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Research Article

Field Efficacy of Commercial *Azotobacter* (Ami *Azotobacter*) on Tomato Crop

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Abstract

Tomato (*Solanum lycopersicum*) cultivation in India faces significant issues due to increased chemical fertilizer use, highlighting the need for sustainable alternatives like biofertilizers. This study aims to assess the efficacy of commercial Azotobacter spp. (*Ami Azotobacte*r) as a biofertilizer for improving tomato growth in the field. *Azotobacter* was identified and purified from soil samples collected at an agricultural farm in Gujarat. The isolated *Azotobacter* was used to create an inoculum for field trials in which tomato seeds were soaked in the *Azotobacter* suspension and use for field trials with various treatments: control (T1), 100% nitrogen (T2), *Azotobacter* (T3), and *Azotobacter* + 50% nitrogen (T4). For 30 days, growth indicators such as plant height stem breadth, root length, and fruit yield were measured to examine the efficiency of the *Azotobacter* treatments. This study showed that tomato plants treated with Azotobacter and 50% inorganic nitrogen (T4) had the highest growth over every parameter compared to the control and other treatments. T4 plants indicated significant increases in height (161.3 cm), stem width (1.2 cm), root length (5.5 cm), and fruit yield (1.62 kg/plant). The results suggest that using *Azotobacter* with inorganic nitrogen fertilizers can significantly enhance tomato plant growth and yield, providing a long-term solution for increasing agricultural output.

Keywords: Ami *Azotobacter*; Field Efficiency; Yield; Bio Fertilizer; Nitrogen Fixation; Solanum Lycopersicum

Introduction

Tomatoes are India's third-largest crop, following potatoes and onions [1]. India is the world's second-largest tomato producer [2]. India is the second-largest producer of tomatoes globally, with an output of 18.74 million tons, following China [3], on average, the highest production occurs at the beginning and end of the year. Tomatoes are cultivated twice yearly, in the rabi season (September) and the kharif season (March to August). The *Solanaceae* family consists of the cultivated tomato, scientifically classified as *Solanum lycopersicum* [4]. Tomatoes serve a major part of Indian vegetable output, making up about 11.04% [5]. It is considered a crucial "protective food" due to its nutritional value [6]. It requires large amounts of nutrients such as nitrogen, phosphorus, and potassium [7]. Yet, relying on inorganic fertilizers is hazardous to the soil and the ecosystem [8].

As the human population develops, their demand for food rises, placing the accessibility of food at risk. To satisfy this need, farmers utilize excessive amounts of chemical fertilizers, which disrupt soil quality by increasing salinity and decreasing biological activity. It not only impacts the environment, but it also threatens human health [9]. Also, it supports eutrophication and soil acidity, damages plant roots, and raises outbreaks of diseases [10]. Thus, sustainable approaches, like employing nitrogen-fixing bacteria and PGPR, offer ways to decrease reliance on nitrogen [11].

Biofertilizers can fix atmospheric nitrogen through biological nitrogen fixation, solubilize plant nutrients like phosphates, and stimulate plant growth by producing growth-promoting chemicals [12]. Organic preparations containing microorganisms increase agricultural production by providing essential nutrients, mainly

phosphorus (P) and nitrogen [13]. Biofertilizers, as environmentally friendly, non-toxic, and hazardous materials, are becoming increasingly critical to agriculture [14]. Crop yield benefits from biofertilizers such as *Azotobacter, Azospirillum*, blue-green algae, *Azolla*, phosphorus-solubilizing microbes, *mycorrhiza*e, and *Sinorhizobium* [15].

In recent years, the use of fertilizers with beneficial bacteria has increased. *Azotobacter* species are essential for improving soil nitrogen levels through nitrogen fixing [16]. They are a highly varied group of non-symbiotic nitrogen-fixing bacteria that are found in soils all over the world [17]. In the rhizosphere, *Azotobacter* species appear naturally, either stimulated by plant signals or fulfilling the bacteria's requirement for nitrogen.

