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Effect of bacterial endophytes - *Azospirillum brasilense and Pseudomonas fluorescens* on growth and yield of Brinjal var. Annamalai in field trial

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Abstract

The trial was carried out in agriculture field, Karaikal during the season of June- September to test the efficacy of isolated bacterial endophytes from the roots of brinjal as microbial inoculants in seeds along with inorganic fertilizers in soil on growth, yield and some biochemical constitutions of brinjal (*Solanum melongena* L.) and considering the growth, yield and the biochemical constitutions, the data revealed that maximum parameters were recorded in T_6 and The results showed that the application of microbial inoculants and inorganic fertilizers and their combinations significantly influenced the growth, yield, quality and biochemical contents of brinjal. The mixed application of 100% Chemical fertilizer with combination of *Azospirillum brasilense* and *Pseudomonas fluorescens* received best performance of brinjal compared to other treatments.

Keywords Endophytes; Microbial inoculants; brinjal; *Azospirillum brasilense* and *Pseudomonas fluorescens*.

1. Introduction

Different kinds of microorganisms, including fungi, actinomycetes and bacteria have been found inside the plants and designated as endophytes. It inhabits plant tissues in their life cycle without causing any apparent harm to their host. These bacteria, that generally colonize the intercellular spaces, have been isolated from all plant tissues and from many plant species constituting a great reservoir of bacterial diversity with a remarkable biotechnological potential (Ulrich *et al*., 2008). Endophytic bacteria have been found in virtually every plant studied, where they colonize in the internal tissues of their host plant and can form a range of different relationships including symbiosis, mutualism, neutralism and commensalism. A diverse array of bacterial species has been reported to be endophytic including *Acetobacter, Arthrobacter, Bacillus, Burkholderia, Enterobacter, Herbaspirillum* and *Pseudomonas* (Lodewyckz *et al*., 2002). In recent years various novel endophytic nitrogen-fixing bacteria have been discovered, such as *Acetobacter seropedicae* (Cavalcante and Dobereiner, 1998), *Herbaspirillum serepedicae* (Baldiani *et al*., 1986), and "*Pseudomonas*", now established as a second species of *Herbaspirillum, Azoarcus* spp. (Reinhold-Hurek *et al*., 1993) and *Alcaligenes faecalis* (Zhou and you, 1988). Also, some strains of *Azospirillum brasilense* have been found to the plant interior (Schloter *et al*., 1994).

1.1. Endophytic colonization

Plant beneficial microorganisms are of interest for application in agriculture either as biofertilizers or as pesticides as well as for phytoremediation applications (Sturz *et al*., 2000; Berg, 2009; Lugtenberg and Kamilova, 2009; Weyens *et al*., 2009). Takuma Gamo and Sang Bae Ahn *et al*., 2013 reported effective colonization and increasing acetylene reduction in *Azospirillum sp*. isolated from several non-graminous crops. Steenhoudt and Vanderleyden, 2000 reported, *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses. Ivan *et al*., 1997 observed, biological nitrogen fixation in non-leguminous field crops, facilitating the evolution of an effective association between *Azospirillum* and wheat. Therefore, not only mechanisms responsible for plant growth promotion have to be investigated, but also a thorough understanding of all steps involved in plant colonization by PGPB is required to improve the efficiency and reliability of inoculant strains. In the last decade it has been repeatedly demonstrated that the plant interior is colonized by a range of endophytes mostly deriving from the rhizosphere and many of them have been reported to improve plant growth or health (Sturz and Nowak, 2000; Hardoim *et al*., 2008).

In the present investigation endophytic nitrogen fixing bacteria were isolated from brinjal. Among the major food crops, vegetables are the most important one by cultivation and consumption in India, particularly in Tamilnadu. Vegetables are nowadays in daily supplements in a variety of ways, as part of main meals and as snacks. As nitrogen is the major element required for its production, focusing on isolation and identification of effective nitrogen fixing bacteria for increasing growth and yield with reduction of the hazardous fertilizer nitrogen use is being an essential need. Most of the studies have explored the properties of these isolates in relation to their as agronomical inoculants fixing bacterial community that inhabits particularly with vegetables has been poorly studied. (Fig 1)

Brinjal or eggplant (*Solanum melongena* L.) is an important solanaceous crop of sub-tropics and tropics. it is commercial vegetable crops grown all over India for its high nutritive value and enumerative price India ranks first both in area (5.1 lakh ha) and production (88 lakh MT) (Anonymous, 2004). The name brinjal is popular

Fig: 1 Solanum melongena var. Annamalai

in Indian subcontinents and is derived from Arabic and Sanskrit whereas the name eggplant has been derived from the shape of the fruit of some varieties, which are white and resemble in shape to chicken eggs. It is known to have ayurvedic medicinal properties and is good for diabetic patients. It has also been recommended as an excellent remedy for those suffering from liver complaints (Shukla and Naik 1993). By keeping its wide cultivation in India and healthy nutrient diet, it has planned to study the effect of endophytic nitrogen fixing bacterial species on growth and yield of brinjal.

