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Selection of effective *Azotobacter* isolates for tomato (*Lycopersicon esculentum* Mill)

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Abstract
A pot experiment to study the selection of effective *Azotobacter* isolates for tomato (*Lycopersicon esculentum* Mill) on yield and biomass accumulation study of tomato, nitrogen fixing efficiency of *Azotobacter* isolates and biochemical parameter of soil was conducted during 2022-2023 at the Department of Agricultural Microbiology, College of Agriculture, Raipur, C.G. Seedlings were transplanted on 28 October 2022. Plant height, number of fruits per plant, total weight of fruits per plant, fruit yield and dehydrogenase activity were determined. Dry matter of fruits per plant and dry matter of shoots per plant at harvest were recorded. It was found that application of local *Azotobacter* isolate AZOT-B-32 was best for obtaining higher values with respect to yield, biomass accumulation study, and nitrogen fixing efficiency and biochemical parameters of soil.

Keywords: Tomato, nitrogen fixing efficiency, plant height, fruit yield, dehydrogenase activity

Introduction
Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crops. It belongs to family solanaceae and is believed to be a native of western South America. This crop is also known as an industrial crop because of its outstanding processing qualities. Tomato (Solanum lycopersicum) is one of the most significant vegetable crops in India, ranking second globally in terms of both production and area. China is the world's greatest producer, accounting for 27.8% of total production, followed by India (11.2%) (Kumar *et al*., 2016; Harisha *et al*., 2019; Gupta *et al*., 2021) [12, 9, 8]. Tomato It is a good source of B, C, and Fe vitamins. Energy (18 kcal), proteins (0.95 g), fats (0.11 g), carbohydrates (4.01 g), total sugars (2.49 g), calcium (11 mg), iron (0.68 mg), magnesium (9 mg), phosphorus (28 mg), potassium (218 mg), sodium (11 mg), zinc (0, 14 mg), vitamin A (1100 I.U.), vitamin C (22.8 mg), thiamin (0.036 mg), riboflavin (0.022 mg), vitamin B6 (0.079 mg), vitamin E (0.56 mg), lycopene (20 -50 mg), titratable acidity (7.5-10 mg /100 ml), total solids (4-7%), fiber (0.6 g), total saturated fatty acids (0.015 g), and total polyunsaturated fatty acids (0.044 g) are all present in an edible portion of tomatoes.Several epidemiological studies indicated beneficial effects of tomato consumption in the prevention of some major chronic disease, such as cancer and cardiovascular disease (Anon., 2013) [4].

In India, it is mainly grown in Bihar, Karnataka, Uttar Pradesh, Orissa, Andhra Pradesh, Maharashtra, Madhya Pradesh, Assam and Chhattisgarh, accounting for growing on 86.5 million hectares, it yields 210.56 million tons of output with a productivity of 24.34 MT/ha. The main states that produce tomatoes are Tamil Nadu, Odisha, and Madhya Pradesh. Gujarat, Karnataka, Andhra Pradesh, Maharashtra, West Bengal, Bihar, and Chhattisgarh (Anon., 2021) [3]. During last one hundred years, large numbers of aerobic and anaerobic bacteria have been identified as free-living nitrogen fixers. Their N fixing potential ranging from 2mg to 25mg per gram of carbon source utilized. Amongst these potential N-fixer *Azotobacter* is one that fixes nitrogen in non-legumes. *Azotobactor* is a heterotrophic free living nitrogen fixing bacteria present in alkaline and neutral soils. *Azotobactor chrococcum* is the most commonly occurring species in arable soils of India. Apart from its ability to fix atmospheric nitrogen in soils, it can also synthesize growth promoting substances *viz*., auxins, and gibberellins and also to some extent the vitamins.

Many strains of *Azotobactor* also exhibit fungicidal properties against certain species of fungus. Response of *Azotobactor* has been seen in rice, maize, cotton, sugarcane, pearl millet, vegetable and some plantation crops. Its population is very low in uncultivated lands. Presence of organic matter in the soil promotes its multiplication and nitrogen fixing capacity.

Area of Chhattisgarh state with is bigger than many states of atmospheric N_2 fixing and P mobilizing microbial inoculation, as has a demand for identified by analysis of soil samples of various district of this state. The low population density of above heterotrophs are mainly due to high air temperature up to 48 ºC, soil surface temperature beyond 60 ºC and low humidity up to 3-4% for prolonged period of summer season resulting to loss of organic matter and population of beneficial microbes. *Azotobacter* spp. is also sensitive to acidic pH, high salts and temperature above 35 ºC, so its population is very poor in soils of Chhattisgarh. The soil of Chhattisgarh is low to medium in available nitrogen thus N is one of the most limiting plant nutrients. In the light of ever increasing prices coupled with increasing demand of chemical fertilizers and depleting soil fertility necessitates developing effective bioinoculant of *Azotobacter* for tomato crop. In this view of above it may worthwhile to develop the specific location effective *Azotobacter* isolates for tomato.

