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## Research Article

# INTERACTION EFFECT OF COMBINED INOCULATION OF PGPR ON GROWTH AND YIELD PARAMETERS OF BHENDI (*ABELMOSCHUS ESCULENTUS L.MOENCH*) ARKA ANAMIKA

Usharani G and Mano B

Department of Microbiology, Annamalai University, Annamalai Nagar, Chidambaram - 608 002, Tamil Nadu, India

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### ABSTRACT

Bhendi (*Abelmoschus esculentus*) belongs to family Malvaceae. It is most important vegetable crop. Which is grown throughout the tropical and sub tropical parts of the world. In the present study, soil samples were collected from ten different location in cuddalore district. The PGPR Organisms viz., *Azospirillum*, *Pseudomonas*, *Azotobacter* and *Bacillus* were isolated from ten different soil samples were purified and maintained for further studies. The selected PGPR isolates viz., AZS-3, PS-2 and AZO-4 were inoculated either individually and along with *Bacillus* BS-3 and interaction effect of bhendi var. Arka anamika was studied under pot culture experiment. All the inoculation of PGPR Organism increased the growth and yield Parameter of bhendi when compared to uninoculated control. PGPR inoculation recorded the maximum vigour index of 1860 and germination percentage of 98.05%. It also recorded the highest plant height of 148.65cm and plant dry weight 21.8g plant<sup>-1</sup>. The yield Parameter such as number of fruits maximum 18 number Plant<sup>-1</sup>. fruit weight 18.5g Fruit<sup>-1</sup> and fruit yield 126.51q/ha was recorded. It was confirmed that the interaction effect was more than microbial inoculation applied as a consortium than individual inoculation.

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### INTRODUCTION

Bhendi (*Abelmoschus esculentus* var. *Arkaanamika*) belongs to family Malvaceae. It is one of the most important vegetable crop of tropics and sub tropics, which is widely grown in India for its mature, tender and green fruits for the culinary purpose. It fruit have high nutritive, medicinal and industrial value and export potential it has high acceptability in Indian market and fetches good price. It fruit are rich in vitamins, calcium, potassium and other mineral matter.

Globally, India contributes one third to world production of bhendi with an area of 5.33 Lakh Hectares and production of 63.4 lakh tonnes with a productivity of 11.9 tonnes. In India, bhendi is extensively grown in the state of Andhra Pradesh, Orissa, Maharashtra, Karnataka, Tamilnadu and West Bengal. A large amount of herbicides, pesticides, and fertilizer is applied every year to achieve maximum productivity of bhendi and to meet the growing demand the use of chemical fertilizer in india has increased 170 times in last 50 years. Plant growth promoting rhizobacteria (PGPR) are free - living, soil - borne bacteria, which enhance the growth of plant either direct or indirect mechanism. The direct mechanism involve nitrogen

fixation, phosphorous solubilization, HCN production, production of phytohormones such as auxins, cytokinins and gibberellins and lowering of ethylene concentration. Indirect growth promotion occurs when PGPR promote plant growth by improving growth restricting conditions. This can happen by producing antagonistic substances, lytic enzymes and siderophore or through competition for nutrient and space. It is also suggested that PGPR can also prevent the deleterious effect of stresses from the environment.

### MATERIALS AND METHODS

A well grown bhendi plant with roots intact was uprooted from the field and excess soil was removed. The soil adhered to root surfaced and in between root was collected and used as rhizosphere soil. The rhizosphere soil was collected in ten different places around the Cuddalore district and is used to isolate the PGPR bacteria such as *Azospirillum*, *Pseudomonas*, *Bacillus* and *Azotobacter*. Based on the sample collection, the isolates were designated as Azs-1 to Azs-10, Ps-1 to Ps-10, Bs-1 to Bs-10 and Azo-1 to Azo-10 respectively. Efficient PGPR isolates were selected and used in field experiment.

\*Corresponding author: Usharani G

Department of Microbiology, Annamalai University, Annamalai Nagar, Chidambaram - 608 002, Tamil Nadu, India

### Preparation of Pots and Treatment Details

Cement pots of size 2'x1'x2' were filled with pot mixture. The pots were watered and treated seeds were sown. The fertilizer schedule of 80: 45: 25 kg of NPK acre<sup>-1</sup> was followed based on the treatments. The pots were arranged according to the treatments. Broth culture of *Pseudomonas* sp., *Azospirillum*, *Bacillus* and *Azotobacter* were prepared. The seeds of bhendi var-Arka anamika were treated with the cultures as per the treatment combination using carboxy methyl cellulose as binder. The treated seeds were shade dried and used for sowing. The experiment was conducted following the completely randomized block design with three replications and twelve treatments.

