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Studies on Effect of Azatobacter Biofertilizer on Growth of Triticum astivum

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Abstract:

The present study was conducted to study effect of *Azatobacter* biofertilizer on growth and yield of *Triticum astivum*. The *Azatobacter* biofertilizer was produced using isolated *Azatobacter* species by applying activated charcoal powder and pretreatment of the same was given to the seeds of *Triticum astivum*. The effect of Azatobacter biofertilizer was observed by performing pot experiment using treated and untreated seeds. Results showed that plant height, grains number per ear and biological yield were significantly higher in inoculated plants than in non-inoculated plants. Plant height, ear length, grains number per ear, biological yield and grain yield were increased in inoculated plant whereas no significant difference was observed in non-inoculated plants.

Key words: - *Triticum astivum*, Biofertilizer, *Azatobacter*, activated charcoal, pot experiment,

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The abuse of chemical fertilizers has led to a number of environmental issues, such as acidification of water, the greenhouse effect, and ozone layer depletion. Biofertilizers can be used to conflict these issues (Solomon and Anjulo 2017). Utilization of life As a result of bacterial-accelerated mineralization of organic wastes in soil, the nutrients more accessible. In addition, because of the impact of the living bacteria in biofertilizer, the absorption reductions in heavy metals. The term "biofertilizer" refers to a chemical that includes live microorganisms and is recognized for aiding in root system expansion improved germination of seeds. The biofertilizer is a potent alternative against chemical fertilizers. Utilization of bio-fertilizers considered as a key part of organic farming, it is minimizing the harmful effects of excessive use of chemical fertilizers through its ability to facilitate elements to plants (Rana *et al.,* 2012). *Azotobacter* biofertilizer has an important role in fixing atmospheric nitrogen in rhizosphere zone of wheat and many other crops, and also contributes to maintenance of soil fertility (Venkatashwarlu, 2008; Rehman *et al.,* 2017).

Nitrogen is an essential element for plant growth and development and a key issue of agriculture. Most studies indicate that nitrogen fertilizers contribute to resolving the challenge the world is facing, feeding the human population. High yield production of agriculture was accompanied by a massive increase in the application of nitrogen fertilizer. Nitrogen enters living organism via nitrogen fixation (Egamberdieva and Kucharova, 2008). This is accompanied by microbial processes, these microbes may be symbiotic or free-living in nature (Reghuvaran *et al.,* 2012).

The process of biological nitrogen fixation is mediated by nitrogenase enzymes and the process of nitrogen fixation is dependent on certain parameters like moisture conditions, amount of oxygen, the supply of organic substrates, and genotypes of microorganisms and plant (Church *et al.,* 2008). Nitrogen fixing organisms are generally active in plant root zone soil. Nitrogen-fixing free-living microorganisms have frequently been reported as plant growth promoters (González-López *et al.,* 2005). Inoculation effect of free-living *Azotobacter* species are largely associated with nitrogen fixation, formation of various physiologically active growth hormones, protection against root pathogens, stimulation of beneficial rhizospheric microorganisms and enhancement of plant yield (Lakshminarayana *et al*. 2000).

In the present study effect of *Azatobacter* biofertilizer was studied by performing pot experiment.

1. Materials and Method

Soil sample collection and characteristics of *Azatobacter* isolates

Four soil samples were collected from different sites in Vasantrao Naik Marathwada Agricultural University, Parbhani,(MS) India. Soil was enriched in nitrogen free mannitol broth (Manitol -1 g Magnesium sulphate - 0.02 g Potassium hydrogen phosphate - 0.05 g Calcium sulfate - 0.01 g Calcium carbonate - 0.50 g Sodium chloride - 0.02 g Distilled water - 100 ml PH - 07) at 28±2 °C for 2– 5 days. Enriched samples were serially diluted and plated on nitrogen free Mannitol agar plates for isolation of free living nitrogen fixing bacteria *Azatobacter*. *Azatobacter* isolates were selected on the basis of morphological and biochemical characteristics.

Inoculation of wheat seeds with *Azatobacter* strains

The effect of *Azatobacter* strains on the growth and yield of wheat (*Triticum aestivum*) was studied under pot house conditions. *Azatobacter* strains were inoculated in nitrogen free mannitol broth and incubated on shaker at 28±2 °C for 3 days. Wheat seeds were inoculated with *Azatobacter* strains (108 cfu ml−1) and sown in pots containing 2.5 kg of unsterilized soil in triplicate. Experiment was done with three treatments using two doses of fertilizer nitrogen (urea) i.e., control (without fertilizer and without culture),. After germination, three plants were kept in each pot. Plant height, shoot dry weight, grain yield and grain per ear in the plants were determined at 135 days after sowing (DAS).

