International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 4, Issue 4 - 2017

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.04.024

Effect of microbial inoculants on Growth and Yield of bhendi [*Abelmoschus esculentus* (L.)Moench] in field trial.

***U.Sivagama sundari[1,2] and A.Gandhi[1]**
[1]Department of Botany, Annamalai University, Tamilnadu, India
[2]Department of Life Sciences, Kristu Jayanti College, Bangalore, Karnataka, India
*Corresponding author: *drusbot@gmail.com*

Abstract

An experiment was conducted in agriculture field, Karaikal during the season of June- September to observe the efficacy of isolated bacterial endophytes from the roots of bhendi as microbial inoculants in seeds on growth, yield and some biochemical constitutions of bhendi cv. *Arkaanamika* [*Abelmoschus esculentus* (L.) Moench] and the results revealed that the height of plant, number of branches, number of leaves, number of flowers, number of fruits, fruit weight, fruit length, fruit diameter and yield/plot were recorded maximum in $T_7$and proved that the application of microbial inoculants and their combinations with inorganic fertilizers significantly influenced the growth, yield, quality and biochemical contents of bhendi. The combined application rates of 75% Chemical fertilizer with *Azospirillum braziliense* and *Pseudomonas fluorescens* gave the best performance of bhendi compared to other treatments. From the results, microbial inoculants willreduce the farmer's budget for crop fertilization and inclusion of organic fertilizer in the combination will surely ensure production of crop under a less polluted environment.

Keywords: Endophytes, Microbial inoculants, okra, *Azospirillum brasilense* and *Pseudomonas flourescens*

Introduction

Okra is a flowering plant in the mallow family Malvaceae, originating from tropical and subtropical Africa and is natural to West Africa (Tindal, 1983). It was formerly considered a species of hibiscus, but is now classified in the genus Abelmoschus. The word okra is of Africa origin and means "Lady's fingers" in Igbo, a language spoken in Nigeria. Okra is mainly cultivated for its "pods" which are cooked and eaten in countries like Sudan, Egypt and Nigeria. It is also important in other tropical areas including Asia central and South America. This crop is suitable for cultivation 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 (70% of the total world production) 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. Vegetables play a vital role in the improvement of the diet of mankind. Okra is a good source of vitamins, minerals, calories and amino acid found in seeds. Hence, it has planned to study the effect of endophytic nitrogen fixing bacterial species on growth and yield of bhendi. In recent years we are creating awareness for eco-friendly organic products. Sustainable and eco-friendly agriculture which minimizes the use of harmful energy intensive inputs is achievable through the use of organic and

biofertilizers. Organic nutrition for vegetables is especially important as they provide quality foods, which are very important for providing health security to people. Since the vegetables are mostly consumed as fresh or partially cooked, they should be devoid of residual effect of chemical fertilizers. Increase in the yield of chilli, okra, tomato and brinjal by application of organic manure was reported by Gaur *et al.*, (1984). Nowadays the practice of using microbial inoculants as biofertilizers as a partial substitute for chemical fertilizers is gaining much momentum. In general bio-fertilizer from associative N_2 fixing bacteria could be used especially for cash crops such as vegetables, fruits, flowers and medicinal or herbal crops. It is a breakthrough technology that promises very significant impact on the country's farmers in terms of increasing farm productivity and income as well saving the country's dollars reserve due to decreased importation of inorganic nitrogenous fertilizers.

It is mainly composed of microorganisms that can convert the nitrogen gas into available form to sustain the nitrogen requirement of host plants. These bacteria once associated with roots of some vegetable plants can enhance their root development growth and yield. In the present agricultural practices there are number of microbial inoculants used as bio-fertilizers. They induce *Azospirllum* and *Azotobacter* and phosphor-bacterium, which have been given much attention as they are responsible to plant growth and yield of crops under field inoculation (Shaheen *et al*., 2007). *Azospirillum* is an associative symbiotic nitrogen-fixing bacterium having high potential for nitrogen fixation and produces growth hormones. *Azospirillum* inoculation is known to increase the yield of crops by 5 to 20 per cent (Dart, 1986). *P. fluorescens* belong to Plant Growth Promoting bacteria, the group of bacteria that play a major role in plant growth promotion, induced systemic resistance, biological control of pathogens etc. With regard to the plant growth promoting potential of *P. fluorescens*, Fernando Dini Andreote, 2009 reported *Pseudomonas* is a novel competent endophyte from potato and causes cultivar-dependent suppression of *Phytophthora infestans* in his study of Endophytic Colonization of Potato (*Solanum tuberosum* L.). In the present investigation theendophytic growth promoting nitrogen fixing bacteria and inorganic fertilizers were studied in combinations to establish the growth and yield and bhendi (*Abelmoschus esculentus*, L.).The results showed that application of microbial inoculants and inorganic fertilizers and their combinations significantly influenced the growth, yield, quality and nutritional contents of bhendi.

