International Journal of Biotechnology and Microbiology
Online ISSN: 2664-7680; Print ISSN: 2664-7672; Impact Factor: RJIF 5.46
Received: 18-01-2021; Accepted: 02-02-2021; Published: 18-03-2021
www.biotechnologyjournals.com
Volume 3; Issue 1; 2021; Page No. 70-73

An *In vitro* effect of agrochemicals on the growth of plant growth promoting rhizobacteria isolated from the soil

Sajid Shaikh[1], BS Naphade[2*]
[1] Department of Microbiology, New Arts, Commerce and Science College, Ahmednagar, Maharashtra, India
[2] Department of Microbiology, Badrinarayan Barwale Mahavidyalaya, Jalna, Maharashtra, India

Abstract
In the present study effect of four commonly used agrochemicals (2, 4-Dichlorophenoxyacetic acid, Diammonium phosphate, Nitrogen-Phosphorus-Potassium Fertilizer and Urea) was studied on the growth of ten previously isolated PGPR. The herbicide 2, 4- D inhibited the growth of isolate-7 completely at 0.01%. On the other hand isolate-5, 8, 9 and 10 showed growth up to 1% concentration. Isolate 4 was the most tolerant to DAP followed by isolate-5 and isolate-10, Isolate 4 tolerated up to 4% of DAP, While isolate-5 and 10 tolerated 3% DAP. Low concentration was beneficial for the growth of all isolates but growth was inhibited with the increase in concentration of urea. Most of the isolates were inhibited at 4% urea except isolate-10. it was able to grow even at 5% urea. NPK also initially enhance the growth of all isolated but higher concentrations were inhibitory for all isolates. Most tolerant isolates to urea were 4, 7 and 10. They survived up to 3% concentration. Among all isolate-10 was most resistant to all agrochemicals studied.

Keywords: PGPR, DAP, NPK, 2, 4-D, Urea

Introduction
Microorganisms play an important role in growth and development of plants. Bacteria which can enhance the plant growth and yield via various plant growth promoting substances are called as plant growth promoting bacteria. Plant Growth Promoting Rhizobacteria (PGPR) are group plant growth promoting bacteria which resides in the rhizosphere area. They use different mechanisms for plant growth promotion. It includes nitrogen fixation, phosphate solubilzation, phytohormone production, antibiotic production, siderophore production, hydrogen cyanide, ammonia production, hydrolytic enzyme production, ammonia production, systemic induced resistance etc. (Kloepper *et al*., 1989; Glick *et al*., 1999) [5, 9]. Plant growth promoting rhizobacteria can be classified into extracellular plant growth promoting rhizobacteria (ePGPR) (Clarke and Cowan, 1952) and intracellular plant growth promoting rhizobacteria (Cheryl *et al*, 2002, Pandey and Maheshwari, 2007) [2]. Effect of PGPR on growth and yield under invitro as well field study has been well studied. But extensive use of agrochemicals like pesticide and fertilizers had changed the microenvironment of the soil. Which is not suitable for the survival and growth of PGPR. These agrochemical not only inhibits the growth of PGPR but also interfere with the plant growth promoting abilities. Agrochemicals have increasing demand for protection of crops. (B.J. Bhadbhade, S.S. Sarnaik and P.P. Kanekar, 2002) [1]. With the increased use of chemical fertilizers there exists as increased the risk of accumulation of chemical fertilizers in soil and may leads to infertility of soil (V. Thamgapandian *et al.*, 2007, Gordon, 1951) [4].
In the present study effect of selected agrochemicals-2,4D (2, 4-Dichlorophenoxyacetic acid), DAP (Diammonium phosphate), NPK (Nitrogen-Phosphorus-Potassium Fertilizer) and urea was studied on previously isolated 10 PGPR bacteria from soil.

Materials and Method
Effect of 2, 4 D on growth of isolates
Effect of 2, 4-D on isolates was studied. For this nutrient broth tubes with different concentration of 2, 4-D were prepared (0%, 0.01%, 0.05%, 0. 1%, 0.5% and 1%). Each tube was then inoculated with 0.1ml of 24 hours old culture of all isolates separately. Uninoculated tubes of each concentration was kept uninoculated as negative control. The tubes were then incubated for 24 hours at 30°C. After incubation the tube were shaken to make uniform suspension if visible growth was observed. Absorbance of each inoculated tube was taken against respective negative control at 600nm using UV-Visible spectrophotometer.

