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The Impact of *Azospirallum* and Nitrogen on Growth, Yield, and Nitrogen Content in Okra (*Abelmoschus esculentus*) Leaves.

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The objective of this study was to reduce nitrogen fertilizer costs and enhance both growth and nitrogen content in okra by applying lower nitrogen doses in combination with *Azospirallum*. A simple randomized pot experiment was conducted at Swami Shukdevanand College, Shahjahanpur from October to December 2023. Two control groups were established: one without nitrogen and *Azospirallum*, and another without nitrogen but with *Azospirallum*, to compare the effects of *Azospirallum* alone and in conjunction with different nitrogen doses. Nitrogen levels were assessed in dry leaves at 15, 30, and 45 days after germination (DAG). All nitrogen and *Azospirallum* treatments resulted in significantly improved growth, yield, and nitrogen content in okra leaves. The highest levels of plant growth, yield, and nitrogen content were observed in plants receiving nitrogen plus *Azospirallum*. Conversely, the lowest values for these parameters were observed in the control group (no nitrogen, no *Azospirallum*). Thus, it is recommended to use lower nitrogen doses along with *Azospirallum* seed inoculation to enhance nitrogen content in okra leaves and subsequently increase crop yield, while also reducing production costs by minimizing nitrogen fertilizer usage.
Key words: *Azospirallum*, Nitrogen Content, Growth Yield, Okra (*Abelmoschus esculentus*)

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INTRODUCTION

Vegetables contribute proteins, fats, carbohydrates, calcium, phosphorus, iron, and magnesium to human nutrition. They are good sources of minerals, vitamins, and dietary fibers, supplying a fair amount of free radicals, antioxidants, and micronutrients essential for a balanced diet. Okra (Abelmoschus esculentus (L.) Moench) is cultivated nationwide year-round, covering 0.35 million hectares and yielding 3.5 million tons of fruit, averaging 97 q/ha. Its commercial yield depends on fruit length, thickness, and quantity per plant.

Biofertilizers are significant in organic farming, especially in India where most farmers are small and marginal. Their use, along with organic and inorganic fertilizers, offers sustainable crop production,

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serving as cost-effective, eco-friendly sources of plant nutrients. They improve crop growth and quality while maintaining soil productivity without non-renewable energy sources. *Azospirallum*, a gram-negative bacterium, is free-living and fixes nitrogen efficiently on a nitrogen-free medium. It is pleomorphic and produces polysaccharides. *Azospirallum* species are sensitive to acidic pH, high salts, and temperatures above 35°C. They fix atmospheric nitrogen in the rhizosphere using carbon and calcium.

Nitrogen, the fourth-ranked element in plant composition, is unique as plants can absorb it from the soil in ammonium (NH4+) or nitrate (NO3-) form. It is present in all living matter as a constituent of protoplasm and is integral to various metabolically active compounds, including amino acids, co-enzymes, purines, pyrimidines, porphyrins alkaloids, and growth hormones. Purines and pyrimidines serve as the basic units of nucleic acids, essential for protein and enzyme synthesis. Nitrogen participates in nearly all physiological plant activities. Additionally, nitrogen is crucial for regulating phytohormone levels like cytokinin's and abscisic acid in leaves. Nitrogen application boosts cytokinin levels in plants.

Nitrogen is supplied through synthetic chemical fertilizers like urea, ammonium sulphate, and diammonium phosphate, among others. However, these fertilizers pose health hazards to humans and disrupt the essential micro-population of soil necessary for soil fertility. Furthermore, chemical fertilizers are costly, increasing production expenses. The urgent need is to replace chemical fertilizers with alternative sources. Biofertilizers could meet this need, potentially replacing chemical fertilizers. Therefore, the study aims to assess the effectiveness of nitrogen doses combined with *Azospirallum* on okra plant growth, yield, and leaf nitrogen content.

MATERIALS AND METHODS

The aim of the present investigation was to study the integrated effect of biofertilizer Le. *Azospirallum* and different doses of nitrogen fertilizers in the form of urea on plant growth, yield and chemical composition of okra (Abelmoschus esculentus (L.) Moench) var, selection-151. The simple randomized experiment was carried out in 09 inches clay pots filled with 4 kg mixture of soil: sand: compost in the ratio of 3:1:1 at the net house of Swami Shukdevanand College, Shahjahanpur during October to December 2023.

Application of nitrogen: Nitrogen was applied in the following treatments.

