TO EVALUATE THE EFFECT OF BIOFERTILIZER ON PLANT GROWTH PARAMETER OF TRITICUM AESTIVUM (WHEAT)

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Abstract
The term biofertilizer or called 'microbial inoculants'/fertilizer can be generally defined as a preparation containing live or latent cells of efficient strains capable of nitrogen fixation, phosphate solubilization which are used for application of seed, soil with the objective of increasing the numbers of such microorganisms and accelerate certain microbial process to enhance the degree of the availability of nutrients to plant. Increasing cost of chemical fertilizers is unaffordable by small and marginal farmers. There is found depletion in soil fertility due to widening gap between nutrient removal and supplies. By considering the threat to sustainable agriculture by the use of chemical fertilizers and human activity there is growing concern about environmental hazard management. Besides above facts, the long term use of organic fertilizers is economical, eco-friendly, more efficient, productive and accessible to marginal and small farmers over chemical fertilizers.
Keyword : [Microbial inoculants, Plant growth promotion, rhizosphere, eco-friendly]

1. Introduction :

The excess application of chemical fertilizer will lead to higher chances minerals to be lost and pollute the environment. Bacterial bio-fertilizer contains soil microorganisms such as bacteria, algae or fungi that increase the uptake of mineral nutrients in the plant. Biological control using microbes is an effective and environmentally friendly strategy for controlling soil-borne fungal pathogens and promoting plant growth (Bargabus RL *et al*., 2003 and Tjamos SE. *et al.*, 2005). To increase production and productivity, maintain soil health, reduce nutrient losses, improve soil environment and minimize energy consumption, it is necessary to use bio-fertilizers. Bio-fertilizers also help in fixing atmospheric nitrogen, dissolve soil phosphorus and stimulate plant growth through synthesis of growth promoting substances. The cultured microorganisms packed in some carrier material for easy application in the field are called biofertilizers. Bio-fertilizers are living microorganisms of bacterial, fungal and algae origin. Biofertilizer can provide an economically viable support to small and marginal farmers for realizing the ultimate goal of increasing productivity.

In the soil with poor agricultural activity, the use of PGPR assumes significant importance because they adapt to diverse environmental conditions, like drought stress (Arshad, M. *et al*., 2008; Arzanesh, MH. *et al*., 2011), salt stress (Mayak, S. *et al*., 2004), high temperatures, dryness or heavy rainfall in tropical countries (Da Mota, FF. *et al*., 2008) and contaminated environments (Burd, GI. *et al*., 2000; Gupta, A. *et al*., 2002; Dell'Amico, E. *et al*., 2008). Although the farmers are now awaking about the importance of biofertilizers uses but still there is unawareness amongst the farmers about the facts regarding utilization, storage and handling of the product. The paramount need of agriculture sector, is to come up with the user friendly and ecofriendly solution. Thus, biofertilizer developed during present investigation could prove to be ecoferiendly, cost effective and could be effectively used in agriculture practices for sustainable agriculture approach. In the present research work, the attempt was made to develop effective bacterial based fertilizer, which have plant growth promotional ability.

2. Material and Method :

2.1. Sampling sites and collection of soil samples

As the study aimed at isolation of Pseudomonas species for the preparation of the biofertilizer and related bioassays, on the basis of thorough analysis of literature review cultivated lands with growing crops were selected, for probable isolation of *Pseudomonas* species. While sampling, undamaged roots and nearby adhered soil samples were collected from the plant's rhizospheric region.

2.2. Isolation of Pseudomonas species

Collected soil samples were processed further for serial dilution by suspending its 1gram (g) into 100 milliliter (ml) sterile distilled water and kept on rotating shaker for 24 hours (hr). Mixing by rotation ensured release of soil from roots and also the associated bacterial population in water. One ml of the sample was further diluted in 10^{-1} to 10^{-6} times and 100 micro liter (µl) of the sample was spread plated on already prepared King's medium B (KMB) agar plate (selective media for *Pseudomonas* species). Plates were incubated at 28 degree celsius (ºC) for 48hr for the development of the colonies.

2.3.Characterization of isolates

Preliminary identified fluorescent *Pseudomonas* species confirmed for their morphological, microbiological and biochemical characters by following standards of Bergey's Manual of Systematic Bacteriology.

