



Quantification of Nitrogen Savings by Efficient *Azotobacter* Isolate in Tomato (*Lycopersicon esculentum* MILL) Cultivation and Evaluation of Biocontrol Efficacy

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Abstract: A pot experiment was conducted to study the selection of effective *Azotobacter* isolates for tomato (*Lycopersicon esculentum* Mill) in terms of nitrogen accumulation, total N uptake, and antifungal activity of *Azotobacter* isolates. The experiment was carried out during 2022-2023 at the Department of Agricultural Microbiology, College of Agriculture, Raipur, C.G. Tomato seedlings were transplanted on 28 October 2022. An interaction study with *Fusarium* was conducted to determine the nitrogen-fixing efficiency of *Azotobacter* isolates, as well as the fruit and shoot nitrogen content, fruit and shoot nitrogen uptake, and total nitrogen uptake at harvest. The application of the local *Azotobacter* isolate AZOT-B-32 resulted in the highest values for nitrogen accumulation in tomato, total nitrogen uptake, and the antifungal activity of *Azotobacter* isolates.

Keywords: *Azotobacter* isolates, Nitrogen accumulation, *Fusarium* interaction, Antifungal activity

Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable crop due to its high nutritional content, low environmental requirements, ease of cultivation, and high yield and quality. Tomatoes are grown worldwide on approximately 5.1 million hectares and global tomato production is estimated at 182.8 million metric tons annually. The average global productivity of tomatoes is about 35.8 tons per hectare FAO (Food and Agriculture Organization 2023). In India, tomatoes are cultivated on approximately 0.9 million hectares with production of t 20.3 million metric tons annually. The average productivity in India is about 22.5 tons per hectare (Ministry of Agriculture and Farmers' Welfare, Government of India, 2023). In Chhattisgarh, tomatoes are cultivated on approximately 45,000 hectares. The state produces about 1.2 million metric tons of tomatoes annually and average productivity is 26.7 tons per hectare (Department of Horticulture, Government of Chhattisgarh, 2023).

The large number of aerobic and anaerobic bacteria have been identified as free-living nitrogen fixers, with their nitrogen-fixing potential ranging from 2 mg to 25 mg per gram of carbon source utilized. Among these potential nitrogen fixers, *Azotobacter* is key bacteria that fixes nitrogen in non-legumes. *Azotobacter* is a heterotrophic, free-living nitrogen-fixing bacterium commonly found in alkaline and neutral soils. *Azotobacter chroococcum* is the most widely occurring species in the arable soils of India. In addition to ability to fix atmospheric nitrogen, it can also synthesize growth-

promoting substances such as auxins, gibberellins, and, to some extent, vitamins. Many strains of *Azotobacter* also exhibit fungicidal properties against certain species of fungi. Its population is relatively low in uncultivated lands, but the presence of organic matter in the soil promotes its multiplication and nitrogen-fixing capacity. The low population density is also due to extreme environmental conditions, including high air temperatures of up to 48°C, soil surface temperatures exceeding 60°C, and low humidity (3-4%) during the prolonged summer season (Bali et al., 2022, Patel et al., 2023). These conditions result in the loss of organic matter and a reduction in the population of beneficial microbes (Chhattisgarh State Agricultural Department 2023).

Broad-spectrum microbes such as *Rhizobium*, *Azospirillum*, *Azotobacter*, phosphorus-solubilizing bacteria (PSB), vesicular-arbuscular mycorrhiza (VAM), and blue-green algae (BGA) are incorporated as active ingredients in biofertilizers and are commercially available on the market (Seenivasagan and Babalola 2021). Some *Azotobacter* isolates influence the synthesis of secondary metabolites and phytohormones, which play a role in tomato yield and quality. These phytohormones affect metabolic pathways (such as amino acids, vitamins, phospholipids, and fatty acids) and induce ethylene synthesis, which, in turn, promotes carbohydrate production and translocation in fruit. Auxins, cytokinins, and gibberellins promote plant growth, biomass accumulation, and agricultural yield (Hindersah et al., 2020, Fusco et al., 2022). *Azotobacter* spp. are sensitive

to acidic pH, high salinity, and temperatures above 35°C, which results in a low population density in the soils of Chhattisgarh. The soils in Chhattisgarh are low to medium in available nitrogen, making nitrogen one of the most limiting plant nutrients. Given the rising prices of chemical fertilizers, coupled with the increasing demand for these inputs and the depletion of soil fertility, there is a need to develop effective bioinoculants of *Azotobacter* for tomato cultivation. The current studies were conducted to develop location-specific *Azotobacter* isolates for tomatoes in Chhattisgarh.