### The Treatments Were

- T<sub>1</sub>- Control;
- T<sub>2</sub>- *Azospirillum*;
- T<sub>3</sub> - *Azotobacter*;
- T<sub>4</sub>- *Pseudomonas*;
- T<sub>5</sub> - *Bacillus*;
- T<sub>6</sub>- *Azospirillum*+*Azotobacter*;
- T<sub>7</sub> - *Azospirillum* + *Bacillus*;
- T<sub>8</sub> - *Azotobacter* + *Bacillus*;
- T<sub>9</sub>- *Pseudomonas*+*Bacillus*;
- T<sub>10</sub>- *Azospirillum*+ *Azotobacter*;
- T<sub>11</sub> - *Pseudomonas* + *Azotobacter*and
- T<sub>12</sub>- *Azospirillum* + *Azotobacter* + *Pseudomonas* + *Bacillus*.

**Biometric Observations:** Five plants were chosen for each treatment for recording the biometric observation. Plant samples were taken at periodic intervals viz., 25<sup>th</sup>, 50<sup>th</sup>, 75<sup>th</sup> and 100<sup>th</sup> days after sowing. The biometric observations such as plant height, plant dry weight and yield of chilly were recorded.

**Germination Percentage:** The germination count of bhendi was recorded on 15 DAS the germination percent arrived at using the formula

$$\text{Germination\%} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 10$$

**Vigour Index:** The vigour index was worked out as on 15DAS by multiplying the germination percentage with plant height and expressed as whole numbers.

**Determination of Plant Height:** The height of the plant from the surface of the soil to top most leaf was measured at periodical intervals viz., 25<sup>th</sup>, 50<sup>th</sup>, 75<sup>th</sup>, days after sowing.

**Determination of Plant Dry Weight:** The weight of the sample was determined in an oven at 60°C until a constant weight was obtained and expressed in (g) on oven dry basis.

**Fruit Yield:** The fruit yield of five plants were taken from each treatment and expressed as g per plant.

**Number of Fruits:** The number of fruits of five plants in each treatment was recorded and expressed as number of fruits plant<sup>-1</sup>.

**Fruit Weight:** The average fruit weight of five plants selected as randomly recorded and expressed as g fruit<sup>-1</sup>.

**Estimation of Nitrogen Content of the Plant:** The nitrogen content of the plant was estimated by Microkjeldahl method.

The plant samples were initially dried and then dried in an oven at 60°C till constant weight was obtained. The dried samples were powdered, sieved and the nitrogen content was estimated.

**Estimation of Phosphorus Content of the Plant:** The phosphorus content of the plant was estimated using vanadomolybdate method.

**Statistical Analysis:** The experimental data were analyzed by following the method of Panse and Sukhatme.

## RESULTS AND DISCUSSION

Plant growth promoting rhizobacteria (PGPR) constitute approximately 2- 5% of the total rhizomicrobial population. Evidence of the beneficial effects of PGPR has been accumulating for the past 150 years. PGPR have been demonstrated to increase growth and productivity of many commercial crops including rice, wheat, cucumber, maize, cotton, black pepper and banana. A few studies have isolated and characterized the PGPR and phosphate solubilizing bacteria from bhendi rhizosphere, the effect of PGPR on bhendi growth and productivity under field conditions has hitherto not been investigated. Co-inoculation of PGPR has been demonstrated as a sustainable approach in plant health management. Prudent application of binary or multiple mixtures of PGPR inoculants can expand the spectrum of biocontrol activity. Therefore, individual and combined effects of PGPR on the yield and productivity of host plant should also be assessed.