Effect of DAP on growth of isolates
For this nutrient broth tubes with different concentration of DAP were prepared (0%, 01%, 0.2%, 0. 3%, 0.4%, 0. 5%, 0. 6%, 0. 7%, 0. 8%, 0. 9%, 1%, 2%, 3%, 4% and 5%). Each tube was then inoculated with 0.1ml of 24 hours old culture of all isolates separately. Uninoculated tubes of each concentration was kept uninoculated as negative control. The tubes were then incubated for 24 hours at 30°C. After incubation the tube were shaken to make uniform suspension if visible growth was observed. Absorbance of each inoculated tube was taken against respective negative control at 600nm using UV-Visible spectrophotometer.

Effect of NPK (19:19:19) on growth of isolates

For this nutrient broth tubes with different concentration of NPK were prepared (0%, 01%, 0.2%, 0. 3%, 0.4%, 0. 5%, 0. 6%, 0. 7%, 0. 8%, 0. 9%, 1%, 2%, 3%, 4% and 5%). Each tube was then inoculated with 0.1ml of 24 hours old culture of all isolates separately. Uninoculated tubes of each concentration was kept uninoculated as negative control. The tubes were then incubated for 24 hours at 30ºC. After incubation the tube were shaken to make uniform suspension if visible growth was observed. Absorbance of each inoculated tube was taken against respective negative control at 600nm using UV-Visible spectrophotometer.

Effect of Urea on growth of isolates

For this nutrient broth tubes with different concentration of urea fertilizer were prepared (0%, 1%, 2%, 3%, 4% and 5%). Each tube was then inoculated with 0.1ml of 24 hours old culture of all isolates separately. Uninoculated tubes of each pH was kept uninoculated as negative control. The tubes were then incubated for 24 hours at 30ºC. After incubation the tube were shaken to make uniform suspension if visible growth was observed. Absorbance of each inoculated tube was taken against respective negative control at 600nm using UV-Visible spectrophotometer.

Result and Discussion

Effect of agrochemicals on growth of isolates

Effect of 2, 4 D on growth of isolates

2, 4-D is commonly used agrochemical in agricultural fields. It is an herbicide. When bacterial isolates were grown in different concentration of 2, 4-D, variable results were observed. The growth of isolate-7 was completely inhibited at 0.01% of 2, 4 D. it was the most sensitive isolate to the herbicide. The growth of isolate-2 and 3 was very poor at this concentration. Their growth was completely inhibited with little increase of 2, 4 –D (0.05%). The isolate-6 was inhibited at 0.1% and isolate-4 was inhibited at 0.5%. Isolate tolerated up to 5% concentration. However it was inhibited at 1%. Only four bacteria that is isolate-5, 8, 9 and 10 showed growth up to 1% concentration.

Effect of DAP on the growth of isolates

DAP is a water soluble the chemical fertilizer. It is the source of nitrogen and phosphorous. It contains about 18% of the nitrogen and 46% of the phosphorous. Effect of different concentrations of DAP on isolates was studied. At initial concentrations DAP exerted a positive effect on the growth of isolates. But after a certain concentration, which varied with the type of isolate, it reduced the growth. The optimum concentration of DAP ranged from 0.4-0.7%. The optimum concentration of isolate-1, 6 and 7 was 0.4%. Isolate-2, 3, 4, 5 and 9 showed maximum growth at 0.5% of DAP. Isolate-8 and 10 preferred little higher concentration that is 0.7%. The growth of isolate-1, 2 and 3 was completely inhibited at 2% concentration. Isolate-6, 7, 8 and 9 were inhibited at 3% of DAP. 4% DAP inhibited isolate-5 and 10 but were able to resist 3%. Isolate 4 was the most tolerant. It showed slow growth at 4%, but was inhibited at 5% of DAP.

Effect of Urea on growth of isolates

Urea is commonly used as source of nitrogen for crops. The urea also exerted positive effect on the growth of isolates initially. All the isolates showed maximum growth with 1% of urea in the media except isoalte-10. It showed maximum growth at 2% of urea. All the isolate resisted 3% urea but at isolate-2, 3, 6 and 7 were inhibited at 4% of urea. Remaining isolate except isolate-10 were inhibited at 5% concentration. Isolate-10 was the only isolate which shown growth at 5 % of urea.

