Enhancement of vegetative parameters of brinjal in proplates by the application of bacterial endophytes – *Azospirillum brasilense* and *Pseudomonas fluorescens*

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**ABSTRACT**

An experiment was carried out with endophytic fixing bacteria *Azospirillum brasilense* and *Pseudomonas fluorescens* isolated from brinjal, in different combinations with inorganic fertilizers by seed inoculation of brinjal to observe preliminary vegetative growth at 15th and 30th day and pigment contents in vegetable nursery bed (proplates). A total number of 28 endophytic bacteria isolated from brinjal from three localities (Annamalai University, Karaikal and Putthur). Further the isolates were subjected to various biochemical tests for their species level identification and nitrogen fixing ability was estimated. Based upon their N-fixing ability and IAA production, two strains, one *Azospirillum* sp. and one *Pseudomonas* sp. isolate was selected and tested for its performance in brinjal. The seeds treated with 75% Chemical fertilizer + *Azospirillum brasilense* + *Pseudomonas fluorescens* (T6) showed maximum plant vegetative characters, followed by others compared with control.

**Keywords:** Brinjal; endophytic bacteria; *Azospirillum brasilense; Pseudomonas fluorescens*

1. **INTRODUCTION**

Raised beds are a wonderful choice for vegetable gardening for a lot of reasons. It is easier to reach and access plants in the garden if it is raised; making it possible for people to grow maximum seeds to seedlings and also avoid wastage of seeds, when it directly sown in agricultural land and of course it has some difficulties too. It is easier to see your viability, productivity, weeds and pests.

Raised beds tend to hold more plants in less space than a traditional vegetable garden layout, which means there is less possibility for weeds. Among various types of microorganisms, the uses of bacterial inoculants are in current trends in agriculture practice especially in cereals and vegetables.

Most of the studies have explored the properties of these isolates in relation to their as agronomical importance in supplying nitrogen by nitrogen fixing ability of bacterial community that inhabits particularly with vegetables has been poorly studied. Among the vegetables, immature fruit, brinjal occupying major area in cultivation and consumption in Tamil Nadu.
Brinjal or eggplant (*Solanum melongena* L.) is an important solanaceous crop of subtropics and tropics. It is commercial vegetable crops grown all over India for its high nutritive value and renumerative price. India ranks first both in area (5.1 lakhs ha) and production (88 lakhs MT) (Anonymous, 2004).

Hence, it has planned to study the native endophytic bacterial species reside in brinjal and inoculates its own guest as inoculants by seed application to explore their potentiality in enhancing the vegetative growth and pigment contents 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**

The isolates of *Azospirillum brasilense* and *Pseudomonas fluorescens* which were identified by biochemical tests and molecular characterization through previous investigations was inoculated in nutrient broth medium and continuously rotated in rotary shaker for 72hrs to obtain mass turbid culture.

Then the liquid broths after population test (16×10^5) were mixed along with seeds, shade dried and immediately sown in proplates. there were three replicates maintained for each treatment.

2.3. **Treatment details of the experiment**

| Treatment | Description |
|-----------|-------------|
| T1        | 100% Chemical fertilizer (Control) |
| T2        | 100% Chemical fertilizer + *Azospirillum brasilense* |
| T3        | 75% Chemical fertilizer + *Azospirillum brasilense* |
| T4        | 100% Chemical fertilizer + *Pseudomonas fluorescens* |
| T5        | 75% Chemical fertilizer + *Pseudomonas fluorescens* |
| T6        | 100% Chemical fertilizer + *Azospirillum brasilense* + *Pseudomonas fluorescens* |
| T7        | 75% Chemical fertilizer + *Azospirillum brasilense* + *Pseudomonas fluorescens* |

2.4. **Inoculants application to seeds**

Seeds of 2 gms (250 seeds approx) per treatment was treated 1.5ml of prepared microbial cultures according to the treatment given above separately. Then they were shade dried and shown in proplates (each plate bearing 96 cups, one seed in each cup) containing cocopeat as substrate.
Table 1. Chemical analysis of the experimental cocopeat in proplates.

| S. No | Parameters         | Value       |
|-------|--------------------|-------------|
| 1.    | pH                 | 6.15%       |
| 2.    | EC (mmhos/cm/25 °C)| 0.482%      |
| 3.    | Organic carbon (%) | 10%         |
| 4.    | Available nitrogen (%) | 0.5%     |
| 5.    | Available phosphorus (%) | 0.022%   |
| 6.    | Available potassium (%) | 0.2%     |
| 7.    | Moisture (%)       | 10.76%      |
| 8.    | Ash (%)            | 5.87%       |
| 9.    | Organic matter (%) | 92.23%      |

2.5. Biometric observation

Random samples from each treated plates were harvested at the intervals of 15th and 30th day to notice germination percentage of seeds, days taken to germinate, plant height (root and shoot length) number of leaves and pigment content in leaves.