Materials and Methods

The Experiments were conducted in soil microbiology laboratory and glass house of department of agricultural microbiology, College of Agriculture, Raipur, during 2022-23. The experimental farm is located at 21º16′ N latitude and 81º36′ 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 is located at 21º16′ N latitude and 81º36′ E longitude with the maximum temperature rising up to 60 ºC during summer. The mean annual rainfall of 1200-1300mm, about 85% is received during third week of June to mid-September. The recommended additional rate of fertilizer for tomato is 120:60:80 kg.ha^{-1} of N, P_2O_5 and K_2O, respectively. The cultivar used in the study was C.V. Pusa Rubi. Eleven treatments, complete in a randomized block design included: T_1 (AZOT-B-35+100:60:80 NPK), T_2 (AZOT-B-32+100:60:80 NPK), T_3 (AZOT-B-18+100:60:80 NPK), T_4 (AZOT-B-39+100:60:80 NPK), T_5 (AZOT-B-123+100:60:80 NPK), T_6 (AZOT-B-33+100:60:80 NPK), T_7 (AZOT-B-109+100:60:80 NPK), T_8 (IARI, *S.C.*+100:60:80 NPK), T_9 (control-I+120:60:80 NPK), T_{10} (control-II+115:60:80 NPK) and T_{11} (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 and were taken for the study. 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, P_2O_5 and K_2O, respectively. The soil was vertisol.

Results and Discussion

In the present study a total of forty local *Azotobacter* isolates and standard *Azotobacter* IARI isolate (standard check) were tested as nitrogen fixing efficiency of *Azotobacter* isolates with respect to plant height, fruit yield and biomass accumulation study of tomato. The results obtained from these studies are as follows

Nitrogen fixing efficiency of *Azotobacter* isolates

The nitrogen fixing ability of local *Azotobacter* isolates and standard check was tested for initial screening of the isolates. The range of nitrogen quantity fixed in the N-free Jensen's liquid medium varied from 2.35 to 13.45 mg N/gm of sucrose (0.0047 to 0.0269% N) after seven days of incubation. Three local *Azotobacter* isolates i.e. AZOT-B-33, 32 and 18 were found at par with standard check (standard *Azotobacter* IARI isolate). Among all isolates, isolate number 33 fixed maximum quantity of nitrogen in the medium i.e. 13.45 mg N/gm sucrose (0.0269% N), followed by isolate No.32 which fixed 13.15 mg N/gm sucrose (0.0263% N) after seven days of incubation. The standard check released 13.10 mg N/gm sucrose (0.0262% N) after seven days of incubation. Agrawal and Singh (2002) [2] also conducted similar type of experiment to select effective *Azotobacter* strains by their nitrogen fixing capacity, growth and survival under stress environment.

Plant height

The data pertaining to plant height study is presented in Table 2. The application of different local isolates and standard check, compared with controls, maximum plant height (53.32 cm) was observed in the plants grown with 100:60:80:: NPK and local isolate AZOT-B-33 which was found at par with treatment CI. Minimum plant height 40.25 cm was recorded in the treatment CIII i.e. 100 kg N. As compared to CIII, significantly highest plant height was observed in AZOT-B-33 with 100:60:80 NPK level, followed by standard check at the same level of nitrogen. All the local *Azotobacter* isolates and standard check with 100:60:80 NPK level showed at par plant growth with control I (120:60:80 NPK).The inoculations with different *Azotobacter* isolates were highly effective in increasing the height of plants. Mahato *et al*. (2009) [13] observed that application of *Azotobacter* increased the shoot length and more number of leaves per plant. This observation was also in line with that of Martinej *et al.* (1993) [14] and Umar *et al.* (2009) [18] who clearly mentioned that application of *Azotobacter* resulted increase of shoot length and more number of leaves.

Fruit number

The data on the influence of different local *Azotobacter* isolates and different levels of nitrogen on fruit number of tomato is presented in Table 1. Maximum number of fruits per plant was recorded due to inoculation of local isolate AZOT-B-33 (22.90) followed by control C-I (120:60:80 NPK) (20.86) and standard check inoculated plants (20.83). The number of fruit increased significantly in plants due to inoculation with local local isolate AZOT-B-33 over standard check at the same level of nitrogen (100kg N) and also over higher nitrogen doses, i.e.115 (C-II) & 120 kg /ha (C-I).