2.4. *In vitro* screening of isolate for Plant Growth Promoting activity

2.4.1. Indol acetic acid (IAA) production assay

Ability of IAA production by the *Pseudomonas* species under *in vitro* conditions was tested. Bacterial species were grown for 48 hr Nutrient agar media at 28 °C and inoculated in 250ml Erlenmeyer flasks containing 100 ml of Nutrient broth and kept incubated in the dark at 28±2 °C for 4 days on a shaker. The production of IAA was determined by Salkowski's reagent method (Patten, C.L. and B.R. Glick. 2002).

2.4.2. Phosphate solubilisation

The *Pseudomonas* isolates were qualitatively tested for phosphate solubilization on Pikovskaya's agar plates. The fresh overnight grown test culture broth of volume 50 µl were separately inoculated on Pikovskaya's agar plates and observed for the clear zone after an incubation period of four days at 28°C. Phosphate solubilization zone observed on the plate was measured in mm.

2.5. Method of Preparation of Bio- fertilizer by using isolated Pseudomonas bacteria

By thorough analysis of the isolated Pseudomonas bacteria through microbiological, biochemical and molecular profiling as well as for its plant growth promotional activity, the said bacterium finally selected for biofertilizer preparation which is called as bacterial fertilizer or biofertilizer. Freshly grown Pseudomonas bacteria culture were inoculated in 500ml conical flask containing Nutrient broth amended with 2% glycerol. This flask was kept on rotary shaker for 48 hrs and then used as biofertilizer.

2.6. Green house study of prepared bacterial based fertilizer on Triticum aestivum (Wheat)

To check the efficacy of above said developed biofertilizers on the plants Triticum aestivum (Wheat), the green house study was performed. A pot culture experiment was conducted to study the effect of developed fertilizers on the germination percentage, growth and development of plants namely Triticum aestivum (Wheat). The experiment was conducted with 3 treatments with 3 plants in each treatment along with un-inoculated control. These three treatments includes ;

a) Un-inoculated control - without any treatment.

b) Three plants was inoculated with Pseudomonas based biofertilizer (5 ml per plant) .

3. Result and Discussion

3.1. Isolation of Pseudomonas strain

The serially diluted (10^{-4}- 10^{-6}) soil sample supernatants when inoculated on the Pseudomonas agar, which is a selective medium for growth of Pseudomonas bacterial sp. According to literature, some Pseudomonas sp. have ability to get fluorescence under ultra violet light which is known as fluorescent Pseudomonas sp. Hence, in the present study, isolated Pseudomonas colonies was observed under ultra violet light in dark and it has been observed that the only few colonies showed fluorescence, which was picked up and use for further experiments. During the course of selection colonies which were Rod shaped with

motile character and rendering Gram negativity were considered for further study [Fig. 1.]

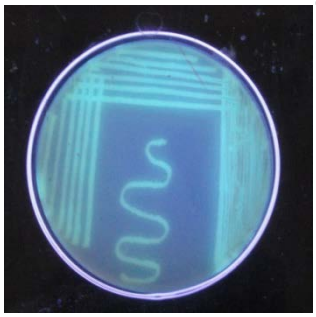


Fig. 1 : Fluorescent Pseudomonas isolate

3.2. Biochemical Characterization

The isolates of fluorescent *Pseudomonas* species have been further characterized biochemically by using standard procedures.

Table 1 : Biochemical Characterization of isolated Pseudomonas bacterium

Sr. No.	Tests	Isolate
1	Oxidase test	+
2	Catalase test	+
3	Gelatin liquefaction	+
4	IAA production	+
5	Phosphate solubilization	+

3.3. Determination of Plant Growth Promoting quality

It was necessary to determine the Plant Growth Promotional activity of the bacteria in order to use it for biofertilizer preparation. Hence the ability of isolate to produce Plant Growth Promotional activity like indol acetic acid and phosphate solubilization was evaluated and the isolate was found to be positive for these activities.

