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Response of liquid biofertilizers on growth and yield of brinjal (*Solanum melongena* L.) crop

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Abstract
Biofertilizer is natural and low cost fertilizer which is containing a large population of specific or group of microorganism. Therefore, the present study was carried out with the aim to examine *Azotobacter sp*. and *Azospirillum sp.* as the liquid biofetilizer on growth, quality and yield of brinjal (*Solanum melongana* L.). The brinjal seeds were treated with liquid biofertilizer before sowing on nursery bed, whereas the NPK was applied in four different rates (25, 50, 75 and 100%). Observation was recorded at different time intervals as 30, 60 and 90 days. Maximum growth was observed in treatment T_{15} which comprised of 100% of NPK along with *Azotobacter* and *Azospirillum*. The experimental results revealed significant variations among the treatments in respect of morphological characters i.e. plant height (58.63 cm) number of branches (13.33), number of leaves (15.33), number of flowers (22.33), number of fruits (13.67), fruit length (14.73 cm), fruit girth (8.00 cm) fruits weight (118.00 g) and physiological characters like leaf area (159 cm^2) and dry weight (9.17 g). Thus, it can be concluded that for obtaining maximum yield as well as profit from brinjal, seeds can be inoculated with liquid bio-fertilizers of *Azotobacter* and *Azospirillum*.

Keywords: *Azotobacter sp*, *Azospirillum sp*, brinjal, crop growth, yield

Introduction
Brinjal eggplant (*Solanum melongena* L.) is a warm-weather crop mostly cultivated in tropical and subtropical regions of the world. Two other cultivated eggplant species, the scarlet eggplant (*S. aethiopicum* L.) and the gboma eggplants (*S. macrocarpon* L.), are less known but have local importance in sub-Saharan Africa Schippers, 2000; Daunay and Hazra, (2012). India contributes 13.44 million tonnes to the global production of brinjal in 2014-2015 and ranks second to China (NHB, at a galance 2015). Based on data from 2014, the global production of eggplant is around 50 million tons annually, with a net value of more than US$10 billion a year, which makes it the fifth most economically important solanaceous crop after potato, tomato, pepper, and tobacco (FAO, 2014) [7]. India occupies a prime position in the world in vegetable production and is the 2nd largest producer of vegetables next only to China; India produces about 125.88 million tons of vegetables from an area of 7.80 million hectares which is far below the desired requirement to fulfil the need of the growing population. (Anonymous, 2008) [2]

They are also a rich source of potassium, magnesium, calcium and iron. Fertilizers application is a necessary condition for good yield of crop due to inherent low status of the soil. The use of fertilizer is reported to be responsible for over 50 percent yield increase in crops. However, the rising cost of chemical fertilizer has further focused attention on the cycling of plant nutrients through organic materials. The crop is widely grown all over India for its immature tender fruits, which are used as vegetable in variety of ways. It has got potential as raw material in pickle making and dehydration industries Singh *et al.* (2006) [15]. The productivity of brinjal can be increased by using several techniques *viz.*, organic farming, integrated nutrient management and good hybrid seeds. Since the nutrient turnover in soil plant system is considerably high in intensive vegetable cultivation, neither the chemical fertilizer nor the organic manure alone can help achieve sustainable production (Khan *et al*. 2008) [8].

Brinjal can be grown in all types of soils. However, it grew best in loose, friable, well- drained silt loam or clay loam soils rich in organic matter. Biofertilizers are the bioinoculants of specific beneficial microorganisms that promote the growth of plant crops by converting the unavailable form of nutrients into available form. These biofertilizers also induce resistance in plants against pests, to improve soil fertility, to help plant growth by increasing the number and biological activity of desired microorganisms in the root surface Sivasakthivelan, and Saranraj (2013) [16]. On the other hand cost of agricultural inputs including chemical fertilizers has risen, due to which marginal farmers are certainly get affected economically. Further, depletion of non-renewable energy sources like petroleum and coal will put a lot of stress on intensive agriculture. Under these circumstances, biofertilizer can definitely come to our rescue as effective, cheap and eco-friendly substitute of costly chemical fertilizers. Therefore, addition of biofertilizers is the preferable remedy for supplementation and improvement of soil fertility as well as productivity at Indian soil. Bio fertilizers improve the quantitative and qualitative features of many plants Yosefi *et al*. (2011) [19].