Many research investigations have proven that utilizing biofertilizers on crops improves the growth of plants, production, and soil fertility [18]. *Azotobacter* was verified to possess a considerable favorable influence on the development of tomatoes and yield, which leads to increases in plant height, root length, stem width, leaf number of fruits, and flower buds [19].

A comparison study was conducted to determine the impact of *Azotobacter* and *Trichoderma*, as well as other fertilizers, on maize yield [20]. Researchers discovered that seeds treated with *Azotobacter* showed significant improvements in stem girth, plant height, root weight, dry shoot weight, and root length and width [21].

The current study aims to isolate commercial *Azotobacter*, produce a biofertilizer using these bacteria, and conduct trials to evaluate its efficacy as a biological fertilizer for tomato growth. The research examines how commercial *Azotobacter* affects the development and production of tomato plants grown in field conditions.

Materials and Methods

Experimental site location

Rhizosphere soil was obtained from a fertile farm in Ode, Ahmedabad, Gujarat (22° 54' 57.7" 72°32'34.3"E) from July 2021 to November 2021. To study the effect of commercial *Azotobacter* on tomato plants. The soil sample was taken with a sterilized trowel, carried in a sterilized container, and then submitted to a laboratory. The Physoco-chemical condition of the soil is mentioned in table 1.

Properties	Values
Sand	29.0
Silt	36.3%
Clay	32.01%
pH	7.2 ± 0.01
EC	1.4
Organic matter	1.21%
Total N	0.03%
Total P	0.02%
Total K	0.03%

Table 1: Physico-chemical properties of soil.

Identifying, isolating, and purifying an isolate

Approximately 2 grams of soil were aseptically introduced into a flask containing Jensen's broth. Jensens Medium is recommended for the detection and cultivation of nitrogen-fixing bacteria [22]. Which contain sucrose, dipotassium phosphate, magnesium sulfate, sodium chloride, ferrous sulfate, sodium molybdate, calcium carbonate, and agar [23] for the isolation of *Azotobacter spp*. The flask containing Jensens medium was subsequently incubated at 28°C ± 2°C for 1-2 weeks until the formation of a pellicle on the broth's surface [24]. A minute quantity of the pellicle was streaked onto Jensen's medium agar plate using a loop. The inoculated plate was then re-incubated at 28 °C ± 2 °C for 5-7 days. Post-incubation, the plates were examined for the presence of mucoid, watery, dewdrop-like colonies of *Azotobacter*.

Preparation of inoculum for field inoculation

Before seeding, the seeds were soaked for six hours. To revive test organisms from stock culture, they were subcultured into enrichment media and incubated for 24 hours at 28 ± 2 °C. Following incubation, mineral compounds in the saline medium were aseptically transferred to four 500-mL conical flasks, each containing 1 L of deionized water. The medium was autoclaved and then allowed to cool. *Azotobacter spp*. was introduced into four conical flasks, one of which contained a mixture of three strains. Flasks were incubated for 24 hours at 28 ± 2 °C.

Test for the viability of the seed

The tomato (*Solanum lycopersicum*) seeds were collected and air-dried for five days. Its viability was tested by sowing around 50

tomato seeds in a tray with five small drainage holes. The seeds were spread all over the tray and lightly coated with dirt. To maintain the humidity level, the tray was wrapped in a plastic bag. After 5-6 days, almost 90% of the seed germinated, which shows seeds are viable.

Inoculation of seed with bacterial isolates

The sterilized seeds were soaked in a suspension of *Azotobacter sp.* (Ami *Azotobacter*), ensuring they were completely immersed in the broth. The seeds were allowed to soak for thirty minutes to two hours. The mixture was then applied to the seeds for about 6 hours, ensuring they were evenly coated. The seeds were air-dried in a shaded area before being planted directly in pots. After drying, the seeds were sown in soil or germinated in a controlled environment, such as sterile soil in pots.

Designing field experiments

The planting pots were 1 meter apart from each other. The treatment consisted of T1: control - soil without biofertilizer; T2: 100% nitrogen; T3: *Azotobacter*; and T4: *Azotobacter* + 50% nitrogen.

