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# Effect of *Azotobacter chroccoccum* on Maize as PGPR

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**Abstract**
Plant Growth Promoting *Azotobacter spp.* (PGPR) improves yield by the production of phytohormones, IAA, ACC-deaminase, phosphate solubilization. *Azotobacter spp.* introduced to cereal crops as PGPR which is economically best alternative to chemical fertilizers. During the present investigation application of *Azotobacter spp.* as PGPR on non-leguminous crops *i.e.* Maize was carried out. *A. chroococcum* was used for present investigation and the effect on vegetative growth of plants was studied. The results were observed after 63 days from the date of sowing and the difference in average results were recorded in observation tables. After studying all the parameters, overall growth of Maize crop after the application of *A. chroococcum* were described. All the parameters were studied in replications showed increased growth in treated plants as compared to control plants. So, it can be summarized that application of *A. chroococcum* as PGPR on Maize showed positive effects in vegetative growth after 14 days of plantation. The increase in maximum percent growth recorded after application of *A. chroococcum was 49.82%*. *A. chroococcum* can be effectively used as PGPR for Maize crop.

**Keywords:** *Azotobacter*, Maize, PGPR, Bioinoculant

## Introduction

Plant Growth promoting bacteria (PGPR) is also known as Plant Health Promoting Bacteria (PHPR). They are involved in plant growth by improving soil as they produce tolerant crops and balance biogeochemical cycles (Gouda *et al.*, 2018) [5]. The PGPR improve in yield by the production of phytohormones. They also produce antibiotics, siderophores which helps in suppression of plant diseases and improves resistance capability of plant. They act as biocontrol agents against various pathogenic bacterial and fungal species in soil. Also, some of the microbes used as PGPR are having capacity to tolerate stress due to salinity, drought, temperature etc. The main purpose of utilizing PGPR is to improve the growth and yield of the crops. PGPR improves nutrients uptake by producing various phytohormones like IAA, gibberellins, auxins, cytokinins etc. which ultimately results in increase in root, shoot length, nodulation, nitrogen content etc. (Gupta *et al.*, 2000) [6].

*Azotobacter* is also introduced to cereal crops as PGPR which can be economically important alternative to chemical fertilizers. Some scientist studied the use of *Azotobacter* as PGPR on popular cereal crops as Antoun *et al.*, in 1998 [3] studied the use of *Azotobacter* and *BradyAzotobacter* together as PGPR showing potential results in non-legumes that is, here, radish. Mahmud *et al.*, (2020) [9] explained the importance to use artificial symbiont to economically crops like rice, maize, wheat etc. Perez-Montano *et al.*, (2014) [11] explained the use of PGPR on legumes and cereal plants, they stated PGPR stimulate the plant growth by their own metabolism or affecting the plant metabolism which ultimately result in increase in root development, increases enzymatic activity and suppresses plant pathogens. Hence, in the present investigation application of *Azotobacter* spp. as PGPR on non-leguminous crops *i.e.* Maize was carried out.

## Materials and methods

*Azotobacter chroccoccum* was used for present investigation *Azotobacter chroccoccum* was applied on a monocot crop *i.e.* Maize to study the effect of application of *Azotobacter* spp. on vegetative growth without formation of root nodules. Initially, the mother culture was prepared from this *Azotobacter* spp. and then, the carrier material was used for observing the PGPR activity.

**1. Mass Production:** Pure culture of isolated *Azotobacter chroccoccum* was used for making starter culture. The selective liquid media *viz*. Mannitol salt agar and Mannitol salt broth without adding agar was used for inoculation of *Azotobacter chroccoccum*. The liquid media was poured in sterile conical flask of 250 ml capacity and sterilized at 15 lbs pressure for half an hour. The isolate from pure cultures was inoculated in conical flask containing respective liquid media aseptically on laminar air flow platform. The cultures were incubated in rotary shaker for 4 days. After incubation, cell count reached up to 108-109 cells/ml and become thicker in consistency which was called mother culture (Ismail *et al.*, 2020) [7]. Mother culture was stored under suitable condition and temperature. The pH of the inoculum was kept in between 6.0 - 7.5 pH. The liquid broth culture can be used as PGPR directly as liquid bio inoculant.

**2. Carrier Material:** Mother culture can be used alone as liquid bioinoculant or it can be converted in to solid form by using carrier material. In experimentation, the carrier material used was activated charcoal. Fine powder of carrier material was weighed 50 gm. and autoclaved at 15 psi at 1210C for 20 min. The mother culture was mixed with carrier in 1:3 ratio (Datta *et al.*, 2015) [4]. After proper mixing, the mixture was spread on plastic sheet in closed room for air drying. Carrier material was incubated for 4-10 days at 22-24$^0$C which help the growth of *Azotobacter spp* in carrier material.