Azotobacter is Gram -ve bacteria, polymorphic having different sizes and shapes. Their size ranges from 2-10x1-2.5 µm, young cell possess peritrichous flagella as locomotive organ. Old population of bacterial cells includes encapsulated forms and have enhanced resistant to heat, desiccation and adverse conditions. The cyst germinates under favorable conditions to give vegetative cells. Also produce polysaccharides. *Azotobacter spp.* is sensitive to acidic pH, high salts, and temperature above 350C. *Azotobacter spp.* is free living bacteria which grow well on a nitrogen free medium. These bacteria utilize atmospheric nitrogen for their cell protein synthesis. This cell protein is then mineralized in soil after the death of *Azotobacter* cells, thereby contributing towards the nitrogen availability to the crop plants.

Under certain environmental and soil conditions, *Azospirillum* can positively influence plant growth, crop yields and N-content of the plant. This plant stimulatory effect exerted by *Azospirillum* has been attributed to several mechanisms, including biological nitrogen fixation and auxin production. *Azospirillum sp.* colonizes the plant roots and stimulates plant growth. The geneus *Azospirillum* has many species such as *A.amazonense, A. halopraeferens, A.braisilense and A.lipoferumare* and are distributed worldwide (Arun, 2007). It is free-living bacteria and widely distributed in soils of tropical and subtropical climate in the roots of grasses of great economic importance. About 30 to 90% of soil samples collected from different part of the globe had *A. brasilense* or *A. lipoferum*. Auxins are the most abundant phytohormone secreted by most plant-associated bacteria. *Azospirillum spp.* are known for the production of indole-3- acetic acid, gibberellic acid and kinetin whereas *Azotobacter chroococcumis* identified to produce, gibberellic acid, indole-3- acetic acid and cytokinnin. PGPR alter root growth in grasses by producing phytohormone. Cassan *et al.* (2009) [4] described the same effect via legume seedlings inoculation with *Azospirillum brasilense* and *Bradyrhizobium japonicum*. PGPR strains especially *Azospirillum spp.*, from wheat rhizosphere soil. Among PGPR, *Azospirillum* is considered as an important genus which is closely-associated with plants and shows potential to degrade organic contaminants, improve the plant health and increase crops yield Rasool *et al*. (2015) [14]. The present study was carried out to determine the effect of liquid biofrtilizer on seed germination and to evaluate the efficacy of liquid biofertilizer on plant growth and yield of brinjal crop.

Materials and Method

Place of the work

The present study entitled "Response of liquid biofertilizer on growth and yield of brinjal (*Solanum melongena* L.) crop" was conducted at Department of Industrial Microbiology, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad. Procurement of bacterial strain Bacterial strain *Azotobacter spp*. (MCCB NO. 0461) and *Azospirillum spp*. (MCCB NO.0463) was procured from Microbial Culture Collection Bank (MCCB) Department of Industrial Microbiology, Sam Higginbottom University of Agriculture Technology and Sciences, (SHUATS) Allahabad.