MATERIAL AND METHODS

The experiments were conducted in at Department of Agricultural Microbiology, College of Agriculture, Raipur, Chhattisgarh, India during 2022-23. The experimental farm is located at 21°16' N latitude and 81°36' E longitude with an altitude of 298.56 meters above mean sea level. The climate of the area ranges from dry-sub humid to semi-arid. It is located at 21°16' N latitude and 81°36' E longitude, with maximum temperatures rising up to 46°C during the summer. The mean annual rainfall is 1200-1300 mm, with about 85% of it received from the third week of June to mid-September.

The recommended fertilizer for tomato is 120 kg/ha of nitrogen (N), 60 kg/ha of phosphorus pentoxide (P₂O₅), and 80 kg/ha of potassium oxide (K₂O), respectively. The cultivar used in the study was C.V. Pusa Rubi, procured from the Agricultural College Seed Storage Lab, Raipur, Chhattisgarh, India. There were eleven treatments, complete in a randomized block design : T₁ (AZOT-B-35+100:60:80 NPK), T₂ (AZOT-B-32+100:60:80 NPK), T₃ (AZOT-B-18+100:60:80 NPK), T₄ (AZOT-B-39+100:60:80 NPK), T₅ (AZOT-B-123+100:60:80 NPK), T₆ (AZOT-B-33+100:60:80 NPK), T₇ (AZOT-B-109+100:60:80 NPK), T₈ (IARI ,S.C.+100:60:80 NPK), T₉ (control-I+120:60:80 NPK), T₁₀ (control-II+115:60:80 NPK) and T₁₁ (control-III+100:60:80 NPK). Forty local *Azotobacter* isolates and standard *Azotobacter* IARI isolate (standard check) were collected from Microbial Culture Bank of Department of Agricultural Microbiology, CoA, Raipur. During this experiment, seven top performing isolates were compared with the same standard check and three uninoculated control contained 100:60:80, 115:60:80 and 120:60:80 kg N, P₂O₅ and K₂O, respectively.

RESULTS AND DISCUSSION

Nitrogen fixing efficiency of *Azotobacter* isolates: The range of nitrogen fixed in the N-free Jensen's liquid medium varied from 2.35 to 13.45 mg N/g of sucrose (0.0047% to 0.0269% N) after seven days of incubation (Table 1). Three local *Azotobacter* isolates: AZOT-B-33, 32 and 18 were at par with standard check (*Azotobacter* IARI isolate). Among all

isolates, isolate AZOT-B-33 fixed the maximum quantity of nitrogen in the medium, i.e., 13.45 mg N/g of sucrose (0.0269% N), followed by isolate No. 32 after seven days of incubation. The standard check released 13.10 mg N/g sucrose (0.0262 % N). *Azotobacter* strains have been evaluated for their nitrogen-fixing efficiency and tolerance to abiotic stressors, which has implications for enhancing sustainable agricultural practices (Singh et al., 2021). The nitrogen-fixing bacteria, *Azotobacter* contribute significantly to soil fertility and plant nutrition (Singh and Yadav 2021, Patel et al., 2022, Kumar et al., 2023).

Nitrogen content in fruit: The inoculation of tomato seedlings with local *Azotobacter* isolates and standard check with 100:60:80 kg of NPK significantly increased the N-content in tomato fruit over control C-III (100:60:80 NPK) (Table 2, 4). Maximum nitrogen content in fruit was 1.95 % in T₆ (AZOT-B-33+100:60:80 NPK) followed by T₉ (standard check +100:60:80 kg NPK). The *Azotobacter* isolate AZOT-B-33 significantly increased the percent N content in fruit over standard check when the plants were fertilized with the NPK level of 100:60:80. However, the N content due to isolates 33 and standard check were at par with the N content of fruits of uninoculated plants grown with 120:60:80 kg NPK (C-I). The nitrogen content in the fruits of plants raised with the 115:60:80 NPK level was statistically comparable to the

Table 1. Nitrogen fixation capacity of local *Azotobacter* isolates and standard check in the N free Jensen's liquid medium