**Table 1** Effect of individual and combined inoculation of PGPR on germination percentage and vigour index of bhendi var Arka anamika

| Treatments      | Germination percentage | Vigour index |
|-----------------|------------------------|--------------|
| T <sub>1</sub>  | 69.20                  | 899.06       |
| T <sub>2</sub>  | 82.42                  | 984.98       |
| T <sub>3</sub>  | 74.30                  | 944.02       |
| T <sub>4</sub>  | 90.42                  | 1265.88      |
| T <sub>5</sub>  | 70.28                  | 980.26       |
| T <sub>6</sub>  | 79.66                  | 1115.24      |
| T <sub>7</sub>  | 91.76                  | 1417.12      |
| T <sub>8</sub>  | 90.08                  | 1376.14      |
| T <sub>9</sub>  | 92.05                  | 1564.85      |
| T <sub>10</sub> | 82.38                  | 1312.28      |
| T <sub>11</sub> | 90.50                  | 1360.11      |
| T <sub>12</sub> | 98.05                  | 1860.00      |

**Table 2** Effect of individual and combined inoculation of PGPR on plant dry weight of bhendi var Arka anamika. Plant dry weight (g / plant ) Sampling period in days

| Treatments      | 25 <sup>th</sup> day | 50 <sup>th</sup> day | 75 <sup>th</sup> day |
|-----------------|----------------------|----------------------|----------------------|
| T <sub>1</sub>  | 40.25                | 86.25                | 110.25               |
| T <sub>2</sub>  | 42.50                | 89.56                | 115.96               |
| T <sub>3</sub>  | 39.00                | 88.37                | 115.80               |
| T <sub>4</sub>  | 45.32                | 96.47                | 116.21               |
| T <sub>5</sub>  | 38.50                | 87.17                | 114.32               |
| T <sub>6</sub>  | 46.30                | 106.28               | 137.10               |
| T <sub>7</sub>  | 47.80                | 110.54               | 138.15               |
| T <sub>8</sub>  | 47.20                | 107.28               | 137.12               |
| T <sub>9</sub>  | 48.24                | 124.25               | 140.25               |
| T <sub>10</sub> | 46.20                | 106.15               | 136.86               |
| T <sub>11</sub> | 45.86                | 106.90               | 136.50               |
| T <sub>12</sub> | 50.30                | 130.25               | 148.65               |

A pot culture experiment was conducted to study the interaction effect of inoculation of efficient isolates of PGPR viz., *Azospirillum azs-3*, *Pseudomonas Ps-2*, *Azotobacter azo-4* and *Bacillus Bs-3on* growth and yield parameters of bhendi.

The individual and combined inoculation effect of PGPR on germination percentage and vigour index of bhendi were studied and the results are given in (Table – 1). It was observed that all the treatment recorded significant difference in germination percentage and vigour index of bhendi var *Arka anamika* over control due to the inoculation of microbial inoculants. The treatment T<sub>12</sub>, Azs + Azo + Ps + Bs recorded the maximum germination percentage of 98.05 and vigour index of 1860.00. Among the individual inoculation, *Pseudomonas* Ps-2 (T<sub>4</sub>) was found to be best in increasing the germination percentage and vigour index followed by *Azospirillum* Azs-3 (T<sub>2</sub>) and *Azotobacter* Azo-4 (T<sub>3</sub>). Among the dual inoculation, the treatment T<sub>9</sub> (Ps 2 + Bs) recorded the maximum germination percentage of 92.05 and vigour index of 1564.85 followed by T<sub>7</sub> (Azs + Bs) and T<sub>8</sub> (Azo + Bs). It was also observed that the interaction effect was more pronounced in the combined inoculation of all PGPR than single and dual inoculation

It was observed that the plant height of bhendi was significantly increased by all the treatments over control. The maximum plant height of 148.65 cm was recorded by the treatment Azs + Azo + Ps + Bs on 75 DAS. Among the individual inoculation, the *Pseudomonas* Ps- 2 was found to be the best and recorded 45.32cm of plant height on 25<sup>th</sup> DAS followed by Azs-3 (42.50) and Azo-4 (39.00). Among the dual inoculation, the treatment T<sub>9</sub> (*Pseudomonas* + *Bacillus*) recorded the maximum plant height of 48.24 cm on 25 DAS followed by Azs + Bs (47.80) and Azo + Bs (47.20). Though increase in plant height on 25 DAS was not statistically significant, but as the advancement of sampling periods, the differences between the treatments were statistically significant. It was also observed that the combined inoculation of all the PGPR was observed to be the best and exhibited maximum interaction effect than individual inoculation and dual inoculation of PGPR (Figure – 1).