Materials and Methods

Collection of seeds

Certified seeds of Okra 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. 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.

Field trial

The trial was carried out in cultivable land, Karaikal during the season of June to September2014, in a randomised block design 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 45 cm for bhendi 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.

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*

Replications: 3 Nos. in each.

Chemical fertilizers: Recommended dosages of NPK for okra **N-20 kg/ha, P-50 kg/ha, K-30 kg/ha**

Biometric observations

Random samples from each treated plots were harvested at the intervals of 30th, 60th and 90th day for biometric observations. Proximal compositions of fruits Total moisture (*FSSAI*, 2012)and total ash (*FSSAI*, 2012), protein (Lowery *et al*., 1951), total sugars (Nelson, 1944), total phenol (Jagadish *et al*., 2009), flavanoids (Jagadish *et al*., 2009), carotenoids (Arnon, 1949), ascorbic acid (Omeye *et al*., 1979) and antioxidant enzymes (Kumar and Khan, 1982) were estimated when the fruits were tender.

Results

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

Treatments	Shoot Length (cm)			Root Length (cm)		
	30 DAYS	60 DAYS	90 DAYS	30 DAYS	60 DAYS	90 DAYS
T_1	20.18±0.03055	42.35±0.0208	55.82±0.0305	5.16±0.1934	11.29±0.2081	15.84±0.0251
T_2	32.26±0.0360	55.52±0.0264	67.20±0.0251	6.13±0.0404	13.82±0.0360	17.24±0.0251
T_3	31.40±0.0602	52.41±0.0251	67.26±0.0208	6.18±0.0251	12.44±0.0351	16.32±0.0152
T_4	32.25±0.0305	53.32±0.5723	66.40±0.0251	6.21±0.0251	13.27±0.0360	16.19±0.0360
T_5	32.19±0.0305	52.58±0.0305	64.37±0.0360	5.29±0.0611	12.24±0.0351	16.12±0.0305
T_6	32.21±0.0321	57.26±0.0264	72.30±0.0251	8.02±0.0351	14.72±0.0305	18.25±0.0251
T_7	34.19±0.0351	60.29±0.0360	75.12±0.0208	8.11±0.0264	16.12±0.0458	20.62±0.1588

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

There is 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 inoculated with *Azospirillum brasilense* and *Pseudomonas fluorescens* with 75% chemical fertilizer was found superior to other combinations. T_7 recorded maximum readings in all parameters, Similarly biochemical constitutions of fruit, i.e. total moisture, total ash, total sugars, protein, flavonoids (major antioxidant in okra), caroteniods, ascorbic acid and antioxidant enzymes have also shown maximum contents in combined inoculation of *Azospiriillum brasilense* and *Pseudomonas fluorescens* with 75% chemical fertilizer followed by other combinations compared with control. 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 Abhisheknaik, 2013 in Okra.

Table 2. Effect of efficient isolates of *Azospirillum* and *Pseudomonas* 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	0.78±0.0565	0.96±0.0208	1.32±0.0251	0.52±0.0503	0.68±0.0305	1.28±0.0568	1.30±0.0450	0.68±0.0305	2.60±0.0450
T_2	0.82±0.0450	1.19±0.0351	1.65±0.0208	0.88±0.0351	1.28±0.0416	1.86±0.0378	1.67±0.0513	1.28±0.0416	1.86±0.0378
T_3	0.87±0.0650	1.24±0.0152	1.8±0.0305	0.88±0.0737	1.57±0.0251	1.88±0.0208	1.75±0.0737	1.57±0.0251	1.88±0.0208
T_4	0.55±0.0832	1.12±0.0251	1.4±0.0416	0.75±0.0416	0.97±0.0264	1.39±0.0351	1.30±0.0655	0.97±0.0264	1.39±0.0351
T_5	0.72±0.5502	1.11±0.0208	1.44±0.0351	0.73±0.5852	1.18±0.0416	1.49±0.0450	1.45±0.0404	1.18±0.0416	2.84±0.0378
T_6	1.18±0.0416	1.26±0.0305	1.92±0.0360	1.02±0.0321	1.76±0.0305	2.33±0.0556	2.20±0.0472	1.76±0.0305	4.15±0.0251
T_7	1.22±0.040	1.31±0.0264	2.02±0.0305	1.12±0.0351	1.89±0.0458	2.38±0.0416	2.30±0.0450	1.89±0.0458	4.40±0.0509