<i>Azotobacter</i> Isolates	Percent N	N – fixed (mg N /gm of sucrose)
Standard check	0.0262	13.10
AZOT-B-33	0.0269	13.45
AZOT-B-32	0.0263	13.15
AZOT-B-18	0.0261	13.05
AZOT-B-39	0.0218	10.90
AZOT-B-123	0.0209	10.45
AZOT-B-35	0.0207	10.35
AZOT-B-109	0.0203	10.15
AZOT-B-46	0.0200	10.00
AZOT-B-51	0.0198	9.90
AZOT-B-126	0.0197	9.85
AZOT-B-144	0.0196	9.800
AZOT-B-34	0.0195	9.75
AZOT-B-156	0.0195	9.75
AZOT-B-146	0.0087	4.34
AZOT-B-121	0.0053	2.62
Rest isolates	0.0047-0.0052	2.35-2.60
CD (p=0.05)	0.0008	0.40

nitrogen content resulting from the inoculation of local *Azotobacter* isolates 18, 32, 39, and 123 with the C-III fertilizer level. Similar results were found by Reddy et al. (2018). The maximum growth of tomato was in T6, which consisted of 75% of the NPK dose along with *Azotobacter* sp. and *Azospirillum* sp. The observed parameters in T6 were germination 90%, plant height 51 cm, leaf area 59 cm², branches per plant 8.66, and leaves per branch 17.33. The study indicate that these strains possess great potential to be developed as biofertilizers to enhance soil fertility and plant growth.

Fruit nitrogen uptake: The inoculation of tomato plants with *Azotobacter* isolates including standard check significantly increased the accumulation of nitrogen by the fruits except isolate AZOT-B-109. The isolate No 33 showed the best performance which was able to uptake 797.26 mg nitrogen per pot in presence of 100:60:80 NPK level followed by the uninoculated fertilization (716.94 mg/pot) containing 120:60:80 kg of NPK (C-I). Minimum N- uptake by fruits was in uninoculated control treatment with 100:60:80 NPK level (C-III). The *Azotobacter* isolate AZOTO-B-33 significantly increased nitrogen uptake in tomato fruits over standard check of *Azotobacter* with the same level of NPK i.e. 100:60:80. The local isolate (33) alone was at par result with control-I which fertilized with 120:60:80 kg of NPK. The amount of nitrogen which was up taken by fruits due to inoculation with three other local *Azotobacter* isolates AZOT-B-18,32 and 39 and fertilization with 100:60:80 kg NPK was at par with nitrogen accumulated under uninoculated fertilizer treatment C-II (115:60:80) . Inoculation of standard check was found significantly superior over uninoculated control C-

II. A similar type of study was also conducted by Reddy et al. (2022) on the production of growth substances by nine *Azotobacter chroococcum* isolates from the sugar beet rhizosphere. This study showed that these isolates have the ability to produce auxins, gibberellins, and phenols, and, in association with tomato plants, increased plant length, biomass, and nitrogen content. *Azotobacter* application significantly increased nitrogen uptake in tomato. This was attributed to better nitrogen fixation resulting from accelerated activity of *Azotobacter* and enhanced root system development, which likely led to higher nitrogen accumulation in tomato shoots (Kumar et al., 2023).

Shoot nitrogen content: The local *Azotobacter* isolates, standard check and different levels of nitrogen exhibited a differential influence to enhance shoot N content of tomato plants, (Table 2, 4). The inoculation of tomato seedlings with local *Azotobacter* isolates and standard check with 100:60:80 NPK significantly increased the N-content in tomato shoot at the time of harvest over uninoculated control C-I (100:60:80). Maximum percent N content in shoot was 0.79 % in local *Azotobacter* isolate AZOT-B-33 followed by uninoculated control C-I (0.77%). Minimum value was recorded in C-III (100:60:80) i.e. 0.52%. The *Azotobacter* isolate AZOT-B-33 significantly increased the percent N content in shoot over standard check when the plants were fertilized with the NPK level of 100:60:80. The level of N due to isolate 33 and standard check was at par with the nitrogen content in uninoculated plants raised under NPK level of 120:60:80 (C-I). Nitrogen content in shoot under another uninoculated control treatment C-II (NPK::115:60:80) was statistically insignificant over local *Azotobacter* isolates AZOT-B-18, 32, 39 and 123 with CIII fertilizer level.

Shoot nitrogen uptake: The inoculation of tomato plants with *Azotobacter* isolates including standard check significantly increased the accumulation of nitrogen by the shoot at the time of harvest. Maximum accumulation of nitrogen in plant shoot was attributed to the inoculation of local *Azotobacter* isolate AZOT-B-33 (595.19 mg/pot) with 100:60:80 NPK level, followed by uninoculated fertilized pot (542.57 mg/pot) containing 120:60:80 kg NPK. Minimum N-uptake was under uninoculated control treatment with 100:60:80 NPK level (C-III). The *Azotobacter* isolate AZOT-B-33 significantly increased the nitrogen uptake in tomato shoots over standard check of *Azotobacter* with the same level of NPK i.e. 100:60:80. However, promising isolate (33) and standard check were at par with that of control-I which received only 120:60:80 kg NPK. The amount of nitrogen which was accumulated by *Azotobacter* isolates AZOT-B-18,32,39 & 123 in presence of 100:60:80 kg NPK was statistically equal to that of nitrogen accumulation under