Preparation of liquid biofertilizers and seed treatment with biofertilizers formulation

For the production of liquid biofertilizers 100 ml of Ashby's broth pH of the broth medium was adjusted to 8.3 by using acid and base, medium was sterilized at 121 °C for 20 min. at 15 lbs and utilized for the production of biofertilizers. The inoculums were prepared in Ashby's broth by using pure culture of *Azotobacter sp.* and *Azospirillium sp*. The loopful of pure cultures of *Azotobacter sp.* and *Azospirillium sp*. were transferred in Erlenmeyer flasks 100 ml of Ashby's broth and flasks were incubated at 28 °C for 120 hour on a rotary shaker (180 rpm). Seeds were treated with 20ml/kg of liquid inoculants when the colony count reached to 10^4-10^5 cfu/ml of the broth and was used as inoculants to treat seeds. For treatment of seeds the concentration of liquid biofertilizers were used as followed *Azotobacter* 0.25ml/10g, *Azospirillum* 0.25ml/10g and *Azotobacter* + *Azospirillum* 0.25ml/1og.

Seed germination test

The number of seedlings emerged in each plot were counted after 25 days of sowing and germination percentage was estimated by using the formula. Abdul Balli and Anderson (1973)

$$\text{Germ. \%} = \frac{\text{No. of germinating seedlings}}{\text{No. of seeds sown}} \times 100$$

Observation Recorded

The following growth, yield and quality parameters of brinjal were observed and recorded with the respective plants under investigation. Every 30 days the plant was measured with the respect to plant height, number of branches, number of leaves, leaf area, number of flowers, number of fruits, fruit length, fruit girth, fresh weight of fruits and dry matter.

Statistical Analysis

The experiment was performed in Completely Randomized Design (CRD) with 3 replications by using the standard statistical method as described by Panse and Sukhatme (1985) [12] for statistical significance.

Results and discussion

Initial microbial count of liquid *Azotobacter sp.* and *Azospirillum sp.*

The data presented in the plate (plate no. 1) initial stage of liquid biofertilizers before transplanting seedlings in pots, number of colony forming unit (C.F.U.) were expressed as logarithms per 1 ml broth and the bacterial activity was evaluated. The average counts of total bacteria *Azotobacter sp.* population at initial stage was ranged from (15.00 × 10^5C.F.U./ml). Whereas, the *Azospirillum sp.* population at initial stage was ranged from (12.00 × 10^5C.F.U./ml) was recorded at initial stage.

Initial microbial population of production medium.

SR. NO.	Bacteria	C.F.U.
1.	*Azotobacter sp.*	15 × 10^5
2.	*Azospirillum sp.*	12 ×10^5

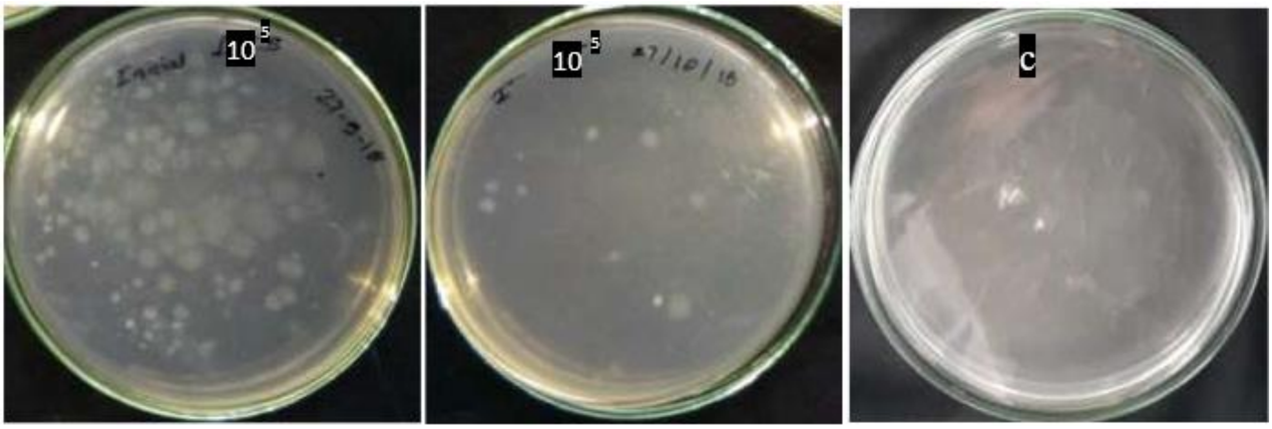

Plate 1: Initial microbial population

Effect of liquid biofertilizers under varying levels of chemical fertilizers on growth and yield parameters