2. RESULTS AND DISCUSSION

Isolation and Identification of *Azatobacter* species

The collected soil samples were enriched in nitrogen free Mannitol broth. After 48 hours brown color thick pellicle was form in the nitrogen free Mannitol broth flask, which then converts into brown colored Azatobacter colony was form on the nitrogen free Mannitol agar plate. The nitrogen free Mannitol broth is selective media for Azatobacter sp. as this media is free of any nitrogen source which is a macronutrient and without which there is no growth of any microorganism, but only free living nitrogen fixing bacteria can grow within this. For the confirmation of the bacterial culture as *Azatobacter* the morphological and colony characteristics were studied. *Azatobacter* forms large, flat, soft, milky, mucoid and gummy colonies. Table 1 represents all the morphological and colony characteristics of *Azatobacter* species.

Table 1 : Colony characteristics of *Azatobacter sp*.

Sr.No.	Characters	Colony Characteristics
1.	Size	2.8 mm
2.	Shape	Circular
3.	Color	Brown
4.	Opasity	Translucent
5.	Consistensy	Viscous
6.	Elevation	Convex
7.	Appearance	Shiny
8.	Motility	Motile
9.	Gram Nature	Gram Negative

Enrichment of *Azatobacter*

Growth of *Azatobacter*

The biochemical characteristics of the isolated Azatobacter sp. was indicated in Table2. Which confirms the presence of *Azatobacter sp.*

Table 2 : Biochemical characteristics of *Azatobacter sp*.

Sr.No.	Biochemical Characters	Colony Characteristics
1.	Indole test	Positive
2.	Methyl red	Positive
3.	Vogous Proskaur	Negative
4.	Citrate utilization	Positive
5.	Catalase	Positive
6.	Sugar fermentation	Positive
7.	Starch hydrolysis	Negative
8.	H_2S production	Negative
9.	Gelatinase	Positive

Inoculation of wheat seeds with *Azatobacter* strains

The initial microbial density was measured using spectrophotometer which is indicated in Table 3. All the seeds were treated with 10 ml of pure microbial biomass except for the control which had treated with 10ml pure water.

Treatment of seeds with *Azatobacter sp.*

The present study was carried to understand the effect of *Azatobacter* biofertilizer on growth of plant. All obtained results were discussed below. Results from the present study indicated that plant height, grains number per ear and biological yield have been affected by inoculation with *Azatobacter*. In the other word, *Azatobacter* could proper part of nitrogen for feed plants in the rhizospheric region. N fertilizer could affect significantly on traits such as: plant height, root and shoot length. These all the parameters were checked time to time and recorded results were showed in the following tables.

Table 3 : Growth parameters recorded after 15 days

Parameters	Treated plant	Control
Root length	10 cm	6cm
Shoot length	3.9cm	2cm
Whole plant length	13.9 cm	8 cm
Number of leaves	3	2

After 15 Days, a pot treated with microbial biomass from TST showed better results rather than control. It has relatively good characteristics of plant parameters like root and soot length when compared to the other strains and control. In addition, broad leaf and thick stem were visualized. Results are shown in Table (3,4 and 5) and photoplate 3. This is because TST isolates can fix nitrogen without the requirement of any association since *Triticum astivum* is nonleguminous plant and it is treared with *Azotobacter* which is free nitrogen fixer. Similar results were reported by some researchers (Hamza *et al.,* 2017; Sadik *et al.,* 2016). The microbial biomass produced from *Azatobacter* bacteria isolated in the present study has plant growth promoting activity.

Table 4: Growth parameters recorded after 40 days

Parameters	Treated plant	Control
Shoot length	22 cm	14 cm
Root length	8.3 cm	4cm
Whole plant length	38.3 cm	25.4cm

Number of leaves	**6**	**4**

Table 5: Growth parameters recorded after 70 days

Parameters	Treated plant	Control
Shoot length	**26.5cm**	**17cm**
Root length	**11.5 cm**	**5cm**
Whole plant length	**47.5 cm**	**27.3 cm**
Number of leaves	**10**	**6**

Accordingly, treatment of *Triticum astivum* seed with 10 ml of microbial biomass from *Azatobacter* isolate have enhanced the root length and the shoot length and number of leaves over water treated control, recorded after 70 days of seed germination.

15 days after (Treated)

40 days after (Control)

40 days after

70 days after (Treated)

70 days after (Treated)

At the time of harvest

The results of this investigation are reliable with earlier examinations (Rajpoot and Panwar, 2013). The results of the current investigation showed that *Azatobacter* treatment of *Triticum astivum* plant seeds showed increased plant growth (root and shoot length). By reducing the input and impact of the dangerous chemical fertilizer in the field, the nitrogen fixation process used by soil microorganisms that naturally occur in the soil plays a significant role. Additionally, it has a favorable effect on the development of sustainable agriculture, particularly in nations like Ethiopia where agriculture plays a significant role in the national economy.

3. Conclusion

The results obtained from this study summarized that wheat yield influenced strongly by Azatobacter inoculation. *Azatobacter* inoculation increased plant height, shoot length, number of leaves, and root length as compare to the untreated seeds of wheat plant. Thus, it is recommended to use a *Azatobacter* biofertilizer to get the highest yield and decrease adverse environmental effects due to chemical nitrogen fertilizers.

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