- No nitrogen + No *Azospirallum* (N_0+B_0)
- No nitrogen + *Azospirallum* (N_0+B)
- Full dose of nitrogen + No *Azospirallum* (N_{90}+B_0)
- Full dose of nitrogen + *Azospirallum* (N_{90}+B)
- ¾ Dose of nitrogen *Azospirallum* ($N_{67.5}$ +B)

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- ½ dose of nitrogen *Azospirallum* (N_{45}+B)
- ¼ Dose of nitrogen *Azospirallum* ($N_{22.5}$+B)

The nitrogen was given in the form of urea of 350 mg, 263 mg 175 mg and 875 mg per pot to provide 90.0, 675, 45.0 and 22.5 kg N/ha soil, respectively. The doses of urea were mixed with the soil before the sowing of the seeds.

Application of *Azospirallum* to seeds: Seeds of okra were inoculated with *Azospirallum* 100 g seeds/litter bacterial culture. One-gram jaggery (Gur) and 5g bacterial culture were dissolved in 20 ml distilled water. Sonds were soaked in the above mixture for one hour to put a coating of the culture on the seeds. Jaggery is used as a fixative. Ten seeds were sown in each pot at equal distance. Uninoculated seeds were used as control. Each treatment was replicated three times.

Determination of growth and yield: Plants from different pots were uprooted after 15, 30 and 45 days after germination (DAG) stages. The growth an" yield data of plant was recorded. The plants were then dried in an oven at 60° C for 7 days. Estimation of nitrogen from dried leaves: Dried leaves were powdered in an electric grinder for the estimation of nitrogen which was estimated by the method of Lindner, (1944) using spectrophotometer.

Statistical analysis of data: Data was analyzed statistically using ANOVAs method for the comparison of the results.

RESULTS AND DISCUSSION

Data given in **Table-1** indicates that various growth parameters viz. shoot and root length, fresh weight of shoot and root, dry weight of shoot and root studied at different growth stages increased significantly when treated with *Azospirallum* with various levels of nitrogen. The treatment comprising inoculation with minimum amount of nitrogen (N+B) resulted in the maximum shoot length which was 20.73 cm, root length 10.53 cm. fresh shoot weight 4.45 g, fresh root weight 1.040 g, dry shoot weight 0.447 g. dry root weight 0.112 g: on the other hand, treatment having no nitrogen and no *Azospirallum* (N+B) gave the minimum shoot length which was 10.53 cm only. All the treatments showed significant difference among themselves. The treatments N_{4}+B N_{ap}+B_{ap} N_{a}+B_{2} N_{a/3}+B_{a} N_{a}+B and NB showed 18.99, 35.13, 57.36, 73.50 and 96.87 percent increase in shoot length over control (N_{2}+B_{2}) respectively at 15 DAG stages. The similar trend was found in shoot length, fresh and dry weight of shoot at all the three stages of growth.

Treatments N_{4}+B N_{ap}+B_{ap} N_{a}+B_{2} N_{a/3}+B_{a} N_{a}+B and NB showed 18.99, 35.13, 57.36, 73.50 and 96.87 percent increase in shoot length over control (N_{2}+B_{2}) respectively at 15 DAG stages. The similar trend was found in shoot length, fresh and dry weight of shoot at all the three stages of growth.

Table 1: Effect of *Azospirallum* and different doses of nitrogen on growth of okra var. selection-151 at three growths Stages.

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	Shoot Length (cm)			**Root Length (cm)**			**Shoot Fresh weight(g)**			**Root Fresh Weight(g)**			**Shoot Dry Weight(g)**			**Root Dry Weight(g)**		
	15	30	45	15	30	45	15	30	45	15	30	45	15	30	45	15	30	45
TREATMENT	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
N_0+B_0	10.53	21.63	31.07	4.43	11.46	18.2	2.06	6.75	14.16	0.405	1.23	3.18	0.206	0.679	1.41	0.044	0.125	0.321
N_0+B	12.53	23.13	33.07	5.03	12.33	19.5	2.44	7.64	15.75	0.550	1.46	3.70	0.251	0.766	1.57	0.062	0.148	0.374
$N_{90}+B_0$	14.23	24.67	35.13	6.26	13.66	21.47	2.80	8.46	16.22	0.665	1.68	4.12	0.285	0.849	1.62	0.070	0.170	0.414
$N_{90}+B$	16.57	25.47	37.20	7.43	14.23	23.47	3.25	9.66	17.51	0.790	1.83	4.51	0.330	0.968	1.75	0.086	0.185	0.454
$N_{67.5}+B$	17.43	26.40	40.07	8.13	15.5	26.23	3.70	10.70	18.62	0.850	2.08	5.08	0.375	1.070	1.86	0.092	0.210	0.510
$N_{45}+B$	18.27	24.76	42.8	9.43	16.56	28.23	4.10	11.90	19.71	0.903	2.37	5.87	0.414	1.190	1.97	0.098	0.240	0.593
$N_{22.5}+B_0$	20.73	29.33	45.37	10.53	17.4	30.20	4.45	12.66	20.20	1.040	2.66	6.42	0.447	1.260	2.02	0.112	0.270	0.644
C.D. at 5%	0.5802	1.0968	0.845	0.49	0.4779	0.59	1.10	0.12	0.1403	0.011	0.044	0.0542	0.009	0.012	0.0135	0.004	0.006	0.0059