The individual and combined effect of PGPR on plant dry weight of bhendi was studied and the results are presented in Table - 2. It was observed that the plant dry weight of bhendi was significantly increased by all the treatments tested over control. The maximum plant dry weight of 21.08 g plant<sup>-1</sup> was recorded by the treatment Azs+ Azo + Ps + Bs on 75 DAS. Among the individual treatments, the inoculation of *Pseudomonas* Ps-2 was found to be the best and recorded 6.48 of plant dry weight on 25<sup>th</sup> DAS followed by Azs-3 (5.98) and Azo-4 (5.87). Among the dual inoculation, the treatment T<sub>9</sub> (Ps+Bs) recorded the maximum plant dry weight of 7.31 25 DAS followed by Azs+ Bs (6.88) and Azo + Bs (6.56). It was also observed that the combined inoculation of all the PGPR was observed to be the best and exhibited maximum interaction effect than individual inoculation and dual inoculation of PGPR. The individual and combined effect of PGPR on number of fruits of bhendi was studied and the results are presented in (Figure – 2). It was observed that the number of fruits in bhendi was significantly increased by all the treatments tested over control. The maximum number of fruits 18.0 plant<sup>-1</sup> was recorded by the treatment Azs + Azo + Ps + Bs on 75 DAS. Among the individual treatments, the inoculation of *Pseudomonas* Ps-2 was found to be the best and recorded 14 number of fruits on 75 DAS followed by Azs -3 (13) and Azo-4 (12). Among the dual inoculation the treatment T<sub>9</sub> (Ps + Bs)

recorded the maximum number of fruits 17.0 number of fruits on 75 DAS followed by Azs +Bs and Azo+ Bs. It was also observed that the combined inoculation of all the PGPR was observed to be the best and exhibited maximum interaction effect than individual inoculation and dual inoculation of PGPR.

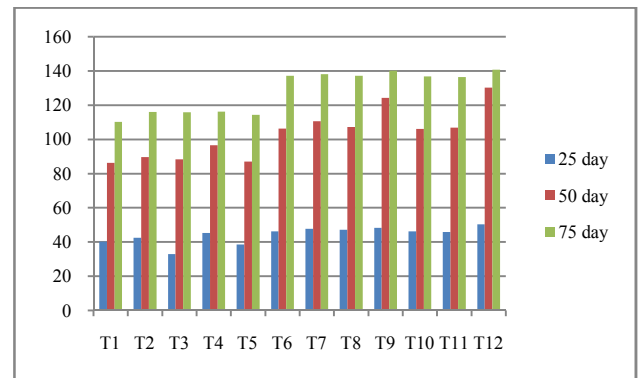


Fig 1 Effect of individual and combined inoculation of PGPR on plant height of bhendi var- Arka anamika

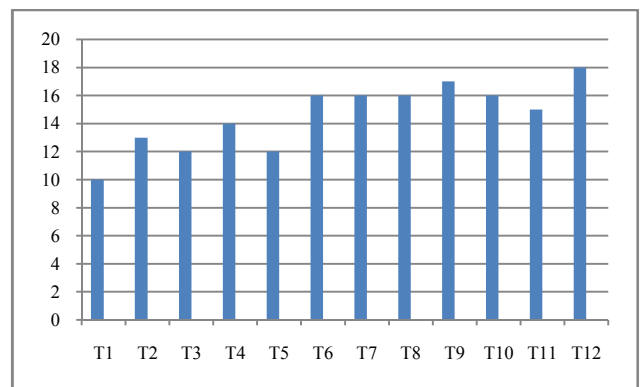


Fig 2 Effect on PGPR isolates on increase in number of fruits per plant

Table 3 Effect of individual and combined inoculation of PGPR on fruit yield in bhendivar- Arka anamika Fruit yield (g /plant)

| Treatments      | Pot culture study |
|-----------------|-------------------|
| T <sub>1</sub>  | 220.28            |
| T <sub>2</sub>  | 256.46            |
| T <sub>3</sub>  | 254.50            |
| T <sub>4</sub>  | 260.72            |
| T <sub>5</sub>  | 250.84            |
| T <sub>6</sub>  | 298.36            |
| T <sub>7</sub>  | 316.96            |
| T <sub>8</sub>  | 310.80            |
| T <sub>9</sub>  | 320.15            |
| T <sub>10</sub> | 309.46            |
| T <sub>11</sub> | 315.50            |
| T <sub>12</sub> | 333.00            |

It was observed that the plant fruit yield of bhendi was significantly increased by all the treatments tested over control. The maximum fruit yield of 333.00 (g plant<sup>-1</sup>) was recorded by the treatment Azs + Azo + Ps + Bs. Among the individual treatments, the inoculation of *Pseudomonas* Ps-2 was found to be the best and recorded the 260.72 (gplant<sup>-1</sup>) of fruit yield followed by Azs-3 (256.46) and Azo-4 (254.50). Among the dual inoculation, the treatment T<sub>9</sub> (Ps + Bs) recorded the maximum fruit yield of 320.15 g plant<sup>-1</sup> followed by Azs + Bs (316.96) and Azo + Bs (310.80). It was also observed that the combined inoculation of all the PGPR was observed to be the best and exhibited maximum interaction effect than individual inoculation and dual inoculation of PGPR (Table 3).

