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Studies on Pomegranate Rhizobacteria for Phosphate Solubilization

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ABSTRACT:

Plant root secretions influence soil microorganisms. The region of the soil that is immediately surrounds the plant root and associated microorganisms is called as known as rhizosphere. There is presence of heterogeneous rhizobacteria which are referred as Plant Growth Promoting Rhizobacteria (PGPR). The bacteria are known for their plant growth promoting activities by synthesis of macronutrient necessary for plant growth. The deficiency of which restricts crop yield. The bacteria from rhizosphere of pomegranate (***Punica granatum***) were isolated and screened in vitro for their potential to solubilize phosphate. Totally 40 bacterial isolates were obtained from the rhizospheric soil samples of the plant collected from different regions of Beed district. The screening was performed on Pikowaskaya's agar plates. Out of 40 bacterial isolates, 11 isolates showing phosphate solubilization activity were screened out.

KEY WORDS- PGPR, Pomegranate plant, Rhizospheric Soil, Pikovaskaya's agar, Phosphate solubilization.

INTRODUCTION:

The demand for food is rapidly increasing all over the world. In has potential to meet the demand as agriculture is the main practice in India and can satisfy ever increasing demand of growing population of food and including fruits. Importantly pomegranate (***Punica granatum***) is a fruit crop from arid and semiarid regions. The plant is believed to have originated from Iran and Turkmenistan (Ram Chandra *et al* 2010). In the world, India is one of the leading producer of pomegranate. (R.K. Pal *et al* 2014). Out of total pomegranate production in India, contribution of Maharashtra is 90 % (Rede Ganeshkumar, 2021)

The agriculture practice in India is giving emphasis on sustainable production system. The ecofriendly influence of the rhizosphere microorganisms on plants is considered as important tool to protect the health of plants. (Govind Gupta *et al*. 2015). Phosphorus, one of the macronutrient, required by plant growth and is a part of basic biological activities such as cell division, nucleic acids synthesis, photosynthesis and respiration, energy transfer and in the formation of oils,

nucleic acids and starches. Phosphate content of the soil naturally is 100–1000 g/acre but this is uavailable for plants to uptake and consequently severely affects crop productivity (Awasthi et al., 2011).

Phosphate solubilizing rhizobacteria play vital role in accumulation, transformation and availability of phosphate to plant roots. Phosphorus is absorbed mostly during vegetative growth. This absorbed form of phosphorus is then located in seeds and fruits (Eftekhari *et al*., 2010).

Sustainable crop development can be achieved with microbe mediated management of phosphorus and that may be considered as an eco-friendly and cost effective approach in India which has about 98% of phosphorus deficient crop land (Krishna and Shalini, 2019).

Present study was aimed to isolate and screened the phosphate solubilizing rhizobacteria from pomegranate rhizosphere.

MATERIAL AND METHODS:

Collection of Soil Samples-

In the present investigation, totally 15 pomegranate rhizospheric soil samples were collected from *Shivni*, *Aherwadgaon* and *Kajala* regions of Beed district for isolation of rhizobacteria of the pomegranate plant. In the rhizosphere a pit was dug using a pointing trowel. Soil samples were collected by scrapping the sides of the pomegranate roots. Approximately 10-15 g of rhizospheric soil samples were collected. Sterile containers were used to for the collection. The samples were transported to the laboratory for isolation of rhizospheric bacteria.

ISOLATION OF BACTERIA-

To each flask containing 99 ml of saline water 1 gm of each rhizospheric soil sample was added. To obtain isolated colonies further tenfold serial dilutions were made up to 10^{-5}. Then 0.1 ml of the dilutions 10^{-4} and 10^{-5} were inoculated on nutrient agar surface by spread plate technique. For each dilution the inoculations were made in triplet. The plates were then incubated at 30 ^{o}C for 24 hours in an incubator. On incubation the plates were observed for isolated bacterial colonies. The isolates from the plates were labeled on the basis of initials of the sites of collection. Analysis was made for the plant growth promoting activity. Pure cultures of the isolates were maintained by inoculation on nutrient agar slants and kept at 4^{0}C in for next studies.

In-vitro screening of bacterial isolates for Phosphate Solubilization

Phosphate solubilization capacity of the bacterial isolates was tested by using Pikovskaya's (PKO) medium (Zhonghua Wang *et al*, 2022). The medium contained the following components (g/L): Glucose 10.0 g, $Ca_3(PO_4)$ 25 g, $(NH_4)_2SO_4$, 0.5 g, NaCl 0.2 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, KCl 0,2 g; Yeast extract 0.5 g, $MnSO_4 \cdot H_2O$ 0.002 g, $FeSO_4 \cdot 7H_2O$ 0.002 g and distilled water 1000 ml. The medium was sterilized by autoclaving at 110 ^{o}C for 40

minutes. On sterilization the medium was mixed well and poured to petri dishes. For screening the bacterial isolates were spot inoculated on these agar plates. The plates were then incubated at 30 °c temp. for 48 to 72 hours. After the incubation period, the plates were observed for the presence of clear zone of various diameters around the colonies those indicate the extent of Phosphate solubilization.

RESULTS:

Isolation of rhizospheric bacteria

Under the investigation, a total of 40 isolates were isolated from the soil samples collected from 3 different places of Beed district. The number of isolates form respective place are as *Shivni* (16 isolates), *Aherwadgaon* (11 isolates) and *Kajala* (13 isolates).

Fig.1. Bacterial isolates from rhizosphere of pomegranate plant.

In-vitro screening of bacterial isolates for phosphate solubilization

Total 40 bacterial isolates were subjected for the screening. Among the isolates, phosphate solubilization activity was shown by 11 isolates. The isolates screened out from different soil samples collected on the basis of their phosphate solubilization activity are as Shivni-4 isolates (S4, S13, S15, S16), Kajala 4 isolates (K1, K4, K7, K9) and Aherwadgaon 3 isolates (A3, A4, A8). were screened for phosphate solubilizing activity.

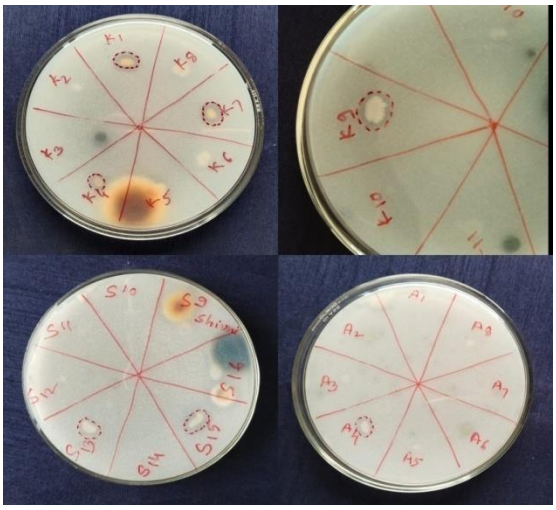

Fig.2. Screened out bacterial isolates from rhizosphere of pomegranate plant showing phosphate solubilization activity

DISCUSSION

Unrecoverable environmental pollution and many health problems are caused by intensive use of inorganic fertilizers (Chandini *et al*, 2019). Soil health is affected by the disproportionate use of chemical fertilizers in agriculture field which also undesirable effect on health of soil, environment, and economy. Phosphate solubilizing rhizobacteria play an important role in solubilizing organic

phosphorus into inorganic form that becomes available for plant uptake without disrupting the agro-ecosystem and environment. Further studies will be made on optimization and application of the phosphate solubilizing bacterial isolates.

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