NB: Each value is a mean of three replicates, DAG=Days after germination, B=Biofertilizer-*Azospirallum*, N =Doses of Nitrogen

The observation from the **Table-2** showed the response of *Azospirallum* with various doses of nitrogen on number of pods /plant of okra at harvest stage. The treatment N_{z=2}+B_{r}N_{a}+B N_{a}+B_{c}N_{a/2}+B_{c} showed 50 percent higher number of pods/plant over the control (N_{a}+\mathbb{B}_{a})_{s} the treatment (N+B) gave the highest total weight of pods which was 22 86 g. On the contrary, the treatment (N_{2}+B_{2}) gave the least total weight of pods which was 3.04 g at harvest. The treatment N_{22x}+B produced

11.30 cm long pods which were maximum in length and significantly different from the control (N_{x}+B_{y}) rest of the treatments. On the other hand, the treatment (N_{g}+B_{g}) gave last length of pods which was 5.80 cm only. The treatments N_{a}+B_{r}N_{w}+B_{w} N_{et}+B_{e} N_{atz}+B_{z} N_{\mu}+B and N_{23}+B gave 18.96, 29.31, 41.38, 56.89, 75.86 and 94.83 percent higher length of pods over the control (N_{2}+B_{3}) Similar trends was found in all the yield parameters.

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Table 2: Effect of *Azospirallum* and different doses of nitrogen on yield of okra var. selection-151 at 90 days after growth stage.

Treatments	Number of Pods/Plant	100 Seed Weight	Total Weight of Pods (g)	Length of Pods (cm)	Number of Seeds/Pod	Weight (g)/Pod
N_0+B_0	2.00	3.01	5.80	15.00	20.27	1.52
N_0+B	3.00	5.22	6.90	17.00	32.57	2.61
N_{90}+B_0	3.00	7.26	7.50	20.00	37.76	3.63
N_{90}+B	4.00	9.06	8.20	23.00	44.37	4.53
$N_{67.5}$+18	4.95	16.32	9.10	26.00	70.64	5.41
N_{45}+B	5.75	19.54	10.20	28.00	74.46	6.51
$N_{22.5}$+B_0	6.50	22.86	11.30	31.00	78.21	7.62
CD at 5%	0.58	1.30	0.30	1.64	1.20	0.65

Data presented in **Table-3** show that the treatment NB (*Azospirallum*) gave the highest nitrogen content in leaves which was statistically different from the rest of the treatments as well as control (N_{a}+B_{a}) The treatments N_{x}+B_{x} N_{w}+B_{w} NB, N_{ab}+B_{a} N_{O}+B and N_{2=1}+B resulted in 11.49, 21.08, 33.64, 44.99, 53.68 and 67.65 percent higher nitrogen content over the control (N_{g}+B_{g})_{s} respectively at 15 DAG. The treatment comprising inoculation of *Azospirallum* with minimum dose of nitrogen (N+B) showed the highest nitrogen content which was 1.33% higher as compared to control (N_{1}+B_{2}) The control gave poorest response and reported 0.82% nitrogen content only at this stage. At 30 DAG the treatments N_{x}+B N_{w}+B_{w} N_{a}+B_{a} N_{65}+B N_{ad}+B and

N_{I12}+B gave 12.50, 22.02, 34.56, 44.99, 53.68 and 67.65 percent higher nitrogen content over the control (N_{i}+N_{i}) respectively. The treatment comprising inoculation with minimum amount of nitrogen (N+B) gave the maximum nitrogen content of 1.48% on the contrary, the treatment having no nitrogen and no *Azospirallum* (\mathbb{N}_{i}-\mathbb{B}_{i}) gave the minimum nitrogen content which was only. All the treatments showed significant difference among themselves. On the other hand, the treatments N+B N_{w}+B_{w} N+B, N_{arg}+B_{z} N_{w}+B and NB gave 12.59, 23.14, 35.79, 46.32, 53.65 and 69.19 percent higher nitrogen content over the control (N_{2}+B_{2}) respectively at 45 DAG. Again the treatment N_{22}+B resulted in maximum nitrogen content in leaves at 90 DAG also.

