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Impact of Bacterial Endophytes on Growth and Yield of Brinjal *Var. Annamalai* Under Pot Experiment

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Abstract

An experiment was conducted during the season of June- September 2014 to observe the efficacy of isolated bacterial endophytes from the roots of brinjal as microbial inoculants through seed treatment along with inorganic fertilizers on growth, yield and biochemical constitutions of brinjal (*Solanummelongena* L.). The results showed significant difference in height of plant, number of branches, number of leaves, number of flowers, number of fruits, fruit weight, fruit length, fruit diameter and yield and proves that the application of microbial inoculants along with inorganic fertilizers and their combinations significantly influenced the growth, yield, quality and nutritional contents of brinjal. The combined application rates of 100% Chemical fertilizer with *Azospirillum brazilense* and *Pseudomonas fluorescens* gave the best performance of brinjal compared to other treatments. From the results, it can be concluded that microbial inoculants will reduce the farmer's budget for crop fertilization and ensure crop production under a less polluted environment.

Keywords

Endophytes, Microbial inoculants, brinjal, *Azospirill umbrazilense*, *Pseudomonas fluorescens*

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INTRODUCTION

Application of microbial inoculants like *Azospirllum* and *Azotobacter* particularly in vegetable crops has been of great significance in terms of yield and quality attributes (Jeevajothi *et* al. 1993; Charta *et al.* 1997). Biofertilizers (also known as bioinoculants), the organic preparations containing microorganisms are beneficial to agricultural production in terms of nutrient supply particularly with respect to N and P. When applied as seed treatment or seedling root dip or as soil application, they multiply rapidly and develop a thick population in rhizosphere (Sartaj *et al*., 2013). In the management of vegetable crops, the application of fertilizers has a major role on yield and growth. Although these fertilizers contribute a lot in fulfilling the nutrient requirement of vegetable crops, but the regular, excessive and unbalanced use may lead to health and ecological hazards, depletion of

physiochemical properties of the soil and ultimately poor crop yields. The problems of nutrient drain from the soil are becoming so acute. Hence there is a need to think of alternate source of safe fertilized which may enhance crop yields without having adverse effects on soil properties, the use of microbial inoculants as bio-fertilizers seen to be a hope in this direction.

They are the carrier-based preparations of microbial inoculants containing mainly effective strains of microorganisms in sufficient number, which are useful for plant nutrients. If they are used in association with macronutrients the expected yields per unit area may be much higher. Among bio-fertilizers, *Azospirillum sp.* and *Pseudomonas sp*. play a key role to trap the atmospheric nitrogen through its fixation in the roots. They have been also reported to improve fertility condition of the soil. Therefore, the present investigation was based on the objective, to find out the effect of microbial inoculation of *Azospirillum brasilense and Pseudomonas fluorescens* on growth and yield of brinjal.

MATERIALS AND METHODS

Collection of seeds

Brinjal var. *Annamalai* was received from the Department of Horticulture, Faculty of Agriculture, Annamalai University, Annamalainagar.

Inoculant preparation

NFB liquid medium for *A. brasilense* and King's B liquid medium for *P. fluorescens* were prepared. The selected *A. brasilense* and *P. fluorescens* were inoculated to the respective growth medium and shaked for 48hrs in rotary shaker at 32ºC. After shaking, the density of the culture was observed by turbidity and the population test was carried out by standard method. Then the cultures were used for seed inoculation.

Seed treatment with bacterial endophytes

The most common way of inoculation is "seed inoculation", in which the grown effective bacterial isolates of *A.brasilense* and *P.flourescens* were mixed with seeds. 2gms of brinjal seeds (approximately 250 seeds) was treated with each 1.5ml of known population of *A. brasilense* and *P. fluorescens* broth as individual and dual form according to the treatment given below. The untreated seeds were maintained as control. The treated seeds were shade dried and immediately sown in proplates at rate of one seed in each cup, containing cocopeat as substrate.