The data presented in Table 1, 2 and 3 revealed that all the vegetative parameters of brinjal were significant variations among the treatments except number of branches were found to be non- significant. Among all the following treatments, the treatment T_{15} (*Azotobacter* + *Azospirillum* + 100% NPK) shows the better results.

Plant height (cm) at 30 DAT was recorded at T_{15} (29.07 cm) followed by T_{14} (25.97 cm) and minimum was recorded in T_8 (22.67cm). At 60 DAT the significant increase in plant height was recorded in the treatment T_{15} (47.80 cm) followed by T_7 (44.93 cm) and the minimum was recorded in T_8 (40.83 cm). At 90 DAT the maximum plant height was recorded in treatment T_{15} (58.63 cm) followed by T_{10} and T_{14} (56.63 and 56.30 cm) which were at par with each other and the minimum was recorded in T_8 (52.17 cm). Similar results were found by Doifode and Nandkar (2014) [5] height of the plant studied from 30 to 90 Days After Planting (DAP). The maximum plant height at 90 DAP was found in (*Azotobacter* + *Azospirillum*+ NPK), whereas the minimum was in control.

The maximum number of branches at 30 DAT was recorded in the treatment T_5 (6.33) followed by T_7 (6.00) and the minimum was recorded in T_0 Control (4.00). At 60 DAT the maximum number of branches was recorded in T_1 (10.67) followed by the treatments T_5 (10.33) and the minimum was recorded in T_0 (7.30). At 90 DAS the maximum number of branches was recorded in T_{15} (13.33) followed by T_1 (13.00) and the minimum was recorded in T_0 (11.33). Similar results were found by Naseeruddin *et al*. (2016) [11] reported the maximum number of branches/plant (8.23) was recorded under treatment T_8 (NPK 100% + *Azotobacter)* followed by (8.15 cm) T_{10} (NPK 50% + Vermicompost), whereas the minimum number of branches/plant (4.65) was recorded under treatment T_0 (control).

The maximum number of leaves at 30 DAT was recorded in T_{15} (6.67) followed by T_1 and the treatment T_{14} (5.67) showed statistically similar and significantly higher number of leaves and the minimum was recorded in T_0 (4.33). At 60 DAT the maximum number of leaves was recorded in T_{15} (12.67) followed by T_9, T_{10} and T_{11} (11.33) showed similar and the minimum was recorded in T_0 control (8.67). At 90 DAT the maximum number of leaves was recorded in T_{15} (15.33) followed by T_5 (14.67) and the minimum was recorded in T_0 (12.67). The maximum leaf area at 30 DAS was recorded in T_{15} (64.93 cm^2) followed by T_{14} (63.83 cm^2) and the minimum was recorded in T0 (42.27 cm^2). At 60 DAT the maximum leaf area was recorded in T_{13} (130.33 cm^2) followed by treatment T_6 and T_{15} and the minimum was recorded in treatment T_0 control (104.17 cm^2). At 90 DAT the maximum leaf area was recorded in the treatment T_{13} (162.37 cm^2) followed by T_{14} (159.00 cm^2) and the minimum was recorded in T_0 control (136.17 cm^2). Similar results were found by Kumar *et al.* (2013) [9] All the growth attributes like plant height, leaf area and number of leaf per branch were significantly influenced by the fertility levels, FYM and *Azotobacter*, Leaf area increased significantly with corresponding increase in fertility levels up to 100% NPK leaf area (181.8 cm^2).

