were sown directly from seed beds and placed in dark place at room temperature. After germination of seeds the treatment of *P. polymyxa* was given as a proper dose and then put the pods in the light as a ratio of 16:8 hrs. The observations were made in three parts such as same day of sowing, after 10 days and finally after 25 days. The parameters of the observation which were taken: Shoot length, root length, total length, no of leaves and size of leaves. Readings were also taken in triplicates.

RESULTS AND DISCUSSION

Soil analysis

The presence of macronutrient in soil samples along with their fertilizer recommendations were made by IFFCO, Phulpur, Allahabad. According to the reports, available potassium (K) in used soil was 269 kg/hectare, greater than its original recommendations whereas *i.e.* phosphorous (P) was 15 kg/hectare, just half of its recommendations. The odd availability of the nutrients might be due to the occurrence of insoluble and non usable form of potassium as well as phosphorous. Moreover, micronutrients analysis states that the availability of copper (1.74 ppm), manganese (4.32 ppm) and zinc (1.34 ppm) were higher contradicting to iron (6.80 ppm) and sulphur (13.00 ppm) which was lesser than their original

recommended concentration as per hectare. The selection of *P. polymyxa* for experimentation was also due to its biochemical activities¹⁴.

Biochemical analysis of *P. polymyxa*

Phosphorous (P) is one of the most important macronutrient present in the soil which is required for the development of crops¹⁰. Its odd availability also makes it important when compared to most abundant

elements in environment. *P. polymyxa* was found to be a very good phosphate solubilizer (Table-1). The solubility of inorganic phosphate is considered as a result of release of some low molecular weight organic acids by the experimented strain. These organic acids convert the inorganic phosphate into soluble forms that can be easily available to the plants.

Table 1
Biochemical characteristics of *P. polymyxa*

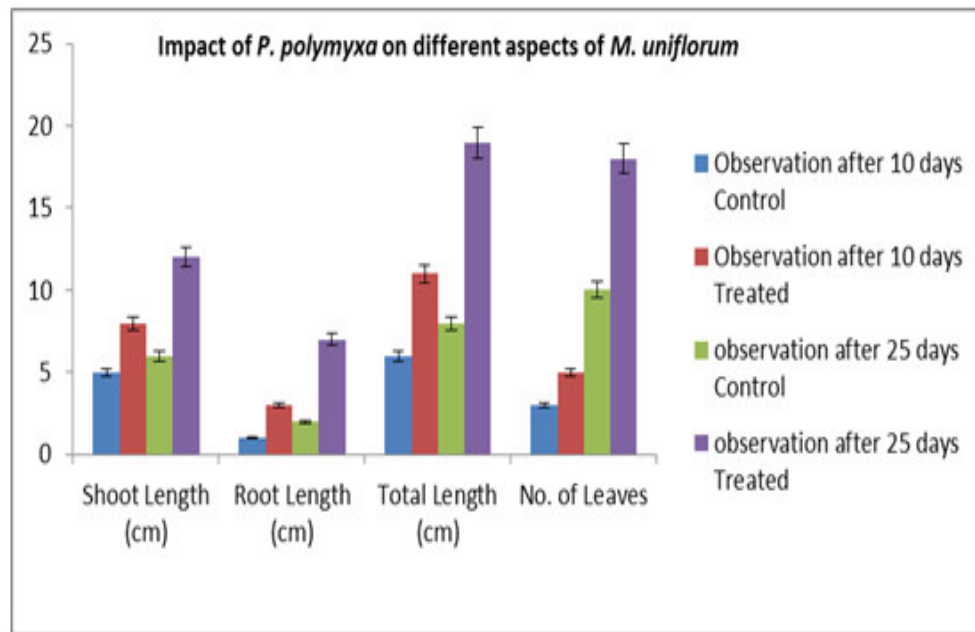
S. No.	Characteristics/Tests	Results
1.	Phosphate Solubilizing test	+
2.	IAA Production	+
3.	Potassium Solubilization test	+
4.	Siderophore production test	+

For the constant growth of plants, hormones are playing a vital role such as IAA. The used bacterium was IAA producing agent (Table-1). The availability of precursors and uptake of microbial IAA by plant are the two main factors that affect the ability of bacteria to produce IAA in the rhizosphere. The causal mechanism of growth promotion may as production of plant growth promoting hormones in the rhizosphere and other plant growth promoting activities. *P. polymyxa* is also a very good Potassium (K) solubilizer which is absorbed in the form of cation. More than 90% of potassium exists in silicate minerals and insoluble rocks¹³. On the basis of halo zones, on solid CAS universal blue agar plates, experimented bacterium was found positive. Iron deficiency made the conditions for cultivation very tough but these PGPB makes the respective plant efficient to combat aforesaid stress situation.

Effect of *P. polymyxa* on the growth of *M. uniflorum*

M. uniflorum, a highly medicinal plant majorly found in the hilly region but cultivation in the plain areas is a little challenging task. The observations were made

as a measurement of 4 growth parameters such as Shoot length, root length, total length and no. of leaves in the interval of zero days, ten (10) days and twenty five (25) days in the form of triplicates (Figure-1). In the same day made no significant change. Next were observed after 10 days and found the increased growth in the all parameters as compared to control (Figure-1). These parameters are clearly giving the signal of positive growth shown reflected by the plant after treatment by *M. uniflorum* might be due to the increased production of hormones especially auxin¹⁵. The final measurement shows excellent growth and development in each treatment. A good amount of enhancement was obtained and all differences were found to be just double as shoot length was found 12 but 6 cm in control, root length was 5 cm greater than control, and so on in case of total length (Figure-1). It was also noteworthy that the enhancement in root and shoot length is due to the production of phytohormones¹⁵ as well as more nutrients were absorbed from rhizosphere of roots as induced by *P. Polymyxa* which is a good P and K solubilizer.



Leaves no also increases parallel with increase in shoot length. This increased growth in shoot length and no. of leaves led to the maximum photosynthesis resulting in formation of ample food which is essential for plant growth¹⁶. Our findings also reveal that use of *P. polymyxa* can increase the cultivation of *M. uniflorum*. Finally it was found that there was significant change in the growth of treated plant as compared with control ones. Approximately 72.8% increase in the growth of treated plant had been seen; while there was 50 % and 133% growth increase had been seen in shoot and root length respectively.

CONCLUSION

Horse gram is one of the most important pulses growing in the dry regions of the country. Its cultivation is tough due to odd availability of arable soil and climatic conditions ultimately creating the situation of stress. In the present study, it was clearly shown the possible ample cultivation by using *P.*

polymyxa. The application of aforementioned soil bacterium made effective improvement in the growth of the plant, as a result of excellent plant nutrient uptake. *P. polymyxa* also plays an important role in root colonization which results in improved nutritional balance increasing plant length below and above the soil. In addition, the use of *P. polymyxa* as biofertilizer has also shown the possible ways for the replacement of chemical fertilizers. In the similar manner, it can solve the foremost global problem of environmental pollution and also fetch a premium in the agricultural market.

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There is no fund granted for present study.

Competing Interests

The authors declare no competing interest.

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