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Potential Use of *Azotobacter* as Biofertilizer and Comparitive Pot Trails in Fenugreek and Mustard Plants

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ABSTRACT

The present study was aimed at isolating bacteria from different soils collected from in and around the city of Hyderabad and few regions and districts from Telangana and Andhra Pradesh states. Most of the soils thus obtained were seem to be supporting agriculture actively. These soil samples thus collected were packeted and brought to the laboratory for further microbiological testing. Characterization and microscopic observation of the culture was done and the bacteria identified were *Streptomyces sp., Staphylococcus sp.,Azotobacter sp., Leptothrix sp., Derxia sp., Bacillus sp., and Sphaerotilus sp.* Of the above isolated organisms *Azotobacter sp.* was selected for pot trials. Pot trails have been conducted on Fenugreek and Mustard plants by soil application of bacteria *Azotobacter* (nitrogen fixing bacteria) and testing them for their applicability as a biofertilizer. Soil inoculation of fenugreek and mustard with *Azotobacter* resulted in higher growth of the crop and is correlated with increased germination and growth of seedlings as compared to without inoculation (control).When the results of the effect of *Azotobacter* on fenugreek and mustard are compared the *Azotobacter* has a slightly greater effect on mustard than compared to fenugreek plants. There by indicating that *Azotobacter* act as a better biofertilizer in both the growth of mustard and fenugreek plants than compared to its respective controls.

Keywords : Biofertilizer, Growth, *Azotobacter*, Fenugreek and Mustard.

I. INTRODUCTION

World agriculture has progressed dramatically as a result of research and development based policy support. After the introduction of chemical fertilizers farmers were happy of getting increase yield in agriculture in the beginning. But slowly chemical fertilizers started displaying their ill effects.[1] Due to these reasons, focus in recent times has been shifted towards the use of inexpensive natural resources such as Plant Growth Promoting Rhizobacteria (PGPR)[2]. The beneficial effects of these rhizobacteria on plant growth can be direct or indirect. A considerable number of bacterial species are able to exert a beneficial effect on plant growth.[3] Among non-nodule forming diazotrophs, *Azotobacter*, a free living nitrogen fixer, discovered and described in 1901 by the Dutch Microbiologist and botanist Martinus Beijerinck, plays an important role in crop improvement by supplying mainly nitrogen (N) to plants.[4] *Azotobacter* promotes plant growth directly by secreting considerable amounts of biologically active substances like B vitamins, nicotinic acid, pentothenic acid, biotin, gibberellic acid, Indole-3 Acetic Acid (IAA) and cytokinin (Ahmad et al., 2005; Lenin and Jayanthi, 2012; Oskar et al., 2014) and ammonia (Narula and Gupta, 1986) or indirectly by protecting the plant from diseases (Saini, 2012).[5] Extensive field experiments in India on *Azotobacter* inoculation of seed or seedling of onion, brinjal, tomato and cabbage under different agro-climate condition founded that its inoculation saves 10-20% nitrogenous fertilizers.[6] So, it is well known fact that bacterial species promotes plant growth.[7] Bacterial strains isolated from the rhizosphere hold great promise as seed inoculants in agricultural fields. For these reasons, in this present investigation we have isolated and identified *Azotobacter spp.* and tested against

fenugreek and mustard seeds. Fenugreek (Trigonella foenum-graecum) is a self pollinated crop belongs to family Fabaceae. It is commonly used in Indian curries and homeopathic medicine and it is a mediterranean herb used for cooking, spice, cosmetics, soaps, healing inflammation, and even improving digestive problems and lowering cholesterol.[8] Being an important rabi spice crop, farmers largely include it in their cropping plan. Mustard (Brassica juncea) belongs to a family Brassicaceae seeds are a rich source of oil and protein. The seed has oil as high as 46-48%, and whole seed meal has 43.6% protein. They are important spice in many regional foods and may come from one of three different plants: black mustard (Brassica nigra), brown Indian mustard (B. juncea), or white mustard (B. hirta/Sinapis alba)[9]

II. METHODS AND MATERIAL

Different soil samples were collected from various places in Hyderabad and surrounding districts of Telangana and Andhra Pradesh states. The soil samples were then studied for the soil type, colour and the crops that they support for cultivation. The soils were then typed as red soil and black soil. Crops like Green leafy vegetables, Onion, Turmeric, Ground nuts, Rice, Pea plants and Tomato were being cultivated on these soils. The soils were then labelled and segregated according to the crops and place from where they were obtained.