Test of seedling growth

The initial seedling proliferation experiment measured the number of leaves, plant height (cm), number of fruit yields recorded at harvest, number of flower buds, stem width (cm), and root length (cm) every five days until the experiment ended (July 2021 to November 2021).

Plant height was measured at harvesting from the base of the plant to the base of the fully opened top leaf with the help of a scale, and the number of branches was also counted. The fruit yield was recorded at harvest. The fruit samples collected from the tomato plant at harvest time were oven-dried.

Statistical analysis

Data analysis in this study utilized one-way analysis of variance (ANOVA) at a significance level of 5%. To identify specific significant differences between treatments, the least significant difference (LSD) test was applied with a significance threshold of $P < 0.05$.

Results and Discussion

Production of Siderophore and IAA

The growth properties of plants that observed are demonstrated in table 2. Under iron-limited conditions, *Azotobacter spp*. and many associated soil bacteria are known to produce catechol-type siderophores such as azotobactin and aminochelin that enhance iron uptake [25]. They also produce indole acetic acid (IAA), which improves plant health [26]. The synergistic activity of IAA and siderophores promotes plant development and resistance; overall, IAA controls growth hormones, while siderophores support iron uptake and control pests [27].

Phytohormones	Result
Indole Acetic Acid	Present
Siderophore Production	Present

Table 2: Evaluate the beneficial properties of investigated bacterial isolates that support plant vegetative development.

Other research has discovered that bacteria in the genus *Azotobacter* produce iron-rich nitrogenases, which reduce nitrogen [28]. Production of the siderophores was stimulated by *Azotobacter sp*. Observations show that growth stage, culture conditions, and substrate availability affect the production of IAA by PGPR, which varies between species [29].

Measuring growth variables

Plant height

Azotobacter has a significant effect on tomato plant height. Figure 1 shows the height of tomato plants (in cm) measured over a 30-day trial period post-planting. The study used a variety of factors to analyse plant height growth over a period of a month. The control group, which had no biofertilizer treatment, exhibited a small rise in height compared to the other treatments. In contrast, treatment T4 had the most dramatic impact on tomato plant height after 30 days. The data showed that tomato plant growth increased over time, with plants treated with Ami *Azotobacter* and supplemented with 50% inorganic nitrogen surpassing all other treatments.

Similar research has found that inoculating plants with *Azospirillum* causes significant alterations in a variety of growth measure-

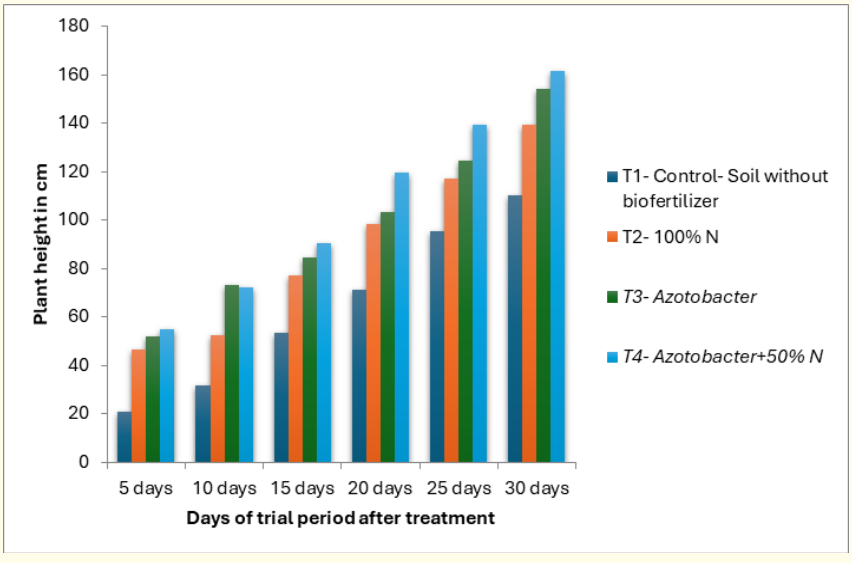

Figure 1: The growth of tomato plants was measured in centimetres from five to thirty days after planting.

ments, including plant height [30]. In their third experiment, farhadi and asghari (2023) found that inoculating maize seeds with different bacterial strains significantly improved plant height, 100-seed weight, number of seeds per ear, and leaf area. The results showed a significant increase in shoot and ear dry weight of maize [31].