Pot experiment

The trial was carried out in pot (Diameter- 12ins, 30cm; depth 11ins, 27cm; distance of soil level from top of the pot 2ins, 5cm) during June-September 2014, in a randomised block design with seven treatments and three replications. 30 days old seedlings from proplates were transferred to pots. There were three replicates for each treatment. Observations were taken from random samples from each treated plant. The data were collected on Plant height, Number of leaves, Number of branches, number of flowers, number of fruits, fruit weight, fruit length, fruit diameter and number of seeds per fruit.

Treatments:

T_1 - 100% Chemical fertilizer (Control)
T_2- 100% Chemical fertilizer + *Azospirillum braziliense*
T_3- 75% Chemical fertilizer + *Azospirillum braziliense*
T_4- 100% Chemical fertilizer + *Pseudomonas fluorescens*
T_5- 75% Chemical fertilizer + *Pseudomonas fluorescens*
T_6- 100% Chemical fertilizer + *Azospirillum braziliense* + *Pseudomonas fluorescens*
T_7- 75% Chemical fertilizer + *Azospirillum braziliense* + *Pseudomonas fluorescens*

Chemical fertilizers:

Recommended dosages of NPK for brinjal **N-100 kg/ha, P-50 kg/ha, K-30 kg/ha**

Biometric observations

Three plants from each treated pot were selected at random to observe the following growth and yield parameters.

Growth parameters

Shoot length

The selected plants were used for measuring shoot length in centimetres from the base of plant to the terminal growing point of the plant at 30th, 60th and 90th day after transplanting (DAT).

Root length

The selected plants were used for measuring root length in centimeters from the base of plant to the tip of the longest root at 30th, 60th and 90th day after transplanting (DAT).

Leaf chlorophylls content (Arnon's 1949)

100mg of leaves was grounded in a mortar and pestle with 20ml of 80% acetone. The homogenate was centrifuged at 3000rpm for 15minutes. The clear supernatant was saved. The pellet was a re-extracted with 5ml of 80% acetone each time, until it became colorless. All the supernatant were pooled and was utilized for chlorophyll determination. Absorbance

was read at 645nm and 663nm in spectrophotometer 20.

Number of leaves per plant

The number of leaves was counted at 30th, 60th and 90th DAT.

Total number of branches per plant

The number of branches was counted at 30th, 60th and 90th DAT.

Number of flowers

The plants were observed for number of flowers appeared in each treatment at 30th, 60th and 90th DAT.

Yield parameters

Number of fruits per plant

The mean fruit number per plant was counted from the total number of fruits harvested at 30th, 60th and 90th DAT.

Fruit weight (g)

Fruit weight was weighed individually in each treatment at 30th, 60th and 90th DAT.

Fruit length (cm)

Length of the fruits was measured individually in centimetres from the base of calyx to tip of fruit using Vernier Callipers and the average was calculated at regular intervals.

Fruit girth (cm)

Fruit girth was measured by using Vernier Callipers and later average was worked out and expressed in centimetres.

Number of seeds per fruit

In each treatment, number of seeds per fruit was counted manually and their average was expressed as mean number of seeds per fruit.

RESULTS

TABLE 1. Effect of Efficient Isolates Of Azospirillumand Pseudomonason Shoot And Rootlength Of Brinjal In Pot Experiment.

Treatments	Shoot Length (Cm)			Root Length (Cm)		
	30 DAYS	60 DAYS	90 DAYS	30 DAYS	60 DAYS	90 DAYS
T_1	10.26±0.0632	16.18±0.0494	25..09±0.0282	2.81±0.0282	8.12±0.0353	12.50±0.0141
T_2	14.20±0.0424	22.16±0.0494	40.05±0.0141	3.50±0.0141	10.12±0.0141	14.22±0.0353
T_3	12.27±0.0212	24.09±0.0212	41.12±0.0424	3.88±0.0353	9.82±0.0353	14.20±0.0141
T_4	15.46±0.0141	23.36±0.0353	37.02±0.0212	3.49±0.0141	8.48±0.0353	13.19±0.0212
T_5	13.31±0.0212	26.30±0.0424	42.12±0.0212	3.58±0.0141	8.69±0.0141	13.29±0.0212
T_6	17.11±0.0212	31.13±0.0212	48.16±0.0494	4.98±0.0353	12.32±0.0353	15.33±0.0565
T_7	16.86±0.0141	30.06±0.0212	46.38±0.0353	4.82±0.0141	11.92±0.0353	15.26±0.0494