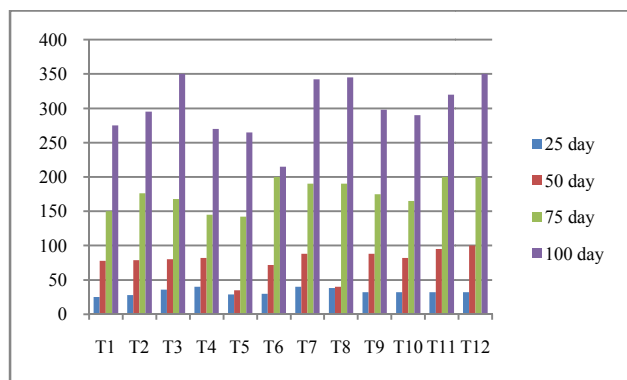


Fig 3 Effect PGPR isolates on the increase in plant nitrogen content

Table 4 Effect of individual and combined inoculation of PGPR on fruit weight in bhendi var -Araka anamika

| Treatments | Fruit weight (g fruit <sup>-1</sup> ) |
|------------|---------------------------------------|
| T1         | 9.50                                  |
| T2         | 11.38                                 |
| T3         | 10.95                                 |
| T4         | 12.25                                 |
| T5         | 10.82                                 |
| T6         | 13.50                                 |
| T7         | 14.98                                 |
| T8         | 14.56                                 |
| T9         | 15.80                                 |
| T10        | 13.96                                 |
| T11        | 13.81                                 |
| T12        | 18.05                                 |

It was observed that the fruit weight of bhendi was significantly increased by all the treatments tested over control. The maximum fruit weight of 18.5 (g fruit<sup>-1</sup>) was recorded by the treatment *Azo + Azo+Ps +Bs*. Among the individual treatments, the inoculation of *Pseudomonas Ps-2* was found to be the best and recorded 12.25 g plant<sup>-1</sup> of fruit weight followed by *Azs-3* (11.38) and *Azo-4* (10.95). Among the dual inoculation of the treatment T<sub>9</sub> (*Ps + Bs*) recorded the maximum fruit weight of 14.56 (g plant<sup>-1</sup>) followed by *Azs +Bs* (15.80) and *Azo + Bs*(14.98). It was also observed that the combined inoculation of all the PGPR was observed to be the best and exhibited maximum interaction effect than individual inoculation and dual inoculation of PGPR (Table 4).

Table 5 Effect of individual and combined inoculation of PGPR on plant phosphorous ( mg / plant) content in bhendi var Arka anamika

| Treatments | Sampling period in days |                  |                  |                   |
|------------|-------------------------|------------------|------------------|-------------------|
|            | 25 <sup>th</sup>        | 50 <sup>th</sup> | 75 <sup>th</sup> | 100 <sup>th</sup> |
| T1         | 2.29                    | 7.96             | 11.37            | 22.48             |
| T2         | 3.16                    | 7.26             | 15.82            | 28.30             |
| T3         | 3.78                    | 8.08             | 17.25            | 21.39             |
| T4         | 5.80                    | 5.60             | 13.80            | 17.34             |
| T5         | 3.09                    | 6.60             | 12.89            | 15.94             |
| T6         | 4.26                    | 5.85             | 13.32            | 27.98             |
| T7         | 6.35                    | 6.87             | 12.96            | 15.78             |
| T8         | 6.21                    | 5.80             | 13.50            | 26.90             |
| T9         | 6.72                    | 7.62             | 16.28            | 28.89             |
| T10        | 6.10                    | 6.50             | 12.97            | 26.73             |
| T11        | 6.98                    | 9.68             | 12.72            | 29.97             |
| T12        | 7.02                    | 8.76             | 18.25            | 40.80             |

It was observed that the Plant nitrogen content of bhendi was significantly increased by all the treatments tested over control (Fig:3). The maximum Plant nitrogen content of (350mg plant<sup>-1</sup>) was recorded by the treatment *Azs+ Azo + Ps + Bs* on 100 DAS. Among the individual treatments, the inoculation of *Azospirillum Azs-3* was found to be the best and recorded 40.50

mg plant<sup>-1</sup> of plant nitrogen content followed by *Ps-2* (32.58) and *Azo-4* (31.38) on 25 DAS. Among the dual inoculation of the treatment *Azospirillum + Bacillus* recorded the maximum plant nitrogen content of 42.56 (mg plant<sup>-1</sup>) followed by *Ps +Bs* (39.59) and *Azo + Bs* (38.26) on 25 DAS. It was also observed that the combined inoculation of all the PGPR was observed to be the best and exhibited maximum interaction effect than individual inoculation and dual inoculation of PGPR.