Table 2. Influence of *Azotobacter* isolates and different levels of nitrogen on N-accumulation by tomato fruits and shoot at harvest (percent)

Treatment number	Treatment	Fruits	Shoot
T ₁	100:60:80 + AZOT-B-35	1.49	0.64
T ₂	100:60:80 + AZOT-B-32	1.77	0.73
T ₃	100:60:80 + AZOT-B-18	1.72	0.72
T ₄	100:60:80 + AZOT-B-39	1.66	0.70
T ₅	100:60:80 + AZOT-B-123	1.60	0.67
T ₆	100:60:80 + AZOT-B-33	1.95	0.79
T ₇	100:60:80 + AZOT-B-109	1.28	0.60
T ₈	N:P:K::100:60:80 + S.C.	1.78	0.73
T ₉	N:P:K::120:60:80 (C-I)	1.91	0.77
T ₁₀	N:P:K::115:60:80 (C-II)	1.69	0.72
T ₁₁	N:P:K::100:60:80 (C-III)	1.06	0.52
	CD (p=0.05)	0.16	0.05

uninoculated fertilizer treatment C-II (115:60:80 NPK). The increment of nitrogen in tomato shoots may be attributed to N-fixation or glutamase synthetase activity. This observation is in close agreement with Mahato et al. (2009) and Tian et al. (2022). They clearly mentioned that *Azotobacter* inoculation either individually or in combination with other crop beneficial microbe significantly increased nitrogen concentration in the root, shoot and whole plant, hence showed better results as compare to that of inorganic fertilizer.

Total nitrogen uptake: The inoculation of tomato seedlings with local *Azotobacter* isolates and standard check significantly enhanced the total nitrogen uptake by the crop. The maximum amount of N was accumulated by tomato crop (1392.44 mg/pot) due to inoculation of local *Azotobacter* isolate AZOT-B-33 followed by uninoculated treatment C-I (1259.52 mg/pot) with 120:60:80 NPK level. Significant

increase in N-uptake by tomato crop varied from 341.10 (C-III) to 1392.44 mg/pot (AZOT -B-33), The local *Azotobacter* isolate AZOT-B-33 alone significantly superior over control treatment C-I and standard check. However, the control treatment C-I was significantly superior over standard check. *Azotobacter* isolates AZOT-B-32 and 18 were par with control treatment C-II (115:60:80 NPK). The study indicate that nitrogen accumulation in the crop was increased by inoculation with local *Azotobacter* isolates and standard check. The isolate AZOT-B-33 was most effective inoculant for enhancing fruit yield of tomato and the plant nitrogen accumulation. The possible mechanisms that facilitated greater nitrogen uptake by crops include nitrogen fixation (N_2), the delivery of combined nitrogen to the plant, production of phytohormone-like substances that alter plant growth and morphology, and bacterial nitrate reduction, which increases nitrogen accumulation in inoculated plants (Zhang et al., 2022). Verma et al. (2019) also reported that the presence of *Azotobacter* increased nitrogen content in plants rather than phosphorus.

Interaction with *Fusarium*: Out of seven local *Azotobacter* isolates studied, four have shown complete inhibition of the growth of the pathogen (*Fusarium oxysporium*) (Table 2, 3, 4 and Plate 1). The standard check has also shown complete suppression of the fungus. The promising local isolates of *Azotobacter* AZOT-B-33 and 32 were most effective for hundred percent inhibition of *Fusarium oxysporium* (Plate 5). Two other local isolates (AZOT-B-18 and 123) also exhibited hundred percent performance to control *Fusarium oxysporium*. The three local *Azotobacter* isolate AZOT-B-35, 39 and 109 although were significantly superior over control with respect to inhibition of fungal growth but were inferior to isolate AZOT-B-32, 18, 123, 33 and standard check.