Characterization and microscopic observation of the culture was done and the bacteria identified were *Streptomyces sp., Staphylococcus sp., Azotobacter sp., Leptothrix sp., Derxia sp., Bacillus sp., and Sphaerotilus sp.* All the isolated bacteria may not act as biofertilizers. Of the above isolated organisms *Azotobacter sp.* was found to be present in both black and red soil of plants like pea, green leafy vegetables and rice fields. Hence *Azotobacter* was selected and it was subcultured in specific media Jenson's broth and it wasused for the soil application in pots.

Mustard and fenugreek seeds were sown in pots. The soil was inoculated with 250ml of culture in each pot and different aspects of the plant cycle were observed and readings noted. Time taken for seed germination and plant length was noted. Simultaneously Control pots (sterilized soil) were maintained along with pots incubated with cultures.

During the pot trails; the soil samples were collected from the pots in which plants are growing. The soil samples were subjected to serial dilutions and inoculated on specific media and microorganisms reisolated successfully.

III. RESULTS AND DISCUSSION

Azotobacter cells are not usually present on the rhizoplane (root surface) but are in abundant in the rhizosphere (the soil immediately surrounding roots.).[10]Ranna and chandel (2003) used biofertilizer and nitrogen to strawberry CV.'Chandler' and found that *Azotobacter* inoculated plants attained maximum plant height (24.92 cm) more number of leaves per plant (26.29 cm).[11]

When *Azotobacter* inoculants applied to the crops either a seed treatment or seedling root dip or soils treatment, large number of *Azotobacter* cells sticks to the seed or roots and multiply rapidly in the soils along with the developing root and form a thick sheath of bacterial population around roots.[12] Dahama (2002) reported that application of *Azotobacter* increased the yield of wheat, rice, maize, pearl millet and sorghum by 0-30% over control. [13]

Application of *Azotobacter* and *Azospirillum* fertilizers either alone and in combination exhibited in general a considerable improvement in above bio-nutrient parameters of seeds as compared to their respective control. A balanced and judicious use of these biofertilizers is recommended to the farmers to enhance nutritive value of fenugreek seeds.[14] Growth and yield attributes as well as seed and straw yields of fenugreek were significantly influenced by INM treatments.[15] The direct application of microorganisms to seed or other plant parts give them a competitive advantage over pathogens that must complete for nutrients and sites for attachment prior to infection. Routine use of biological systems in controlling plant diseases and high yield have become more attractive due to the added benefits of enhanced plant growth.[16] Positive reports on application of *Azotobacter* and *Azospirillum* on the yield of mustard (Brassica juncea) are available (Tilak and

Sharma, 2007).[17] Seed inoculation with *Azotobacter* or/and PSB along with 100% RDF (40:20:00 NPK kg ha-1) on mustard had significantly increased plant height, number of branches, dry matter and leaf area plant-1, number of siliquae plant-1, number of seeds siliqua-1, test weight, seed and straw yield ha-1.[18] Seed inoculation with *Azotobacter* and *Azospirillum* bacteria in mustard has significantly increased the number of branches, pods/plant, seeds/ pod and yield of seed and stover yield. [19] The increase in seed yield over the control was up to 14.34-20.19% and 16.61-22.61% during 2001-02 and 2002-03 respectively. This favorable effect of bacterial inoculation could be attributed to increase in N supply in inoculated plots due to N-fixation ability of these bacteria.