Effect of NPK (19:19:19)

NPK fertilizers are also very commonly used in agriculture. This study showed that NKP also enhanced the growth of isolates at certain concentration. After a particular concentration it reduced the growth. The optimum concentration ranged between 0.4 to 0.6 % of NPK. Isolate-2 and 3 resisted only up to 0.9% of NPK. At 1% their growth was completely inhibited. Maximally tolerable concentration for isolate-6, 7 and 9 was 1%. Isolate-1 and 5 resisted little higher concentration that is 2%. But the most tolerant were 4, 7 and 10. None of the isolate resisted concentration above 3%

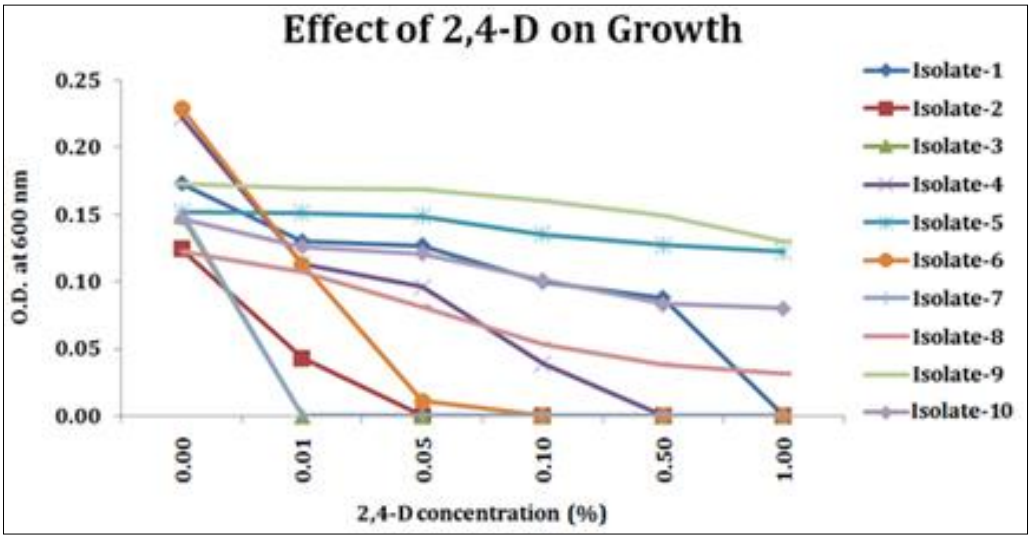

Fig 1

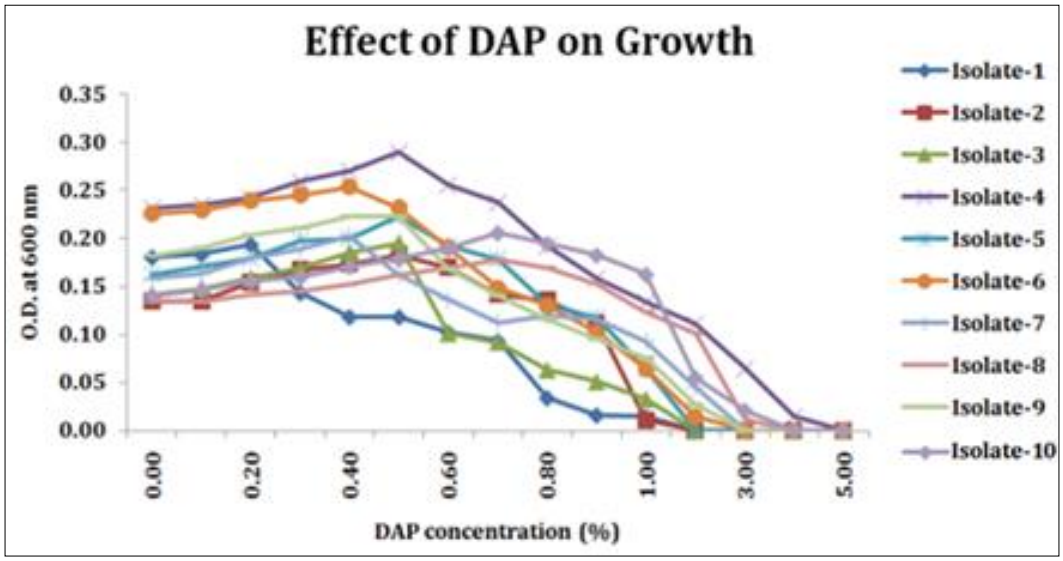

Fig 2

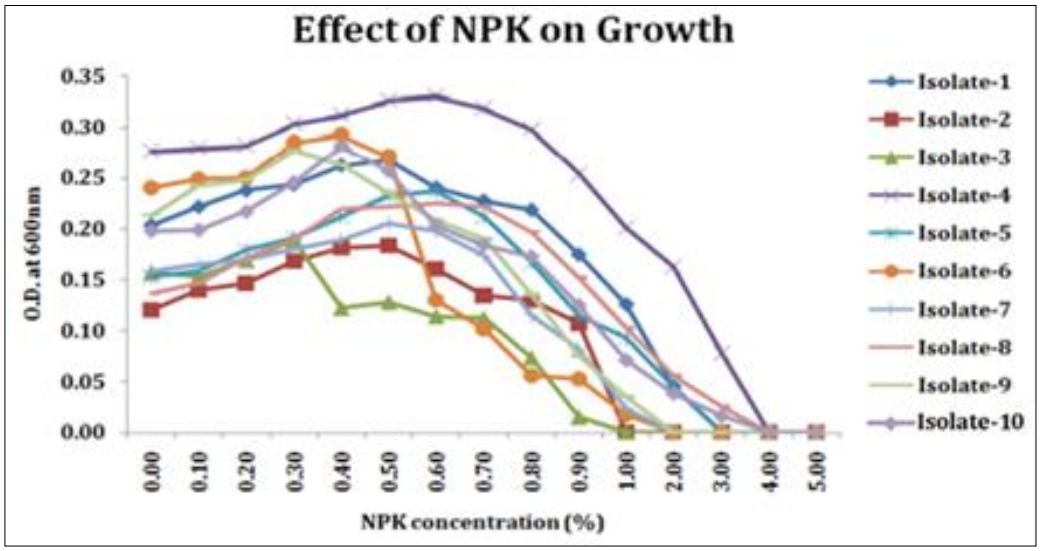

Fig 3

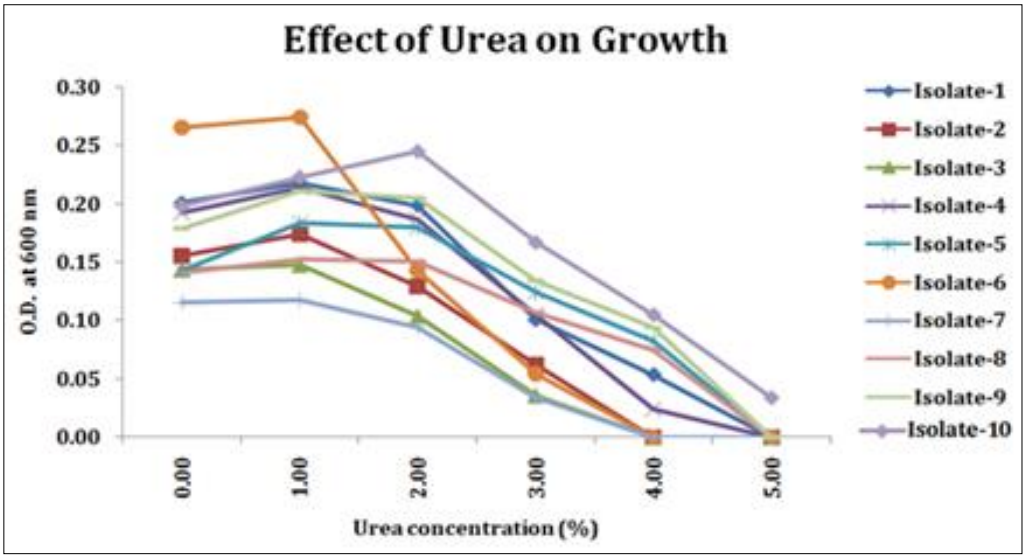

Fig 4

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