Stem width (Cm)

Ami Azotobacter had a considerable effect on tomato plant stem width. The results revealed that the stem width of plants treated with Ami *Azotobacter,* in addition to 50% inorganic nitrogen, increased significantly compared to the control. Measurements taken on the 5th day of inoculation, as shown in figure 2, indicated that T1 developed by only 0.3 cm, T2 grew by 0.6 cm, T3 increased by 0.7 cm, and T4 exhibited the highest growth, rising by 0.9 cm. Previous research has shown that providing nitrogen enhances stem width [32]. *Azotobacter* treatment may have a significant effect on maize stem girth due to adequate nitrogen fixation [33]. El-Beltagi., *et al*. (2022) observed a similar result on stem diameter [34]. Bacterial inoculation was also shown to increase leaf and shoot dry weight and leaf surface area in both sterile and non-sterile soil. Their findings revealed that inoculation with bacterial treatments had a higher stimulating effect on plant development and growth in non-sterile soil compared to sterile soil [35].

Root and internode length

The current research examines Root and internode lengths varied significantly in response to treatment with commercial biofertilizer (Ami *Azotobacter*). The root size was recorded on day thirty of the experiment. The control group (T1) measured 1.8 cm, T2 (100% N) recorded 3.1 cm, T3 (treated with Ami *Azotobacter sp.)* recorded 4.2 cm, and T4 (treated with *Azotobacter* and 50% N) recorded 5.5 cm. From these outcomes, the control had the smallest measurement (1.8 cm), while T4 (treated with *Azotobacter* + 50% N) had the highest measurement (5.5 cm).

Figure 3 represents the internode length (cm) of *Solanum lycopersicum* observed from 5 to 30 days after planting. The impacts of commercial *Azotobacter* (Ami *Azotobacter*) on tomato plants were seen. Inoculation with *Azotobacter* + 50% N increased plant development and germination rates, increased seedling growth, improved responses to prolonged stresses, and protected plants from disease. Previous research revealed that using the inorganic fertilizer NPK reduced root length in T5 and T7 by 20.5% and 12.7%, respectively, compared to the control. *Azotobacter* (T2) had the greatest growth in root length, measuring 12.0% longer [36].

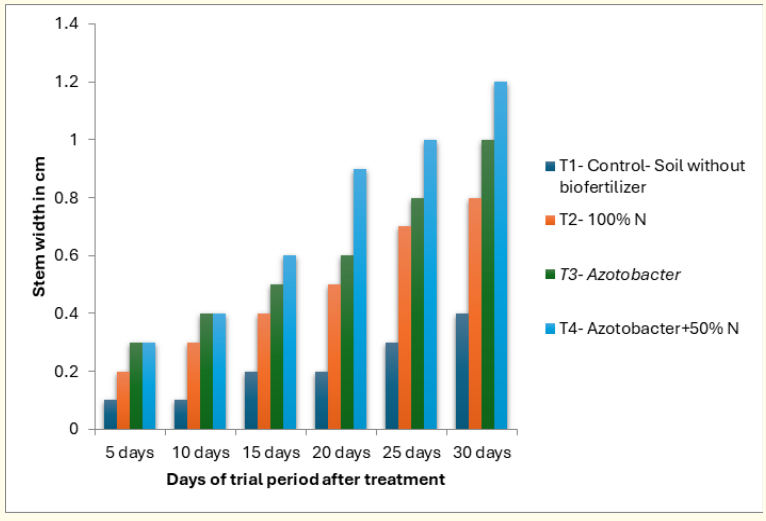

Figure 2: Stem width (cm) variation in tomato plants under four different treatments from five to thirty days after planting.