Fruit weight

The data on fruit weight of tomato presented in Table 1 and illustrated, which revealed that inoculation with all local *Azotobacter* isolates significantly increased the fruit weight over control CIII (100:60:80). The highest fruit weight was observed under treatment 100:60:80 NPK + AZOT-B-33 (552.02 gm/plant) followed by treatment C–I (120:60:80NPK) (518.58 gm) and standard check (505.13 gm) with100:60:80 NPK level. Higher fruit yield of tomato in *Azotobacter* inoculated plants is associated with the efficient fixation of nitrogen as well as metabolic products of *Azotobacter* like gibberellins, indole acetic acid and cytokinin might have helped in inducing early flowering, fruit setting, fruit picking and also increased number of flowers and fruits per cluster (Bhadoria *et al.*, 2007) [5]. This view was corroborated with the observations of Jackson *et al.* (1964) [11] and Aeon & Barea (1975) [1] who mentioned that favorable environment, as the roots provide through proper aeration for better bacterial activity resulting in more nitrogen

fixation and higher growth attributes with seedling inoculation with *Azotobacter* as compared to soil inoculation with *Azotobacter*. Tilak *et al.* (2005) [17] through their detailed study on soil health supporting bacteria concluded that yield improvement of crops is attributed more to the ability of *Azotobacter* to produce plants growth promoting substance such as phytoharmonce IAA and siderophore azotobactin, rather than to diazotrophic activity.

Fruit dry matter

The results on the effect of *Azotobacter* inoculation with local isolates as well as standard *Azotobacter* check on fruit dry matter yield are presented in Table 1. The results clearly elucidate that inoculation of tomato seedlings with local *Azotobacter* isolates and standard check with NPK level of 100:60:80 significantly increased the dry matter accumulation by fruit over only application of fertilizer @ 100:60:80 (C-III). Highest dry biomass of fruit was found with isolate No. AZOT-B-33 (40.85 gm/pot), followed by uninoculated control C-I (37.34 gm) with fertilizer dose of 120:60:80 kg NPK. The local *Azotobacter* isolate AZOT-B-33 significantly accumulated higher fruit dry matter over inoculation of standard check. Simultaneously the same isolate AZOT-B-33 and standard check was found at par with the performance of highest nitrogen dose i.e. 120 kg/ha to increase the fruit dry matter yield.

Shoot dry matter

The effect of inoculation with different local *Azotobacter* isolates vis-à-vis different levels of nitrogen on shoot dry matter yield was recorded and tabulated in Table 1. Data showed that Highest biomass accumulation (75.10 gm/pot) was recorded in treatment T_6 (Plate) which received local *Azotobacter* isolate AZOT-B-33, followed by control CI (120:60:80) (70.50 gm/pot), standard *Azotobacter* check (68.72gm) and treatment T_2 with local isolate AZOT-B-32(68.00gm/pot). The dry matter yield of shoot increased significantly in plants due to inoculation with local isolate AZOT-B-33 over standard *Azotobacter* check in presence of same level of nitrogen. This increase in plant biomass might be due to the impact of *Azotobacter* on tomato plants. Plant growth promoting rhizobacteria use one or more of direct or indirect mechanisms of action to improve plant growth and health. Biological N-fixation, P- solubilisation, improvement of other plant nutrients uptake and phytohormone production like indole-3-acetic acid are some examples of mechanisms that directly influence plant growth (Glick *et al.*, 1995) [7]. Similar findings were also reported by Puertas and Gonzales (1999) [15] who clearly mentioned that the inoculation with *Azotobacter* alone significantly increased the root depth, shoot height, fresh and dry weights of root and shoots of tomato. Biological control of plant pathogens and deleterious microbes, through the production of antibiotics, lytic enzymes, hydrogen cyanide and siderophores or through competition for nutrients and space can improve significant plant health and promote growth as evidenced by Increases in seedling emergence, vigor and yield (Hilal *et al.*, 1997) [10].

Dehydrogenase activity

The analysis data related to biochemical property of soil due to inoculation of local *Azotobacter* isolates and standard check of *Azotobacter* are presented in Table 2. It is apparent from the data that inoculation of tomato seedling roots with crop beneficial bacterium *Azotobacter* significantly increased the activity of dehydrogenase enzyme (DHA) in soil at 30 DAT over uninoculated control C-III. It is clear from the data that highest value of DHA was found due to local *Azotobacter* isolate AZOT-B-33 (42.95 µg TPF/h/g soil), followed by standard check of *Azotobacter* (41.34 µg TPF). Lowest DHA was recorded in uninoculated control pot C-III. Significant increase in DHA of soil was noticed which varied from 25.57 µg TPF (C-III) to 42.95, 41.34, 40.21, 37.46, 35.91, 35.36, 33,50 and 32.63 µg TPF /h/g soil due to inoculation of crop with AZOT-B-33, standard check, AZOT-B-32, 18, 39, 123, 35 and 109, respectively. The local *Azotobacter* isolate AZOT-B-33 was found significantly superior over all the three uninoculated control C-I, II & III but at par with the standard check. The dehydrogenase activity of local *Azotobacter* isolate AZOT-B-32 was found significantly superior over uninoculated control C-II & C-III but found at par with C-I (120:60:80 NPK).Enzymes in the soil are biologically significant as they participate in various transformations and influence the availability of plant nutrients. The dehydrogenase enzyme systems apparently fulfill a significant role in the oxidation of soil organic matter as they transfer hydrogen from substrates to acceptors. Many different specific dehydrogenase systems are involved in the dehydrogenase activity in soils; these systems are an integral part of the microorganisms. Therefore, the result of the assay of dehydrogenase activity would show the average activity of the active population. also expressed the similar views and mentioned that in soil microorganisms, active roots and dead cells are the principal sources of enzymes. were also of the opinion that the increase in dehydrogenase activity was mainly due to the higher microbial population. The earlier studies revealed that the enzyme activities are often used as indices of microbial growth rather than the microbial number, which further may reflect the microbial respiration and the potential capacity of soil to perform biological transformations of importance to soil fertility.