2. Materials and methods

2.1. Collection of seeds

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

2.2. Inoculant preparation

Nitrogen free liquid medium was prepared for *A. brasilense* and King's B liquid medium for *P. fluorescens* were prepared, the cultures were inoculated and kept 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 and applied as seed inoculation.

2.3. 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. fluorescens* were mixed with seeds. 2gms of brinjal seeds was treated with 1.5ml 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. (Fig 2)

Fig: 2 Seed Treatment

Field trial

The trial was carried out in a randomized block design in cultivable land, Karaikal during the season of June to September 2014, with seven treatments and three replications. Immediately after uprooting of 30 days old seedlings from proplates, the roots of the seedlings with cocopeat were dipped in solution prepared with *A. brasilense* and *P. fluorescens* cultures as per treatments for 60 seconds. Then the seedlings of bhendi from proplates was transplanted at the rate of two seedlings per hill with spacing of 75 cm for brinjal between rows and plants respectively in field as per treatments and irrigation was done. Five seedlings were planted per pot as per the treatments and irrigated immediately.

2.4. Treatments

T_1 - 100% Chemical fertilizer (Control)

T_2- 100% Chemical fertilizer + *Azospirillum brasilense*

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*

2.5. Replications: 3 Nos. in each.

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

3. Biometric observations

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

3.0.1. 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 centimetres 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 colourless. All the supernatant was 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.

3.1.2 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 centimeters from the base of calyx to tip of fruit using Vernier Calipers and the average was calculated at regular intervals.

Fruit girth (cm)

Fruit girth was measured by using Vernier Calipers and later average was worked out and expressed in centimeters.

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.

3.1.3. Biochemical constitutions of fruit

Total moisture and Total ash (Lab Manual 5: fssai, Manual methods of analysis of foods for fruits and vegetables), Protein (Lowry *et al*., 1951), Flavonoids (Jagadish *et al*., 2009), Total sugars (Nelson, 1944), Total Anthocyanin measurement (Christopher Beggs and Wellman, 1985), Total phenolic content (Jagadish et al., 2009) and Ascorbic acid content (Vitamin 'C') (Omaye, 1979) and antioxidant enzymes (Kumar and Khan, 1982) were estimated.

Fig: 3 Field trial of Brinjal var. Annamalai

Results

The results indicated significant difference among the kinds of biofertilizers and their combinations with inorganic fertilizers. 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.

Here combined seed inoculation with 100% chemical fertilizer with *Azospirillum brasilense* and *Pseudomonas fluorescens* was found superior to other combinations. similarly, biochemical constitutions of fruit like total moisture, total ash, protein, flavonoids, total sugars, total phenols, anthocyanin and vitamin 'C' (ascorbic acid) have also shown maximum contents in combined inoculation of 75% chemical fertilizer with *Azospirillum brasilense* and *Pseudomonas fluorescens* followed by other combinations compared with control.

The increase in growth characters might be due to the fact that the Azospirillum inoculated plants were able to absorb nutrients from solution at faster rates

Table 1. Effect of efficient isolates of Azospirillum sp. and Pseudomonas sp. on shoot and root length of brinjal in field experiment.

Treatments	Shoot Length (cm)			Root Length (cm)		
	30 DAYS	60 DAYS	90 DAYS	30 DAYS	60 DAYS	90 DAYS
T_1	10.27±0.0754	21.28±0.0305	32.99±0.0360	9.82±0.0404	13.45±0.0602	13.09±0.0458
T_2	12.19±0.0351	22.26±0.0360	42.15±0.0208	12.09±0.0360	17.20±0.0550	15.29±0.0585
T_3	12.37±0.0264	28.66±0.0208	47.32±0.0305	11.61±0.0404	16.89±0.0493	17.16±0.0450
T_4	10.56±0.0305	23.16±0.0305	37.62±0.0152	10.78±0.0435	15.12±0.0251	16.08±0.0550
T_5	10.41±0.0321	25.30±0.0351	42.22±0.0208	11.68±0.0416	16.66±0.0305	15.06±0.0351
T_6	16.21±0.0264	32.83±0.0251	56.18±0.0305	12.87±0.0404	15.66±0.0709	16.82±0.0360
T_7	14.16±0.0264	30.26±0.0264	53.12±0.0351	12.74±0.0450	14.70±0.0450	15.77±0.0472

Values are mean ± S.D of three samples of mg/g of fresh weight

Table 2. Effect of efficient isolates of Azospirillum sp. and Pseudomonas sp. on Chlorophyll content of leaves of brinjal in field experiment.