The numbers of flowers at 60 DAT were non-significant and at 90 DAS the maximum number of flowers was recorded in treatment T_7 followed by T_{11} and the minimum was recorded in treatment T_0 control.The maximum number of fruits observed at 60 DAT was recorded in T_6 (7.33) followed by T_{14} (6.67) and the minimum was recorded in T_0 (3.67). At 90 DAT the maximum number of fruits was recorded in T_{14} (14.67) followed by T_7 (13.67) and the minimum was recorded in T_0 (8.00). The maximum fresh weight of fruit was recorded in T_{15} (118 g), followed by T_{13} (116.86 g) and the minimum weight was found in T_0 (99.37 g). Similar findings were observed by Wange and Kale (2003) [18] and Sengupta *et al*. (2002) the increase in fruit weight may be due to the effect of liquid biofertilizer which stimulate the higher production of fruits. This is in accordance with the results reported by in okra and tomato respectively. The maximum fruit length was recorded in T_{15} (14.73 cm) followed by T_{11} (14.67) and the minimum was found in T_0 (9.33 cm). However, the findings are in accordance with the results of Ercan Y. *et al.* (2012) [6] and Patiel *et al.* (2000) in brinjal and okra respectively. Different levels of inoculums i.e. *Azotobacter*, *Azospirillum* and NPK also gave similar response towards fruit length. The maximum fruit girth was recorded in T_{15} (8.43 cm), followed by T_7 (8.00 cm) and the minimum was found in T_0 (6.30 cm). The results were similar to that of Narayan *et al.* (2007) [10] showed treatment with *Azotobacter*, PSB along with application of chemical fertilizers in tomato and Singaravel *et al*. (2008). It is evident from the results that fruit yield per kg was significantly influenced by the treatment with liquid inoculums along with NPK. Among different treatments, the highest fruit yield per plant was recorded in T_{15} (1767.67 g) followed by T_9 (1666.67 g). Whereas, the minimum fruit yield per plant was obtained in treatment T_0 Control (933.33 g). The results of the present study are in conformity with those of Bashan *et al*. (1989) [3], Raut and Ghosikar (1980) and Mishra and Patjoshi (1995) in tomato, brinjal and okra, respectively. The maximum total dry matter was recorded in T_{15} (9.17 g), followed byT_{11} (*Azospirillum* + 100% NPK) (9.07 g), T_{14} (6.97 g) and the minimum dry matter was found in T_0 (6.77 g)

Table 1: Effect of bio-fertilizer with chemical fertilizer on vegetative growth of the plant of brinjal (*Solanum melongena* L.)

Treatment details	Plant height (cm) per plant			Number of branches per plant			Number of leaves		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T_0 (Uninoculated control)	25.23	44.33	55.00	4.00	7.33	11.33	4.33	8.67	12.67
T_1 (*Azot.*)	25.20	42.43	52.90	5.00	10.67	13.00	5.67	9.33	12.67
T_2 (*Azos.*)	24.10	42.83	53.40	4.33	9.33	13.00	5.33	9.67	14.33
T_3 (*Azot.* + *Azos.* (1:1 ratio)	25.30	43.27	54.80	4.00	9.33	12.00	5.33	10.33	13.33
T_4 (*Azot.* + 25% NPK)	25.03	44.03	54.67	4.67	9.67	12.33	4.33	10.33	12.67
T_5 (*Azot.* + 50% NPK)	25.63	44.17	54.83	6.33	10.33	11.67	5.67	10.67	14.67
T_6 (*Azot.* +75%NPK)	25.60	44.30	55.73	4.67	9.33	12.67	4.67	10.67	14.33
T_7 (*Azot.* + 100% NPK)	25.57	44.93	55.00	6.00	10.00	11.67	5.33	11.33	14.67
T_8 (*Azos.* + 25% NPK	22.67	40.83	52.17	5.00	9.00	12.33	4.67	10.67	13.67
T_9 (*Azos.* + 50% NPK)	25.13	42.97	54.83	6.00	10.33	13.00	4.67	11.33	14.33
T_{10} (*Azos.* +75%NPK)	25.40	43.30	56.63	6.00	10.00	11.33	4.33	11.33	13.33
T_{11} (*Azos.* + 100% NPK)	25.57	43.87	55.67	5.00	9.67	12.00	5.33	11.33	14.33
T_{12} (*Azot.* + *Azos.* + 25% NPK)	25.63	44.87	55.23	5.33	8.67	11.67	5.33	10.67	13.67
T_{13} (*Azot.* + *Azos.* + 50% NPK)	25.57	43.33	55.60	6.00	10.00	12.67	4.67	10.67	14.33
T_{14} (*Azot.* + *Azos.* + 75% NPK)	25.97	43.97	56.30	4.67	9.00	11.69	5.67	11.67	14.00
T_{15} (*Azot.* + *Azos.* + 100% NPK)	29.07	47.80	58.63	6.00	9.67	13.33	6.67	12.67	15.33
F test	S	S	S	NS	NS	NS	S	S	S
S. Ed	0.314	0.349	0.601	0.842	0.687	0.667	0.333	0.333	0.382
CD (5%)	0.905	1.005	1.732	2.424	1.980	1.920	0.960	0.960	1.100