Table 2. Vegetative parameters observed at 15th and 30th day in nursery bed.

| SL.No | Treatments | Germination % of seeds | Germination day | Root length | Shoot length | No. of Leaves/plant |
|-------|------------|------------------------|----------------|-------------|--------------|---------------------|
|       |            |                        |                | 15th day    | 30th day     | 15th day           | 30th day           | 15th day | 30th day |
| 1.    | T1         | 89.5%                  | 8th day        | 1.96 ±0.035355 | 2.20 ±0.035355 | 2.39 ±0.021213 | 3.82 ±0.021213 | 2.10 ±0.049497 | 3.28 ±0.021213 |
| 2.    | T2         | 90.8%                  | 6th day        | 2.48 ±0.035355 | 2.62 ±0.014142 | 3.16 ±0.028284 | 4.54 ±0.028284 | 2.19 ±0.042426 | 3.68 ±0.049497 |
| 3.    | T3         | 88.2%                  | 8th day        | 2.50 ±0.028284 | 2.70 ±0.042426 | 3.10 ±0.056569 | 4.28 ±0.035355 | 2.23 ±0.014142 | 4.21 ±0.035355 |
| 4.    | T4         | 86.4%                  | 8th day        | 2.21 ±0.014142 | 2.59 ±0.035355 | 2.88 ±0.035355 | 4.42 ±0.035355 | 2.20 ±0.035355 | 3.82 ±0.028284 |
| 5.    | T5         | 87.6%                  | 8th day        | 2.18 ±0.028284 | 2.38 ±0.028284 | 3.02 ±0.035355 | 4.37 ±0.021213 | 2.14 ±0.042426 | 3.70 ±0.035355 |
| 6.    | T6         | 88.6%                  | 9th day        | 2.81 ±0.042426 | 3.07 ±0.049497 | 3.22 ±0.056569 | 5.04 ±0.06364 | 2.31 ±0.035355 | 4.20 ±0.028284 |
| 7.    | T7         | 92.8%                  | 4th day        | 3.00 ±0.021213 | 3.22 ±0.014142 | 3.36 ±0.028284 | 5.10 ±0.014142 | 2.48 ±0.021213 | 4.28 ±0.035355 |

Values are mean ± S.D of three replicates.
Table 3. Effect of microbial inoculants on combination with inorganic fertilizers on chlorophyll content in leaves.

| S. No | Treatments | Chlorophyll ‘a’ 15 days | Chlorophyll ‘a’ 30days | Chlorophyll ‘b’ 15 days | Chlorophyll ‘b’ 30days | Total Chlorophyll (a+b) 15 days | Total Chlorophyll (a+b) 30days |
|-------|-------------|------------------------|------------------------|------------------------|------------------------|-------------------------------|-------------------------------|
| 1.    | T1          | 0.6±0.028284           | 0.33±0.0152            | 0.3±0.021213           | 0.28±0.0321            | 0.9±0.036056                  | 0.61±0.0264                  |
| 2.    | T2          | 0.12±0.03555           | 0.60±0.0264            | 0.7±0.021213           | 0.57±0.0360            | 0.19±0.028284                 | 1.17±0.0360                  |
| 3.    | T3          | 0.10±0.028284          | 0.64±0.0251            | 0.6±0.028284           | 0.49±0.0305            | 0.16±0.035355                 | 1.13±0.0208                  |
| 4.    | T4          | 0.15±0.042426          | 0.46±0.0208            | 0.13±0.021213          | 0.38±0.0264            | 0.28±0.035355                 | 0.84±0.0305                  |
| 5.    | T5          | 0.14±0.042426          | 0.51±0.0305            | 0.12±0.014142          | 0.32±0.0305            | 0.27±0.06364                  | 0.83±0.0152                  |
| 6.    | T6          | 0.9±0.028284           | 1.09±0.0208            | 0.6±0.0282840          | 1.14±0.0152            | 1.5±0.028284                  | 2.23±0.0152                  |
| 7.    | T7          | 0.8±0.035355           | 1.04±0.0360            | 0.9±0.028284           | 1.10±0.0305            | 1.7±0.014142                  | 2.14±0.0305                  |

Values are mean ± S.D of three replicates

3. RESULTS AND DISCUSSION

There is significant increase and difference in each treatment combination. Seed inoculation with representative endophytic nitrogen fixing bacterial strains significantly enhanced the germination well in all treatments on par with control.

However, the rate of enhancement varied with different combination percentage of fertilizers with NPK. Here T7 75% chemical fertilizer + Azospirillum + pseudomonas was found superior to other combinations.

T7 recorded the maximum value of root length (3.00 at 15th and 3.22 at 30th day), shoot length (3.36 at 15th and 5.10 30th day) and number of leaves per plant (2.48at 15th day and 4.28 at 30th day). Germination of seeds in all treatments was observed well. Seeds shown in T7 bed were germinated earlier at 4th day from DOS.

T3, T4, T5 and T1 germinated next at 8th day from DOS. It was observed that, T2 seeds germinated earlier next to T7 at 6th day from DOS whereas T6 germinated at 9th day from DOS (Table 2).

Similar trend was observed in pigment content of leaves in brinjal (Table 3). Chlorophyll contents were significantly increased throughout the course time of germination. Maximum chlorophyll content was observed in T7 followed by T6, T3, T4, T5 and T1.

Similar results were reported in Brinjal CV.GOB-1 (Chaudhari and Vihol, 2010) & (Kamim et al., 2002). and tomato (premsekar and Rajashree, 2009).

4. CONCLUSION

So the present study was just an initiative in commonly used vegetable to identify effective strains of plant growth promoting bacterial endophytes for further remarkable biotechnological potential in the field of environmental/ ecofriendly agriculture particularly vegetable production.
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(Received 20 September 2014; accepted 30 September 2014)