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Research Article

EFFECT OF AZOSPIRILLUMANDPSEUDOMONAS ISOLATES AS MICROBIAL INOCULANTS ON GROWTH AND YIELD OF BHENDI [ABELMOSCHUS ESCULENTUS (L.) MOENCH] UNDER POT EXPERIMENT

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ABSTRACT

An experiment was conducted in pot during the season of June- September, 2014, to observe the efficacy of bacterial endophytes isolated from the roots of bhendi as microbial inoculants in seeds along with organic fertilizers on growth and yield of bhendi cv. *Arka anamika* [*Abelmoschus esculentus* (L.) Moench].This study clearly indicated that the combined application of Azospirillum brasilense and Pseudomonas flourescens along with inorganic fertilizers at 75% of the recommended dose of N, P and K favourably influenced the growth and yield of bhendi (*Abelmoschus esculentus*).The present investigation reveals that, microbial inoculants will reduce the farmer's budget for crop fertilization and inclusion of inorganic fertilizer in the combination with plant beneficial endophytic bacteria for sustainable crop production under a less polluted environment.

INTRODUCTION

Bhendi is a flowering plant in the mallow family Malvaceae, originating from tropical and subtropical Africa and is belongs to West Africa (Tindal, 1983). Okra is mainly cultivated for its "pods" which are cooked and eaten in variety of delicious way. This crop is suitable as a garden crop as well as on large commercial farms. It is grown commercially in India. India ranks first in the world with 3.5 million tonnes of okra produced from over 0.35 million ha land (FAOSTAT 2008). It is quite popular in India because of easy cultivation, dependable yield and adaptability to varying moisture conditions. Okra is a good source of vitamins, minerals, calories and amino acid found in seeds and also balanced nutrient diet. For achieving high level of productivity, nutrient management plays an important role. High yielding varieties required high amount of nutrients, which has lead to increase in input cost on fertilizers. Excessive use of fertilizers and continuous cropping has caused adverse effect on Physico-chemical properties of soil. This has resulted in decline of yield. These effects have made it necessary to search for alternate sources of fertilizers called bio-fertilizers which are eco-friendly and cost efficient. Thus, the experiment was carried out to find the suitable combination between chemical and bio-fertilizers to minimize the input cost and improve productivity.

MATERIALS AND METHODS

Collection of Seeds

Certified seeds of bhendi variety *Arka anamika* were collected from Agriculture Department, Karaikal.

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. 1.5gms of bhendi seeds (approximately172) 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.

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Table 1 Effect of efficient isolates of *Azospirillum sp.*and *Pseudomonas sp.*on shoot and root length of bhendi

Treatments	Shoot Length (cm)			Root Length (cm)		
	30 DAYS	60 DAYS	90 DAYS	30 DAYS	60 DAYS	90 DAYS
T_1	24.12±0.0212	44.37±0.04949	56.22±0.0494	5.09±0.0636	10.49±0.0141	14.84±0.0424
T_2	33.16±0.0141	56.52±0.06364	60.13±0.0282	6.16±0.04949	13.32±0.0565	18.34±0.0424
T_3	32.48±0.0565	54.31±0.0424	66.20±0.0424	5.78±0.03535	12.24±0.0424	18.37±0.0494
T_4	34.45±0.0141	53.52±0.0494	65.42±0.0212	6.11±0.03535	13.17±0.0494	18.09±0.0494
T_5	33.29±0.0848	53.68±0.0470	65.17±0.0494	6.39±0.01414	14.44±0.0634	19.12±0.0353
T_6	32.32±0.0494	58.16±0.0141	70.20±0.0282	8.22±0.04949	14.62±0.0424	19.25±0.0424
T_7	34.22±0.0282	58.29±0.0270	75.02±0.0282	7.21±0.0636	16.02±0.0270	19.29±0.0424

Values are mean ± SD 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 bhendi