Note: CFU/ml of liquid biofertilizers concentration were used in treatments *Azotobacter* = 12×10^5 cfu/ml, 0.25ml/10g. *Azospirillum* = 15×10^5 cfu/ml, 0.25ml/10g. and *Azotobacter* + *Azospirillum*, 0.25ml/10g.

Table 2: Effect of bio-fertilizer with chemical fertilizer on vegetative growth of the plant of brinjal (*Solanum melongena* L.)

Treatment details	Leaf area cm^2			Numbers of flowers per plant		Numbers of fruits per plant	
	30 DAT	60 DAT	90 DAT	60 DAT	90 DAT	60 DAT	90 DAT
T_0 (Uninoculated control)	42.27	104.17	136.17	3.00	12.67	3.67	8.00
T_1 (*Azot.*)	49.33	119.53	147.97	4.33	14.33	5.00	9.67
T_2 (*Azos.*)	48.63	112.60	138.70	4.00	14.67	4.67	11.33
T_3 (*Azot.* + *Azos.* (1:1 ratio)	43.53	124.10	153.70	4.33	14.67	5.00	9.67
T_4 (*Azot.* + 25% NPK)	49.20	104.40	138.67	5.33	15.33	5.33	12.33
T_5 (*Azot.* + 50% NPK)	53.63	123.27	143.40	5.67	15.33	4.67	12.67
T_6 (*Azot.* +75%NPK)	50.87	124.10	138.87	5.67	16.33	7.33	11.67
T_7 (*Azot.* + 100% NPK)	52.93	119.27	143.80	6.00	22.33	6.00	13.67
T_8 (*Azos.* + 25% NPK	46.73	120.77	148.30	7.00	17.67	4.67	9.00
T_9 (*Azos.* + 50% NPK)	56.77	122.23	153.53	5.33	17.33	5.67	10.67
T_{10} (*Azos.* +75%NPK)	41.83	123.03	146.07	5.67	17.67	4.33	10.67
T_{11} (*Azos.* + 100% NPK)	48.63	111.83	149.53	5.00	20.67	6.00	11.67
T_{12} (*Azot.* + *Azos.* + 25% NPK)	53.97	118.27	159.00	5.00	18.33	5.33	9.33
T_{13} (*Azot.* + *Azos.* + 50% NPK)	54.97	130.33	162.37	6.67	18.33	5.33	9.67
T_{14} (*Azot.* + *Azos.* + 75% NPK)	63.83	114.67	149.33	7.00	20.00	6.67	14.67
T_{15} (*Azot.* + *Azos.* + 100% NPK)	64.93	119.33	149.27	6.00	17.67	5.33	10.67
F test	S	S	S	NS	S	NS	S
S. Ed	0.734	3.420	3.187	0.812	0.645	0.661	0.425
CD (5%)	2.116	9.851	9.181	2.340	1.859	1.905	1.224