**3. *in-vivo* Experimental Design:** Liquid inoculants were stored in sterile plastic bottles and used directly to the desired plant. Each experiment was having control and treated seeds for comparison and each experiment were repeated for three times and the average of all these 03 observations was considered for further investigation. Actual application of the PGPR or inoculants to crops was done by using two methods as seed treatment and soil treatments.

a. **Seed Treatment:** Seeds of Maize were used for studying the effect of bioinoculant. First, 50 gm. seeds were surface sterilized before sowing using 0.1% Murcuric chloride in petriplates for 2 min. and then washed using distilled water. Sterilized seeds were soaked in respective PGPR for 1 hr. in laminar air flow chamber. Inoculated seeds were then immediately sowed in pots containing one third of soil (Purwaningsih *et al.*, 2020) [12]. Along with treated seeds, normal seeds without treatment were also sown as control. The seeds in each pot were watered daily as per requirement to retain the moisture in soil

b. **Soil Treatments:** Along with seed treatment, soil treatments were also done for getting better results. Liquid inoculums as well as water mixed solid inoculums were directly used as PGPR to soil. The liquid PGPR was taken in 20ml sterile syringe and applied drop wise to the pots containing treated seeds. The observations were recorded after 14 days from the date of plantation.

The treated as well as control plants were observed for its vegetative growth in respect to the parameters like root length, shoot length, total plant length and number of leaves. The experiments were designed by selecting 03 plants of treated and control each. Among the cultivated crops, healthier plants were selected for observations. The results were observed after 14 days from the date of sowing and the difference in average results were recorded in observation tables. Percent growth was calculated in every parameter of plant for comparing the results obtained from various parameters.

**Results and Discussion**

During the present investigation, *Azotobacter chroccoccum* under investigation was applied as bioinoculant on Maize. Effect of *Azotobacter* spp. on Maize was studied after 14 days from the date of sowing.

1. **Effect on root length of Maize:** After the treatment of *Azotobacter chroccoccum* as plant growth enhancer, maximum difference in average root length of Maize was observed after 21 days of plantation with maximum percent growth of 57.14 with respect to control.
2. **Effect on shoot length of Maize:** The next parameter was shoot length and the results were recorded in Table 02. Maximum percent growth in shoot length of Maize was observed after 56 days with the treatment of *Azotobacter chroccoccum* 59.69 as compared to control.
3. **Effect on total plant length of Maize:** Total plant length was depended on shoot and root length, if root and shoot length increased ultimately the total height of plant also increased. After the application of *Azotobacter chroccoccum* as PGPR, maximum percent growth in total plant length of Maize was recorded 58.76 as compared to control after56 days.
4. **Effect on number of leaves of Maize:** The average numbers of leaves were varied in treated and control plants due to the application of *Azotobacter chroccoccum*, in Maize after 63 days of plantation. The maximum number of leaves were observed after the application of *Azotobacter chroccoccum* with maximum percent growth of 56.26 as compared to control.
5. **Effect on leaf length of Maize:** The leaf length in treated and control plants varied due to the application of *Azotobacter chroccoccum*, in Maize after 63 days of plantation. The maximum leaf length was observed after the application of *Azotobacter chroccoccum* with maximum percent growth of 41.64 as compared to control plants.

After studying all the parameters, overall growth of Maize crop after the application of *A. chroccoccum*, was explained in Table 01. All the parameters showed increased growth in treated crops as compared to control crops. So, it can be summarized (As per Table No 02 and Fig.01) that application of *A. chroccoccum* as PGPR on Maize showed positive effects in vegetative growth after 63 days. Mehboob *et al.*, (2011) [10] investigated increase in growth and yield in respect to root and shoot height after the application of various *Azotobacter* strains. They also explained effect of *chickpea* isolates showed more growth rate than other crop isolates. Afzal *et al.*, in 2014 [2] inoculated wheat plant with *R. leguminosarum* and *Pseudomonas* sp. that showed prominent increase in grain yield than non-inoculated crops. Other scientist like Kavimandan (1986) [8], Afzal and Bano (2008) [1] etc. also studied effect of rhizobial isolates on Maize crop and explained increase in grain yield of Maize.