Treatments	Chlorophyll 'a'			Chlorophyll 'b'			Total Chlorophyll		
	30 DAYS	60 DAYS	90 DAYS	30 DAYS	60 DAYS	90 DAYS	30 DAYS	60 DAYS	90 DAYS
T_1	0.76±0.0503	1.26±0.0702	1.62±0.0360	0.28±0.0321	0.79±0.0305	1.32±0.0351	1.55±0.0655	2.31±0.0458	3.77±0.0602
T_2	1.18±0.0321	1.37±0.0360	1.73±0.1026	0.57±0.0360	1.06±0.0208	2.04±0.0450	1.17±0.0360	3.27±0.0503	3.34±0.0550
T_3	1.03±0.0305	1.58±0.0305	1.86±0.0458	0.49±0.0305	0.79±0.0493	1.48±0.0611	1.13±0.0208	2.80±0.0351	3.16±0.0458
T_4	1.00±0.0351	1.46±0.0450	1.82±0.0351	0.38±0.0264	0.69±0.0404	1.34±0.0503	0.84±0.0305	2.64±0.0351	2.20±0.0529
T_5	0.87±0.0513	1.32±0.0351	1.64±0.0556	0.32±0.0305	0.88±0.0351	1.28±0.0503	1.75±0.0964	2.44±0.0351	4.25±0.0650
T_6	1.47±0.0602	1.62±0.0450	2.03±0.0458	1.14±0.0152	1.67±0.0378	2.22±0.0351	3.14±0.0351	3.79±0.0458	4.21±0.0351
T_7	1.39±0.0585	1.64±0.0360	2.07±0.0416	1.10±0.0305	1.55±0.0351	2.14±0.0264	2.94±0.0416	3.72±0.0503	2.94±0.0650

Values are mean ± S.D of three samples of mg/g of fresh weight

Table 3. Effect of efficient isolates of Azospirillum sp. and Pseudomonas sp. on number of leaves, branches and flowers/plant of brinjal in field experiment.

Treatments	Number of Leaves/Plant			Number of Branches/Plant			Number of Flowers/Plant		
	30 DAYS	60 DAYS	90 DAYS	30 DAYS	60DAYS	90 DAYS	30 DAYS	60 DAYS	90 DAYS
T_1	4.00±0.0305	12.36±0.0305	20.06±0.0305	0	0	3.06±0.0305	0	4.89±0.0351	12.29±0.0351
T_2	5.09±0.0264	14.47±0.0360	22.22±0.0360	0	1.77±0.0360	4.71±0.0458	0	7.52±0.0360	18.23±0.0251
T_3	5.18±0.0378	15.12±0.0251	24.36±0.0305	0	2.12±0.0251	3.25±0.0450	0	8.05±0.0208	18.70±0.0360
T_4	4.38±0.0378	14.45±0.0251	20.44±0.0251	0	0	3.18±0.0702	0	6.78±0.0321	15.87±0.0360
T_5	4.12±0.0208	15.10±0.0251	22.10±0.0305	0	2.00±0.0321	4.29±0.0450	0	7.00±0.0305	19.00±0.0458
T_6	7.22±0.0264	16.10±0.0351	29.18±0.0360	0	3.16±0.0305	5.00±0.0450	0	9.16±0.0351	23.08±0.0378
T_7	6.36±0.0208	15.22±0.0152	26.23±0.0305	0	3.12±0.0305	3.88±0.0435	0	8.35±0.0251	20.63±0.0152

Values are mean ± S.D of three samples of mg/g of fresh weight

Table 4. Effect of efficient isolates of Azospirillum sp. and Pseudomonas sp. on yield parameters of brinjal in field experiment.