Values are mean ± S.D 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 in field 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	5.09±0.0351	10.36±0.0251	20.12±0.0351	0	0	1.52±0.0251	0	5.17±0.0251	11.88± 0.0378
T_2	7.21±0.0251	16.18±0.0378	30.28±0.0305	0	0	2.04±0.0251	0	7.18±0.0305	16.06±0.03052
T_3	7.32±0.0264	17.17±0.0321	27.42±0.0264	0	1.09±0.0208	3.13±0.0208	0	8.03±0.0321	16.12± 0.0208
T_4	6.42±0.0251	16.56±0.0321	24.53±0.0281	0	1.00±0.0404	3.44±0.1537	0	6.62±0.0321	15.71 ± 0.0208
T_5	7.16±0.0404	14.23±0.0251	25.19±0.0435	0	0	2.18±0.0378	0	7.05±0.0208	14.20±0.03055
T_6	10.26±0.0251	19.27±0.0251	32.14±0.0251	0	1.02±0.0378	4.36±0.0305	0	9.12±0.0208	17.22± 0.0208
T_7	12.31±0.0360	19.81±0.0264	34.09±0.0351	0	1.1±0.0305	5.11±0.0264	0	10.07±0.0360	18.32± 0.0305

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

Table 4. Effect of efficient isolates of *Azospirillum* and *Pseudomonas* microbial on yield of bhendi 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	9.82±0.0152	1.39±0.0251	7.55±0.0404	5.78±0.0305	72.75±0.0305	4.86±0.0305
T_2	10.13±0.0208	1.56±0.0208	8.48±0.0305	6.10±0.0251	80.12±0.0321	7.43±0.0251
T_3	13.17±0.0251	1.62±0.0251	8.53±0.0208	6.18±0.0305	82.36±0.0321	6.28±0.0321
T_4	13.17±0.0452	1.78±0.0251	7.68±0.0378	5.82±0.0305	80.18±0.0378	7.60±0.0450
T_5	10.36±0.0208	1.55±0.0208	8.25±0.0351	4.16±0.0352	86.20±0.0351	6.04±0.0251
T_6	15.18±0.0321	2.40±0.0264	9.41±0.0404	7.27±0.0404	86.13±0.0321	8.10±0.0416
T_7	17.24±0.0264	2.88±0.0305	9.62±0.0251	7.36±0.0208	88.21±0.0493	8.27±0.0360

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

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

Treatments	Total Moisture (%)	Sugar (mg/gm)	Total ash (%)	Total Phenols (mg/gm)	Protein (mg/gm)	Flavanoids (mg/gm)	Carotenoids (mg/gm)	Ascorbic acid (%)	Peroxidase (mg/gm)	Polyphenol Oxisdase (mg/gm)
T_1	82±4.163	3.66±0.0264	0.28±0.030	4.14±0.0305	1.55±0.3511	1.82±0.0503	0.77±0.0251	3.435±0.003	4.2±0.03055	4.78±0.0416
T_2	87±3.605	4.86±0.0305	0.37±0.051	4.74±0.0360	1.60±0.0305	2.48±0.0321	2.02±0.0305	6.225±0.003	4.89±0.0351	5.30±0.0305
T_3	86±3.517	5.38±0.1193	0.30±0.045	4.86±0.0351	1.77±0.0321	3.04±0.0305	2.20±0.0251	6.624±0.007	5.42±0.0351	5.71±0.0264
T_4	86±3.517	4.66±0.1171	0.40±0.055	4.70±0.0360	1.72±0.0321	2.52±0.0416	2.09±0.0635	6.210±0.007	5.26±0.1835	5.52±0.0305
T_5	87±4.725	5.12±0.0305	0.36±0.056	4.58±0.0360	1.62±0.0346	3.19±0.0472	1.87±0.0321	6.122±0.005	4.67±0.0321	5.19±0.0351
T_6	89±6.244	5.70±0.0450	0.42±0.036	5.08±0.0378	1.86±0.0251	4.09±0.0416	2.48±0.0321	8.106±0.003	5.77±0.0321	5.82±0.0360
T_7	89±4.725	5.92±0.0305	0.59±0.020	5.18±0.0305	1.89±0.0264	4.17±0.0351	2.66±0.0264	8.118±0.004	5.86±0.0208	6.02±0.0251

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

Fig.1 Field trial study

Conclusion

From the above findings and results, it was 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 okra 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 okra. 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 biofertilizers.

Acknowledgments

The author wish to thank UGC-SAP, BSR – Fellowship, New Delhi for financial support for this research work.

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	Website: www.ijarbs.com
Quick Response Code	Subject: Agricultural Sciences
DOI:10.22192/ijarbs.2017.04.04.024	

How to cite this article:
U.Sivagama sundari and A.Gandhi. (2017). Effect of microbial inoculants on Growth and Yield of bhendi [*Abelmoschus esculentus* (L.)Moench] in field trial. Int. J. Adv. Res. Biol. Sci. 4(4): 182-188.
DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.04.024