Values are mean ± SD of three samplesof mg/g of fresh weight

TABLE 2. Effect of Efficient Isolates Of Azospirillum And Pseudomonason Chlorophyll Content Of Leaves Of Brinjal In Pot Experiment.

Treatments	Chlorophyll 'a'			Chlorophyll 'b'			Total Chlorophyll		
	30days	60days	90days	30days	60days	90days	30days	60days	90days
T_1	0.28±0.0494	0.89±0.0378	1.17±0.0494	0.58±0.0353	0.72±0.0494	1.12±0.0212	0.56±0.0636	1.61±0.0282	2.29±0.0212
T_2	0.62±0.0282	1.22±0.0212	1.61±0.0353	0.50±0.0141	1.14±0.0282	1.54±0.0353	1.12±0.0212	2.36±0.0565	3.15±0.0636
T_3	0.54±0.0565	1.17±0.0494	1.32±0.0353	0.49±0.0282	1.10±0.0141	1.20±0.0282	1.03±0.0141	2.27±0.0565	2.52±0.0141
T_4	0.42±0.0353	1.09±0.0282	1.38±0.0212	0.37±0.0634	0.97±0.0353	1.20±0.0212	0.79±0.0353	2.06±0.0494	2.58±0.0353
T_5	0.54±0.0282	1.05±0.0282	1.29±0.0353	0.42±0.0282	1.03±0.0282	1.23±0.0353	0.96±0.0212	2.08±0.0494	2.52±0.0212
T_6	1.10±0.0353	1.68±0.0353	2.12±0.0353	1.06±0.0212	1.42±0.0565	2.17±0.0270	2.16±0.0141	3.1±0.0212	4.29±0.0565
T_7	1.07±0.0141	1.55±0.0494	2.06±0.0212	1.01±0.0282	1.47±0.0565	2.08±0.0565	2.08±0.0565	3.02±0.0212	4.15±0.0494

Values are mean ± SD of three samples of mg/g of fresh weight

TABLE 3. Effect of Efficient Isolates of Azospirillum And Pseudomonason Number Of Leaves, Branches And Flowers Of Brinjal In Pot Experiment.

Treatments	Number of Leaves/Plant			Number of Branches/Plant			Number of Flowers/Plant		
	30 DAYS	60 DAYS	90 DAYS	30 DAYS	60 DAYS	90 DAYS	30 DAYS	60 DAYS	90 DAYS
T_1	6.08±0.0565	14.26±0.0353	22.46±0.0353	0	0	1.12±0.0353	0	3.99±0.0141	7.39±0.0353
T_2	8.02±0.0212	18.07±0.0494	28.12±0.0212	0	0	2.41±0.0212	0	5.02±0.0282	12.33±0.0353
T_3	9.18±0.0141	17.42±0.0636	27.06±0.0494	0	0	2.27±0.0494	0	5.45±0.0636	12.00±0.0282
T_4	7.28±0.0227	16.35±0.0565	29.44±0.0424	0	0	1.86±0.0424	0	5.28±0.0353	12.47±0.0282
T_5	6.32±0.0212	15.60±0.0424	28.10±0.0141	0	0	2.29±0.0141	0	5.00±0.0141	11.00±0.0282
T_6	9.22±0.0424	20.10±0.0141	31.18±0.0212	0	0	3.38±0.0212	0	5.86±0.0141	12.87±0.0212
T_7	9.26±0.0353	19.72±0.0424	30.63±0.0212	0	0	3.00±0.0212	0	5.75±0.0141	12.75±0.0424

Values are mean ± SD of three samples of mg/g of fresh weight

TABLE 4. Effect of Efficient Isolates of Azospirillumand Pseudomonas on Yield Parameters Ofbrinjal In Pot Experiment.