Table 1: Influence of various *Azotobacter* isolates and different levels of nitrogen on fruit yield and dry matter yield of tomato

Treatment Number	Treatment	No. of fruit per plant	Fruit weight per plant (gm)	Fruit dry matter (gm/pot)	Shoot dry matter (gm/pot)
T_1	100:60:80 + AZOT-B-35	15.16	325.33	15.94	59.69
T_2	100:60:80 + AZOT-B-32	19.30	460.78	29.03	68.00
T_3	100:60:80 + AZOT-B-18	18.83	430.45	24.97	67.49
T_4	100:60:80 + AZOT-B-39	19.00	428.07	23.54	62.13
T_5	100:60:80 + AZOT-B-123	17.16	379.06	20.09	60.83
T_6	100:60:80 + AZOT-B-33	22.90	552.02	40.85	75.10
T_7	100:60:80 + AZOT-B-109	13.83	292.37	13.74	56.89
T_8	100:60:80 + *S.C.*	20.83	505.13	34.35	68.72
T_9	N:P:K::120:60:80 (C-I)	20.86	518.58	37.34	70.50
T_{10}	N:P:K::115:60:80 (C-II)	19.55	454.54	27.73	67.54
T_{11}	N:P:K::100:60:80 (C-III)	10.16	211.63	9.74	45.25
	C.D. (0.05)	2.02	45.78	3.78	6.34

Table 2: Influence of various *Azotobacter* isolates and different levels of nitrogen on plant height and dehydrogenase activity in soil at 30DAT of tomato

Treatment Number	Treatment	Height of tomato shoot (cm)			Dehydrogenase activity (µg TPF / h / g soil)
		At 30 DAT	At 60 DAT	At 90 DAT	At 30DAT
T_1	100:60:80 + AZOT-B-35	12.58	45.85	70.65	33.50
T_2	100:60:80 + AZOT-B-32	13.25	49.25	77.43	40.21
T_3	100:60:80 + AZOT-B-18	13.00	47.67	71.23	37.46
T_4	100:60:80 + AZOT-B-39	13.08	48.25	71.97	35.91
T_5	100:60:80 + AZOT-B-123	12.87	47.10	71.27	35.36
T_6	100:60:80 + AZOT-B-33	13.10	53.32	79.67	42.95
T_7	100:60:80 + AZOT-B-109	12.67	44.56	69.43	32.63
T_8	100:60:80 + *S.C.*	13.25	51.25	76.37	41.34
T_9	N:P:K::120:60:80 (C-I)	14.17	51.02	74.00	37.84
T_{10}	N:P:K::115:60:80 (C-II)	13.65	48.42	72.43	33.63
T_{11}	N:P:K::100:60:80 (C-III)	9.65	40.25	63.68	25.57
	C.D. (0.05)	N.S.	6.66	8.15	4.45

Conclusion

The present study evaluated the nitrogen-fixing efficiency of forty local Azotobacter isolates and a standard Azotobacter IARI isolate in tomato plants, focusing on their impact on plant height, fruit yield, and biomass accumulation. The results indicate that several local Azotobacter isolates, particularly AZOT-B-33, demonstrated nitrogen fixation comparable to the standard check. In terms of plant height, AZOT-B-33 significantly increased growth parameters, surpassing both uninoculated controls and standard check treatments. Moreover, inoculation with AZOT-B-33 led to a substantial increase in fruit number and weight, as well as enhanced fruit and shoot dry matter accumulation compared to controls. Additionally, soil enzyme activity, specifically dehydrogenase, was significantly enhanced by AZOT-B-33 inoculation, indicating improved soil health. These findings underscore the potential of local Azotobacter isolates, especially AZOT-B-33, as effective bioinoculants for enhancing tomato productivity through nitrogen fixation and growth promotion mechanisms. Further studies could explore broader applications and field trials to validate these results across different agro-ecosystems.

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