Note: CFU/ml of liquid biofertilizers concentration were used in treatments *Azotobacter* = 12×10^5 cfu/ml, 0.25ml/10g. *Azospirillum* = 15×10^5 cfu/ml, 0.25ml/10g. and *Azotobacter* + *Azospirillum*, 0.25ml/10g.

Table 3: Effect of bio-fertilizer with chemical fertilizer on vegetative growth of the plant of brinjal (*Solanum melongena* L.)

Treatments details	Average Fresh weight (g) of fruit per plant	Average Fruit length (cm) per plant	Average Fruit girth (cm) per plant	Average Fruit yield (g) per plant	Average Total dry matter (g) per plant
T_0 (Uninoculated control)	99.37	9.33	6.30	933.33	6.77
T_1 (*Azot.*)	103.67	12.33	7.63	1400.00	6.97
T_2 (*Azos.*)	106.67	11.53	7.03	1333.33	7.57
T_3 (*Azot.* + *Azos.* (1:1 ratio)	106.40	11.23	6.97	1533.33	7.33
T_4 (*Azot.* + 25% NPK)	107.83	10.00	6.87	1500.00	8.07
T_5 (*Azot.* + 50% NPK)	107.00	11.23	7.87	1500.00	7.30
T_6 (*Azot.* +75% NPK)	105.43	11.33	7.97	933.33	8.47
T_7 (*Azot.* + 100% NPK)	115.43	14.00	8.00	1600.00	8.03
T_8 (*Azos.* + 25% NPK	114.53	12.67	7.40	1100.00	7.70
T_9 (*Azos.* + 50% NPK)	105.33	12.83	7.43	1666.67	7.43
T_{10} (*Azos.* +75% NPK)	112.90	13.67	7.97	1566.67	8.60
T_{11} (*Azos.* + 100% NPK)	114.83	14.67	7.97	1533.33	9.07

T_{12} (*Azot.* + *Azos.* + 25% NPK)	111.67	11.77	7.17	1566.67	7.70
T_{13} (*Azot.* + *Azos.* + 50% NPK)	116.83	12.67	7.43	1433.33	7.67
T_{14} (*Azot.* + *Azos.* + 75% NPK)	113.43	14.33	7.63	1533.33	8.67
T_{15} (*Azot.* + *Azos.* + 100% NPK)	118.00	14.73	8.43	1766.67	9.17
F test	S	S	S	S	S
S. Ed	3.713	0.703	0.186	93.169	0.294
CD (5%)	10.697	2.772	0.535	268.390	0.847

Note: CFU/ml of liquid biofertilizers concentration were used in treatments *Azotobacter* = 12×10^5 cfu/ml, 0.25ml/10g. *Azospirillum* = 15×10^5 cfu/ml, 0.25ml/10g. and *Azotobacter* + *Azospirillum*, 0.25ml/10g

Conclusions
From the study, it is concluded that the inoculation of liquid biofertilizer singly or combined inoculation of *Azotobacter* and *Azospirillum* with full dose of NPK could enhance the germination percentage of seeds, plant growth and improvement in yield attributes in brinjal over un-inoculated control. Dual inoculation of *Azotobacter* and *Azospirillum* along with 50% and 100% NPK fertilizers gave the satisfactory results and better improvement in plant height, number of leaves, number of leaves, number of fruits etc. respectively. Seed inoculation of *Azotobacter* and *Azospirillum* was found to be best when used with NPK. Thus, it can be concluded that for obtaining maximum yield as well as profit from brinjal, seeds can be inoculated with liquid biofertilizers of *Azotobacter* and *Azospirillum*

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