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	0.52±0.0141	0.88±0.0141	1.36±0.0353	0.28±0.0565	0.73±0.0353	1.74±0.0141	0.82±0.0565	1.61±0.0353	2.43±0.0404
T_2	0.70±0.0353	1.21±0.0212	1.68±0.0353	0.72±0.0494	1.33±0.0494	1.68±0.0353	1.42±0.0494	2.55±0.0470	3.36±0.0494
T_3	0.76±0.0070	1.27±0.0212	1.8±0.0282	0.79±0.0353	1.61±0.0353	1.70±0.0282	1.55±0.06364	2.88±0.0353	3.50±0.0212
T_4	0.62±0.0212	1.17±0.0141	1.44±0.0424	0.61±0.0282	1.05±0.0141	1.43±0.0492	1.23±0.0494	2.22±0.0636	3.45±0.0351
T_5	0.65±0.0494	1.20±0.0424	1.47±0.0353	0.66±0.0494	1.42±0.0353	1.77±0.0565	1.31±0.0141	2.62±0.0636	3.24±0.0494
T_6	1.26±0.0353	1.30±0.0141	1.90±0.0141	1.14±0.0424	1.72±0.0141	2.10±0.0141	2.40±0.0212	3.02±0.0141	4.00±0.0141
T_7	1.19±0.0282	1.35±0.0251	2.10±0.0212	1.16±0.0424	1.91±0.0282	2.12±0.0424	2.35±0.0424	3.26±0.0565	4.22±0.0424

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

Table 3 Effect of efficient isolates of *Azospirillum* and *Pseudomonas* on Number of leaves, branches and flowers/plant of bhendi

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	6.09±0.0141	11.76±0.0353	20.02±0.0282	0	0	0	0	0	3.10± 0.0282
T_2	8.11±0.0494	14.18±0.0494	24.48±0.0353	0	0	1.14±0.0494	0	1.02±0.0142	4.00±0.0141
T_3	6.22±0.3394	12.97±0.0353	22.72±0.0212	0	0	1..03±0.0212	0	2.02±0.0141	4.19± 0.0565
T_4	6.42±0.0353	15.16±0.0494	24.13±0.0494	0	0	1.08±0.0565	0	1.08±0.0212	3.85 ±0.0353
T_5	7.06±0.0494	14.33±0.0494	25.09±0.0565	0	0	1.00±0.0212	0	1.05±0.0141	4.24±0.0636
T_6	9.46±0.0494	16.17±0.0494	26.84±0.0424	0	0	2.12±0.0141	0	2.07±0.0212	6.03± 0.0212
T_7	10.01±0.0282	15.91±0.0141	27.09±0.0565	0	0	2.15±0.0353	0	2.12±0.0212	6.12±0.0212

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

Table 4 Effect of efficient isolates of *Azospirillum* and *Pseudomonas* on yield parameters of bhendi

Treatments	Number of Fruits/Plant	Fruit Weight (gm)	Fruit length (cm)	Fruit girth (cm)	Number of Seeds/Fruit
T_1	4.22±0.0141	1.09±0.0212	8.65±0.0424	3.78±0.0141	60.12±0.0141
T_2	7.03±0.0424	1.26±0.0634	10.89±0.0212	6.22±0.0212	64.05±0.0141
T_3	8.17±0.0141	1.52±0.0494	11.23±0.0212	5.38±0.0494	62.16±0.0494
T_4	7.27±0.0636	1.58±0.0494	10.66±0.0282	5.22±0.0282	62.18±0.0212
T_5	7.36±0.0494	1.55±0.0636	11.58±0.0212	4.76±0.0634	60.20±0.0282
T_6	9.14±0.0353	2.18±0.0282	11.74±0.0494	6.77±0.0636	68.05±0.0494
T_7	8.98±0.0424	2.10±0.0565	12.01±0.0212	7.16±0.0494	70.02±0.0212

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

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.

Transplantation in Pots

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 plants. The data were collected on Plant height, Number of leaves, Number of branches, number of flowers, number of fruits, fruit weight, fruit length and fruit diameter.

Treatments

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

Chemical Fertilizers

Recommended dosages of NPK for okra N-20 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 centimeters 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.

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 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.

RESULTS

There was a significant increase and difference among both the inoculants in combination and sole application of either. Seed inoculation with representative endophytic nitrogen fixing bacterial strains significantly enhanced seed germination, growth and yield of bhendi. However, the rate of enhancement varied with bacterial strains. Here combined seed inoculation of *Azospirillum brasilense* and *pseudomonas fluorescens* with 75% chemical fertilizer was found superior to other combinations. Similar results were reported by Asha K. Raj and V.L Geethakumari, 2009, Shaheen *et al*., 2007, Anant Bahadur and R.K. Manohar, 2001, JK Singh, *et al*., 2010, Paramhans Prasad and Abhishek naik, 2013 in Okra.

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 bhendi 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 bhendi. 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 more comprehensive understanding of plant colonization by bacteria has to be developed in order to better predict how bacteria interact with plants interiors and whether they are likely to establish themselves in the plant environment after field application as inoculants.

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