Table 3: Percentage nitrogen content in okra leaves

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Nitrogen Content in Okra leaves						
Treatment	**15 DAG**	**% Increase**	**30 DAG**	**% Increase**	**45 DAG**	**% Increase**
N_0+B_0	0.82	0.00	0.89	0.00	0.91	
N_0+B	0.92	11.49	1.00	12.59	1.03	12.59
N_{90}+B_0	1.00	21.08	1.08	22.02	1.13	23.14
N_{90}+B	1.10	33.61	1.19	34.56	1.25	35.79
$N_{67.5}$+B	1.19	44.99	1.28	44.99	1.35	46.32
N_{45}+B	1.25	53.68	1.36	53.68	1.41	53.65
$N_{22.5}$+B_0	1.33	67.65	1.48	67.65	1.55	69.19
C.D at 5%	0.02		0.02		0.018	

N.B: Each value is a mean of three replicates., DAG =Days after germination, B=Biofertilizer-*Azospirallum*

Growth of plant is defined as irreversible increase in size which may be expressed in terms of length, fresh weight as well as dry weight. Growth is an end result between anabolic and catabolic reactions. The growth of plant organ result from orderly cell division, expansion and differentiation. These processes are dependent on proper supply of mineral nutrients (Moorby and Besford, 1983), hormones as well as the genetic makeup of the plant. A good combination of all these factors brings about healthy growth of plant. Gauhar (2002) reported an increase in growth and yield. He attributed this increase to the secretion of some growth promoting substances by the microbial inoculants. These substances in turn lead to better development of plant parts, increased translocation of water and mineral uptake in potato (Solanum tuberosum). Secretions of plant growth promoting substances like IAA (Indole acetic acid) gibberellins, cytokinin etc. by *Azospirallum* in addition to the fixation of atmosphere nitrogen has also been reported by Pandey and Kumar (1989). Arumugam et al., (2001) reported that root length was significantly affected by combined application of DAP (Di-ammonium phosphate), Ethrel and Azospirillum. This may be due to the response of Azospirillum and DAP on root growth. The increase in dry weight of shoot and root might be due to enhanced nutrients uptake under this treatments. Tilak and Singh (1988) noted that the combined application of VAM and Azospirillum showed increased dry matter production in pearl millet. Similar finding have also been reported by Kumar et al., (2009) in davana and Shaheen et al., (2007) in okra. Similar observations have also been recorded in the present study.

Yield is a genetic term to describe the amount of part of a crop plant of interest that is harvested on maturity of crop. The final yield of a crop, therefore, depends upon vegetative growth, formation of storage organs including all ancillary characteristic and the seed filling. The higher number of fruits per plant has been reported in the plant treated with *Azospirallum*, FAM (Farmyard manure) and chemical fertilizers (Haripriya et al., 2003) in baby corn. Our results also confirm the aforesaid studies.

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The higher availability of atmospheric elemental nitrogen to the growing plant by non-symbiotic *Azospirallum* in the presence of organic manure and low doses of chemical fertilizers might be responsible for

Promoting better yield of crop. Similar results have also been reported in a number of other studies for example Khare and Singh (2008) reported that the increase in yield and yield attributing characteristic could be because of some growth promoting substances secreted by the *Azospirallum* which is turn might have lead to better root enhancement, better transportation of water, uptake and deposition of enhanced food accumulation. Gandora et al., (1998) reported that seed inoculation of maize with *Azospirallum* increased cob length and grain yield. Gajbhiye et al. (2003) reported increase in weight/pod might be associated to better inorganic nitrogen utilization in the presence of biofertilizers which resulted in enhanced biological nitrogen fixation, better development of root system and possible higher synthesis of plant hormones in tomato.

Nitrogen is a main constituent of chlorophyll, amino acids, proteins, vitamins, hormones etc. and is, therefore, involved in various important physiological processes including photosynthesis. Therefore, nitrogen is responsible for the enhanced vegetable growth of plants. It is essential for the formation of protoplasm, thus enhancing the cell division and cell enlargement. Yadav et al. (2005) reported that plant height increased with the application of nitrogen. Furthermore, nitrogen is important for vital body functions such as acid-base and water balance in okra, (Shaheen et. Al., 2007). Similarly, there are many reports regarding the effect of seed treatments with *Azospirallum* on enhancing the nitrogen content in different crops for example Mohiuddin et al. (2000) in wheat and Sarna et al. (2008) in chick-pea which are in agreement with the result of our experiment.

CONCLUSIONS

All the treatments of nitrogen and *Azospirallum* resulted in higher growth and yield due to increased nitrogen content in okra leaves. The application of lower doses of nitrogen along with *Azospirallum* saves the nitrogen fertilizer as well as the cost of production of the crop.

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