Treatments	Number of Fruits/Plant	Fruit Weight(gm)	Fruit Length(cm)	Fruit Girth(cm)	Number of Seeds/fruits
T_1	8.11±0.0212	4.16±0.0494	7.15±0.0141	134.05±0.0494	1274.02±0.0282
T_2	13.10±0.0141	6.12±0.0424	8.05±0.0494	143.16±0.0212	1306.00±0.0141
T_3	12.16±0.0212	6.05±0.0494	8.46±0.0141	133.14±0.0212	1317.17±0.0494
T_4	12.07±0.0212	6.10±0.0141	7.80±0.0212	140.08±0.0565	1329.14±0.0353
T_5	11.18±0.0212	6.06±0.0565	8.28±0.0353	133.24±0.0353	1409.04±0.0212
T_6	13.22±0.0353	7.02±0.0282	9.09±0.0212	147.26±0.0353	1507.02±0.0282
T_7	13.09±0.0212	7.00±0.0282	8.71±0.0141	138.03±0.0282	1413.06±0.0353

Values are mean ± SD of three samples of mg/g of fresh weight

The results indicated that there were significant difference among the treatments. The inoculation with bacterial treatments had a more stimulating effect on growth and development of plants in nonsterile soil than sterile condition**.** Seed inoculation with elite endophytic nitrogen fixing bacterial strains significantly enhanced seed germination, growth and yield of brinjal. However, the rate of enhancement varied with bacterial strains. Here combined seed inoculation of *Azospirill umbrasilense* and *Pseudomonas fluorescens* with 100% chemical fertilizer was found superior to other combinations.

The increase in growth characters might be due to the factthat the *Azospirillum* inoculated plants were able to absorb nutrients from solution at faster rates thanuninoculated plants resulting in accumulation of more dry matter, N, P and K in the stems and leaves (Nantha kumar and Veera ragavathatham, 2001).

Azospirillum inoculation benefits plant growth and increases yield of crops by improving root development, mineral uptake and plant water relationship. In addition to nitrogen fixation, *Azospirillum* also produces growth promoting substances like IAA and GA and these hormones go a long way in enhancing the crop growth. Thesource of IAA from *Azospirillum* might have increased the various endogenous hormonal levels in plant tissue, that was responsible for the enhanced pollen germination and tube growth, which ultimately increased the fruit set. The N availability and N content of the plants was enhanced due to the application of*Azospirillum*. The increased uptake of available N influences the growth characters, since N is the chief constituent of protein essential for the formation of protoplasm, which leads to cell division, cell enlargement and ultimately resulting is increased plant growth and yield. *Azospirillum* fixes the atmospheric nitrogen in the soil enhances the production of phytohormone like substances and increased uptake of nutrients such as phosphorus and potassium. The biological activity of the microorganisms would have helped the soil status to

become a ready to serve zone for essential nutrients to plant's root system. Similar results were reported in Brinjal CV.GOB-1 (Chaudhari and Vihol, 2010), (Kamim, Zargar and Chatoo, 2002) and tomato (premsekar and Rajashree, 2009).

CONCLUSION:

From the present investigation, it is clear that the microbial inoculants and the process of nitrogen fixation have greater contribution in soil fertility in agricultural field. The combination of both the inoculants effectively influence the growth and yield attributes of brinjal in all observed parameters in the study. So it was an attempt in vegetable crops to isolate and identify effective strains of plant growth promoting bacterial endophytes in brinjal. It is our aim and objective to isolate and trace more nitrogen fixers exist as endophytes for further biotechnological potential for sustainable, eco-friendly agricultural production. Generally, a morecomprehensive understanding of plant colonization by bacteria has to be developed in order to better predict how bacteria interactwith plants interiors and whether they are likely to establish themselves in the plant environment after field application as inoculants.

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