**Table 1:** Effect of *A. chroococcum* on the vegetative growth of Maize

| No. of Days | Root Length | | | Shoot Length | | | Total Length | | | No. of Leaves | | | Leaf Length | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Average results After Treatment | Diff.against control | % Growth | Average results After Treatment | Diff.against control | % Growth | Average results After Treatment | Diff.against control | % Growth | Average results After Treatment | Diff.against control | % Growth | Average results After Treatment | Diff.against control | % Growth |
| 14 Days | 10.2 | 3.5 | 34.31 | 14.3 | 5.2 | 36.36 | 9 | 5 | 55.56 | 2 | 1 | 50.00 | 15.2 | 3.1 | 20.39 |
| 21 Days | 10.5 | 6 | 57.14 | 18.6 | 7 | 37.63 | 13 | 6 | 46.15 | 5 | 2 | 40.00 | 17.8 | 5.8 | 32.58 |
| 28 Days | 8.9 | 4.9 | 55.06 | 21.23 | 9.3 | 43.81 | 15.2 | 7.3 | 48.03 | 8 | 3 | 37.50 | 20.5 | 4.9 | 23.90 |
| 35 Days | 12.1 | 6.2 | 51.24 | 26.3 | 9.1 | 34.60 | 17.6 | 8 | 45.45 | 9 | 4 | 44.44 | 23.5 | 6.7 | 28.51 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 42 Days | 13.2 | 5.6 | 42.42 | 28.9 | 9.2 | 31.83 | 18.4 | 8.5 | 46.20 | 10 | 4 | 40.00 | 26.3 | 7.4 | 28.14 |
| 49 Days | 13.3 | 4.6 | 34.59 | 32.5 | 10.2 | 31.38 | 22.7 | 12.7 | 55.95 | 12 | 5 | 41.67 | 28.9 | 11.5 | 39.79 |
| 56 Days | 17 | 5.6 | 32.94 | 39.2 | 23.4 | 59.69 | 29.1 | 17.1 | 58.76 | 14 | 5 | 35.71 | 31.2 | 12.5 | 40.06 |
| 63 Days | 18.3 | 8.3 | 45.36 | 44.6 | 22 | 49.33 | 32.2 | 18.2 | 56.52 | 16 | 9 | 56.25 | 36.5 | 15.2 | 41.64 |

**Table 2:** Effect of *A. chroococcum* on the vegetative growth of Maize after 63 days

| Sr. No. | Parameter | Average results After Treatment | Difference | Percent Growth |
|---|---|---|---|---|
| 1 | Root length | 18.3 | 8.3 | 45.36 |
| 2 | Shoot Length | 44.6 | 22 | 49.33 |
| 3 | Total plant length | 32.2 | 18.2 | 56.52 |
| 4 | No. of leaves | 16 | 9 | 56.25 |
| 5 | Leaf length | 36.5 | 15.2 | 41.64 |
| Average Growth | | 29.52 | 14.54 | 49.82 |

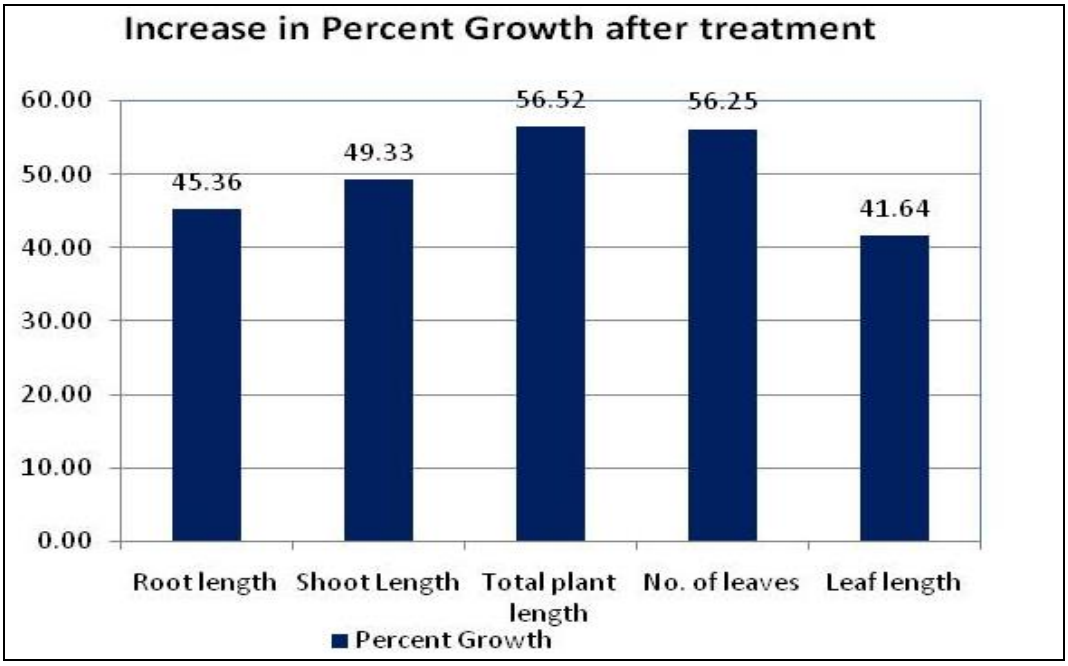


**Fig 1:** Effect of *A. chroococcum* on the vegetative growth of Maize

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