Keeping this in view a field experiment was conducted to study the effect of *Azotobacter* on growth of fenugreek and mustard plants. *Azotobacter* was inoculated into nutrient broth and incubated for 24-48 h. It is then added to the soil in which the crops are grown. A control is also maintained to check for the effect of *Azotobacter* on the crop plants (control = soil – organisms). In the initial days medium sized garden pots were bought. Then the soil was filled into these pots up to the brim. The soil was watered regularly so as to not allow it to dry completely. Then fenugreek and mustard seeds were bought and preserved. These seeds were sown in the pots when the conditions were favourable for the crop plants to grow. From the day the seeds were sown, the crop plants were watered and monitored. The experiment was carried out for over a period of 6 days. The growth of the crop plants was monitored on a day to day basis. The height of the plant was measured everyday and results were tabulated. The health of the crops was also observed in order to ensure that no adverse effects occur. The number of seeds that germinated in each pot was also observed so as to make a comparative analysis between the effect of *Azotobacter* on fenugreek and mustard crop plants.

The control fenugreek seeds (In the pot where there is no inoculum (*Azotobacte*r) is added and to analyse the effect of *Azotobacter* as an effective biofertilizer for the growth of fenugreek) it took 5 days to germinate. Since then the height of the plant was measured. When the control crop for fenugreek was measured the growth at the end of day 6 was 2.5cm. In presence of *Azotobacter*, fenugreek seeds reduced the time of germination and took 3 days and the plant showed growth up to 3.2 cm by the end of 6th day. The plant remained healthy over this period. The analysis from the Table 1 is that fenugreek in the presence of *Azotobacter* took less time for germination and there was an enhance growth than compared to the control. The difference in the height of the crop is 0.7 cm in the presence of *Azotobacter* thereby indicating that the organism has enhanced the growth of plant. Hence can be employed as an effective biofertilizer.

The control mustard seeds (In the pot where there is no inoculum (*Azotobacte*r) is added and to analyse the effect of *Azotobacter* as an effective biofertilizer for the growth of mustard) it took 8 days to germinate. Since then the height of the plant was measured. When the control crop for mustard was measured the growth at the end of day 6 was 4cm. In presence of *Azotobacter*, mustard seeds reduced the time of germination and took 5 days and the plant showed growth up to 4.9 cm by the end of 6th day. The plant remained healthy over this period. The analysis from the Table 1 is that mustard in the presence of *Azotobacter* took less time for germination and there was an enhance growth than compared to the control. The difference in the height of the crop is 0.9 cm in the presence of *Azotobacter* thereby indicating that the organism has enhanced the growth of plant. Hence can be employed as an effective biofertilizer.

IV. CONCLUSION

Present study revealed that soil inoculation of fenugreek and mustard with *Azotobacter* resulted in higher growth of the crop and is correlated with increased germination and growth of seedlings as compared to its respective controls. The number of seeds germinated in the mustard pot were more than the fenugreek seeds. When the results of the effect of *Azotobacter* on fenugreek and mustard are compared the *Azotobacter* has a slightly greater effect on mustard than compared to fenugreek plants. There by indicating that *Azotobacter* act as a better biofertilizer in both the growth of mustard and fenugreek plants than compared to its respective controls.

Table 1 : Comparative Pot Trails In Fenugreek and Mustard Plants

CROP		GERMINATION TIME	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
FENUGREEK	CONTROL	5 DAYS	0.5 cm	1.3 cm	1.5 cm	2.0 cm	2.3 cm	2.5 cm
	CONTROL + AZOTOBACTER	3 DAYS	1.5 cm	2.0 cm	2.3 cm	2.5 cm	3.0 cm	3.2 cm
MUSTARD	CONTROL	8 DAYS	1.0 cm	1.5 cm	2.2 cm	3.0 cm	3.5 cm	4.0 cm
	CONTROL + AZOTOBACTER	5 DAYS	2.5 cm	3.0 cm	3.5 cm	4.0 cm	4.5 cm	4.9 cm

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