The individual and combined effect of PGPR on plant phosphorus content of bhendi was studied and the results are presented in (Table – 5). It was observed that the Plant phosphorus content of bhendi was significantly increased by all the treatments tested over control. The maximum Plant phosphorus content of 40.80 (mg plant<sup>-1</sup>) was recorded by the treatment *Azs-3 + Azo-4 + Ps-2 + Bs-3* on 100DAS. Among the individual treatments, the inoculation of *Pseudomonas Ps-2* was found to be the best and recorded 5.80 mg plant<sup>-1</sup> of plant phosphorus content followed by *Azs-3*(3.78) and *Azo -4* (3.10) on 25 DAS. Among the dual inoculation of the treatment T<sub>9</sub> - *Pseudomonas + Bacillus* recorded the maximum plant Phosphorus content of 6.72 (mg plant<sup>-1</sup>) followed by *Azs+Bz*(6.31) and *Azo+Bz* (6.21) on 25DAS. It was also observed that the combined inoculation of all the PGPR was observed to be the best and exhibited maximum interaction effect than individual inoculation and dual inoculation of PGPR.

Ahamad *et al.*, (2005) reported the production of IAA by 11 isolates of *Pseudomonas* from different crop plants in the range of 5.34 to 22.4 mg/ml. Similarly Karnwal (2009) also reported the varying amounts of IAA production by fluorescent *Pseudomonas*. Anandaraj and Sarma (2003) reported that growth-promoting strains of fluorescent *Pseudomonas* were found to synthesize phytohormones viz., IAA and Gibberellic Acid (GA). *Azospirillum* inoculation enhanced shoots and root growth with increase in nitrogen assimilation and were attributed to growth substances produced by the associated bacteria (Tian *et al.*, 1979). Positive effects of *Azospirillum* inoculation were demonstrated in various root parameters including increased in number and length of root, root dry weight (Hadas and Okon, 1987), increased in the number, density and appearance of root hairs, increased in root surface area (Bashan, 1986) and stimulation of root exudation (Heulin *et al.*, 1982), increased root hair development, root branching and root surface area (Fallik *et al.*, 1988).

*Azospirillum* inoculation increased the soil nutrient status especially in macro and micronutrients. This is mainly due to N fixation by *Azospirillum* strains as biofertilizer. In all the *Azospirillum* treatments, the soil total N was increased over control. This increase in the soil nutrient level was responsible for plant growth and development. *Azospirillum* inoculation increased the total nitrogen content and yield of cereal and forage grasses (Nur *et al.*, 1980; Kundu, 1988). *Azospirillum* inoculated plants showed increase in the level of total nitrogen in different parts of the plant and the inoculated soil (Saxena *et al.*, 1990).

Verma *et al.* (2010) who found that high proportion of rhizomicroorganisms are able produce plant growth hormone, i.e., indole acetic acid, which acts to stimulate plant growth and provides it with more branching and larger surface area. Thus

these IAA producing bacteria like *Pseudomonas fluorescens* and *Bacillus subtilis* were further studied or their effect on plant growth under controlled conditions. Data obtained from the pot experiments demonstrated positive effect on root elongation of treated plants over the control. This indicates both bacteria have the efficiency to improve the root and shoot length of development of the plant and these can be considered as plant growth promoters.

## CONCLUSION

From the present study, it was concluded that the plant growth promoting rhizobacteria *Azospirillum*, *Pseudomonas*, *Azotobacter* and *Bacillus* have the capacity to produce plant growth promoting substances and induce the growth of bhendi plant. Among the twelve treatments the treatment 12 gave the greatest results to induce the growth of plant and fruits. Combined application of a *P. fluorescens* along with *Bradyrhizobium* in groundnut significantly enhanced groundnut root and shoot dry weight, nodule number, nodule dry weight, and per cent nitrogen content of shoot (Vikram *et al.*, 2007).

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