Treatments	Number of Fruits/Plant	Fruit Weight (gm)	Fruit Length (cm)	Fruit Girth (cm)	Number of Seeds/Fruit	Average Fruit Yield/Plot
T_1	11.21±0.0152	6.06±0.0351	8.15±0.0208	130.09±0.0208	1317.17±0.0360	5.27±0.0472
T_2	11.30±0.0305	7.08±0.0152	9.10±0.0503	133.56±0.0264	1436.02±0.0404	7.42±0.0351
T_3	12.16±0.0360	6.14±0.0173	8.46±0.0416	130.14±0.0251	1334.07±0.0360	6.24±0.0606
T_4	12.27±0.0305	6.12±0.0264	8.70±0.0513	140.18±0.0251	1429.24±0.0251	6.17±0.0251
T_5	11.38±0.0416	7.06±0.0251	8.78±0.0416	135.34±0.0251	1589.34±0.0305	7.27±0.0513
T_6	13.19±0.0351	7.21±0.0208	9.09±0.0351	147.16±0.0305	1607.19±0.0067	8.09±0.0351
T_7	13.12±0.0264	7.18±0.0305	8.91±0.0351	138.23±0.0305	1433.06±0.0251	8.02±0.0458

Values are mean ± S.D of three samples of mg/g of fresh weight

Table 5. Effect of efficient isolates of Azospirillum sp. and Pseudomonas sp. on biochemical constituents of fruits of brinjal at harvest in field experiment.

Treatments	Total Moisture (%)	Total Sugar (mg/gm)	Total ash (%)	Total Phenols (mg/gm)	Protein (mg/gm)	Anthocyanin (mg/100gm of peel)	Ascorbic acid (%)	Peroxidase (mg/gm)	Polyphenol Oxiodase (mg/gm)
T_1	80±4.163	3.75±0.0305	0.35±0.043	4.20±0.0305	0.61±0.0251	0.036±0.0171	4.835±0.003	4.12±0.0305	4.45±0.0208
T_2	85±7.637	5.10±0.0351	0.52±0.035	5.02±0.0351	0.84±0.0251	0.052±0.0072	7.335±0.003	4.83±0.0208	5.03±0.0251
T_3	85±2.156	5.50±0.0416	0.44±0.045	5.05±0.0152	0.73±0.0264	0.048±0.0053	6.914±0.007	5.26±0.0305	5.48±0.0305
T_4	88±8.386	5.17±0.0321	0.53±0.055	4.68±0.0321	0.82±0.0264	0.530±0.0454	6.211±0.007	4.79±0.0351	4.82±0.0305
T_5	82±6.110	4.29±0.0351	0.50±0.043	4.80±0.0305	0.61±0.0251	0.048±0.0055	6.102±0.005	5.06±0.0305	5.28±0.0305
T_6	91±4.725	5.81±0.0208	0.72±0.055	5.32±0.0360	0.93±0.0152	0.756±0.0040	8.146±0.003	5.71±0.0251	5.82±0.0152
T_7	90±4.163	6.22±0.0251	0.68±0.068	5.19±0.0351	0.84±0.0305	0.628±0.0046	8.518±0.004	5.52±0.0305	5.60±0.0351

Values are mean ± S.D of three samples

than uninoculated plants resulting in accumulation of more dry matter, N, P and K in the stems and leaves (Nanthakumar and Veeraragavathatham, 2001).

Sole inoculation of *Azospirillum sp.* benefits plant growth and productivity 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.

The source 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*.

Azospirillum fixes the atmospheric nitrogen in the soil enhances the production of phytohormones 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) (Table 1 to Table 5).

Discussion

Today beneficial plant–microbe interactions that promote plant health and development have been the subject of considerable study in the field of agriculture particularly vegetable production which is our main nutritional diet in common food. Many plant-associated bacteria are well known for their capacity to confer plant growth promotion and to increase resistance towards various diseases as well as abiotic stresses.

Nevertheless, they often fail to confer these beneficial effects when applied in the field, which is often due to insufficient rhizo and endosphere colonization. A better understanding on how beneficial bacteria colonize different plant niches will not only result in increased knowledge on plant microbe interactions but will also lead to a more successful and reliable use of bacterial inoculants.

Conclusion

The present study was just an initiative in commonly use vegetable to identify more effective strains of plant growth promoting bacterial endophytes for further remarkable biotechnological potential in the field of environmental eco-friendly agriculture particularly vegetable production. Generally, a more comprehensive understanding of plant colonization by bacteria has to be developed in order to better predict how bacteria interact with plants and whether they are likely to establish themselves in the plant environment after field application as bio fertilizers.

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