Table 3. Effect of different local isolates & standard check of *Azotobacter* on *Fusarium oxysporium*

<i>Azotobacter</i> isolates	<i>Fusarium oxysporium</i> growth in millimeters (mm)
AZOT-B-35	18.00
AZOT-B-32	00.00
AZOT-B-18	00.00
AZOT-B-39	12.00
AZOT-B-123	00.00
AZOT-B-33	00.00
AZOT-B-109	15.00
Standard check	00.00
Control	90.00
CD (p=0.05)	1.05

Table 4. Influence of *Azotobacter* isolates and different levels of nitrogen on N-accumulation by tomato fruits and shoot at harvest ((mg / pot)

Treatment	Treatment	Fruits	Shoot	Total (fruit+ shoot)
T ₁	100:60:80 + AZOT-B-35	238.43	381.27	619.71
T ₂	100:60:80 + AZOT-B-32	512.91	496.68	1009.59
T ₃	100:60:80 + AZOT-B-18	430.97	487.14	918.12
T ₄	100:60:80 + AZOT-B-39	391.71	433.94	825.65
T ₅	100:60:80 + AZOT-B-123	322.45	407.89	730.35
T ₆	100:60:80 + AZOT-B-33	797.26	595.19	1392.44
T ₇	100:60:80 + AZOT-B-109	174.22	340.84	515.06
T ₈	N:P:K::100:60:80 + S.C.	610.88	503.33	1114.22
T ₉	N:P:K::120:60:80 (C-I)	716.94	542.57	1259.52
T ₁₀	N:P:K::115:60:80 (C-II)	466.83	486.69	953.52
T ₁₁	N:P:K::100:60:80 (C-III)	105.76	235.33	341.10
CD (p=0.05)		96.93	90.01	102.32

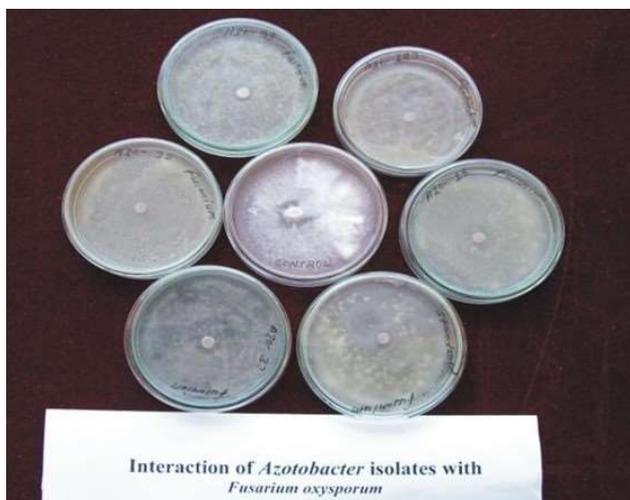


Plate 1. Antifungal activity of promising *Azotobacter* isolates and standard check in dual culture against *Fusarium oxysporium*

Mahalakshmi and Reetha (2009) also observed that six out of nine isolates of *Azotobacter* of tomato rhizosphere positive towards IAA production, phosphate solubilization, siderophore production, HCN production, ACC deaminase activity and antifungal activity. The two above isolates were effective in inhibiting the growth of fungal pathogen *Fusarium oxysporium*, causing wilt of tomato. Sharma et al. (2023) also reported that seed bacterization with *Rhizobium* inhibited the growth of *Sclerotium* spp. The fungal inhibition by *Rhizobium* isolates may be due to production of secondary metabolites with antimicrobial activities under different environment (Kaur and Seema, 2002). Similarly, *Rhizobium* and *Bradyrhizobium* strains were also significantly suppress the mycelial growth of *Fusarium* and other soil-borne pathogenic fungi under in vitro conditions (Verma et al. 2022, Singh et al., 2023, Kumar et al., 2023, Patel et al., 2023). Keeping in view of above mentioned findings, local *Azotobacter* isolate AZOT-B-33 was the most effective isolate for tomato as its inoculation showed best results. The performance of local *Azotobacter* isolate AZOT-B-33 was also significantly superior over standard check to increase yield, dry matter accumulation and nitrogen uptake by tomato crop. However, the performance of both AZOT-B-33 and standard check was at par with CI (120:60:80 NPK level), which means that these organisms were able to supplement 20 kg nitrogen per hectare. *Azotobacter* isolates AZOT-B-32 and 18 were also efficient to save 15 kg of mineral nitrogen per hectare. Similar views were also expressed by Yadav et al. (2023).

CONCLUSION

The local *Azotobacter* isolate AZOT-B-33 was identified

as the most effective for improving nitrogen content and uptake in tomato plants, as well as for inhibiting *Fusarium oxysporium*. It outperformed the standard check and other local isolates in enhancing plant growth and nitrogen utilization. About 20 kg nitrogen could be saved per hectare by using the above bio-inoculant. The findings suggest that AZOT-B-33 could be a valuable biofertilizer for tomato cultivation, potentially reducing the need for chemical fertilizers and improving disease resistance.

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