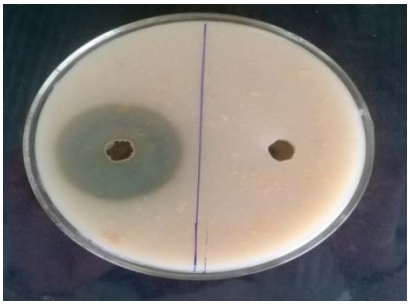


Fig. 2 : Phosphate solubilization by isolated *Pseudomonas strain*

Fig. 3 : Indol Acetic Production by isolated *Pseudomonas strain*

3.4. Preparation of Bio- fertilizer by using isolated *Pseudomonas strain*

The protocol was developed for potential biofertilizer production by using *Pseudomonas* strain. A liquid formulation was

prepared with a addition of certain defined chemical and suitable additive which proved to be effective for a sustaining a shelf life of bacteria.

Fig. 4 – Microbial based fertilizer by using *Pseudomonas* strain

3.5. Prepared Microbial fertilizer based green house study on Triticum aestivum (Wheat)

The prepared biofertilizer was applied at the time of seed sowing and after 15 days interval till harvest. The plants were watered regularly and routein care was taken during study. Likewise, a study was done to prove the usefulness of biofertilizer as compared to control, for its plant growth promoting characteristic. Further testing of liquid biofertilizer on Triticum aestivum (Wheat) was done along with the various parameter study like plant height, number of branches per plant, number of leaves per plant, length of leaf etc. during Green house experiments and finally germination percentage was determined.

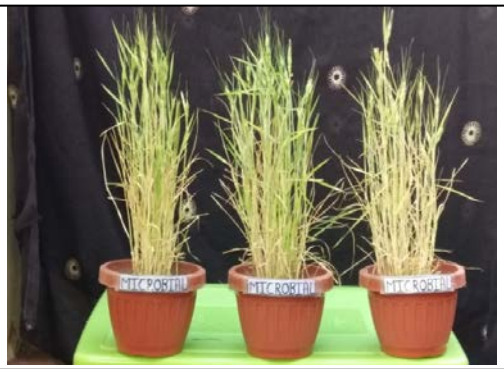
Control plant – 15 days

Microbial fertilizer treated plant – 15 days

Control plant – 30 days

Microbial fertilizer treated plant – 30 days

Control plant – 60 days



Microbial fertilizer treated plant – 60

Plant growth parameters of Triticum aestivum in control and fertilizer treated plant

Control	Shoot Length (cm)	No. of leaves/plants	No. of branches/ plant	Leaf length (cm)	Fresh weight (gm)/plant	Dry wt gm/plant
1	15	40	40	15	2.2	0.5
2	22	45	45	22	2.0	0.7
3	20	52	52	20	2.1	0.6
2. Influence of biofertilizer on plant growth parameters of Triticum aestivum in microbial biofertilizer applied plants						
1	26	75	75	26	2.3	0.6
2	29	70	70	29	2.4	0.7
3	32	72	72	32	2.5	0.7

During the study, isolated bacteria identified by biochemical, morphological and Gram nature was further screened for their potential to become potent agent for biofertilizer development. . According to the Ahmed, *et al.*, 2010 production technology for biofertilizers is relatively simple and installation cost is very low compared to chemical fertilizer plants.

The production of Indole acetic acid and phosphate solubilization ability of the isolate was assessed for their possible use in biofertilizer. The work of Shaharoona, B. *et al*., (2007), were reported that *Pseudomonas* species *Burkholderia caryophylli* was found to posses characteristic like auxin production, P solubilisation, root colonization and hence selected for biofertilizer development. In the present study, the isolated Pseudomonas strain also capable of production of Indole acetic acid and phosphate solubilization, hence use in biofertilizer preparation. The biofertilizer application had stimulated nutrient accumulation and plant growth comparable to the non treated plants (Amir et al., 2003).

Today's agriculture demand is to feed the growing population without disquieting the natural resources of ecosystem. The overuse of pesticides and chemical fertilizers causes toxic impact on production potential and ultimately on consumer of agricultural products. It is also associated with environmental and health problems. Due to decline in soil fertility, the overall agriculture production has gone down which put a questions on the profitability and sustainability of the agriculture system. On the other hand, biofertilizers can be used to **s**timulate plant growth, Activate the soil biologically, Restore natural soil fertility**,** Build up soil fertility in the long term, Cost effective, i.e. reduces the costs toward fertilizers use especially regarding nitrogen and phosphorus, Supplement to fertilizers. They are eco-friendly and pose no damage to the environment.

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