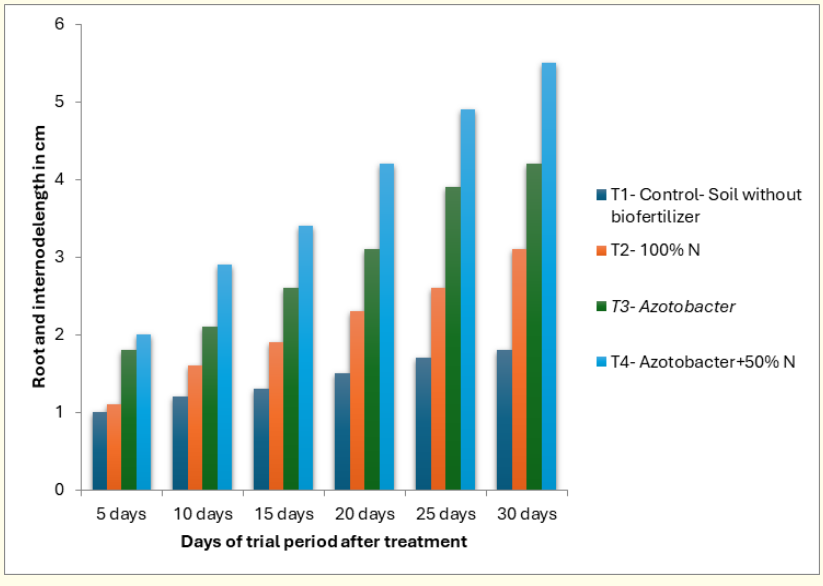

Figure 3: Analysis of root and internode lengths (cm) in tomato plants from five to thirty days post-planting across four treatment conditions.

Fruit yield (kg)

The use of Ami *Azotobacter* in combination with 50% inorganic fertilizer increased fruit yield over the control group (Figure 4). The treatment of *Azotobacter* +50% N produced the most fruit (1.62 kg/plant). The untreated plants yielded a minimum of 0.21 kilograms per plant. When Ami *Azotobacter* and inorganic nitrogen are added to tomato plants, their yields increase.

One of the main production goals for generating economic benefits was to yield heavier and larger fruits. It was discovered that PGPR inoculation enhanced tomato weight and fruit size in both varieties. It was observed that inoculating with biofertilizers such as *Pseudomonas fluorescens* and *Azospirillum brasilense* at the seedling stage resulted in a greater number of ripe fruits and a better

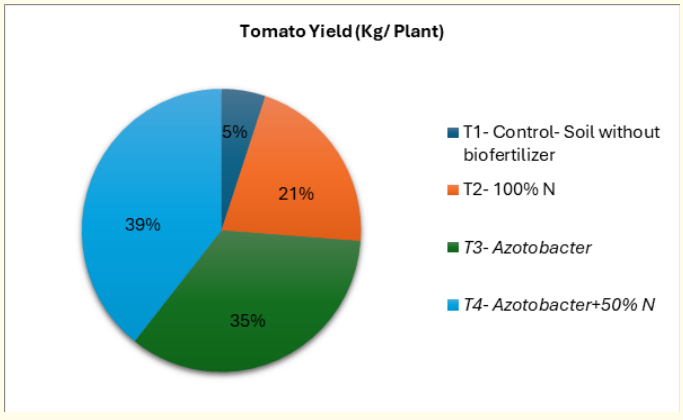

Figure 4: Yield performance (Kg/Plant) of tomato plants cultivated in field conditions with various fertilization treatments.

yield compared to the control group. Plants treated with the PGPR inoculant demonstrated the largest benefit, with a 35 percent increase in the quantity of fruits [37].

Conclusion

An application of *Azotobacter sp,* particularly in combination with 50% inorganic nitrogen, effectively enhances tomato plant growth and yield compared to both untreated controls and other treatments. This approach offers a sustainable agricultural solution by reducing the reliance on full nitrogen fertilization while maintaining high productivity. The use of Ami *Azotobacter* as a biofertilizer presents a promising strategy for improving tomato cultivation, potentially leading to more efficient and environmentally responsible farming practices.

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