International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 12 (2014) pp. 445-448**
http://www.ijcmas.com

Original Research Article

Effect of endophytic bacteria isolated from bhendi as microbial inoculants on vegetative parameters of bhendi in proplates

U.Sivagama sundari* and A.Gandhi

Department of Botany, Annamalai University, Annamalainagar, Tamilnadu, India
**Corresponding author*

Keywords

Bhendi, Endophytic bacteria, *Azospirillum brasilense*, *Pseudomonas fluorescens*

A B S T R A C T

An experiment was conducted at Neravy in Karaikal, Pondicherry, during the month of September to October, 2014, with the objectives to observe the efficiency of isolated bacterial endophytes - *Azospirillum brasilense* and *Pseudomonas fluorescens* from bhendi by seed inoculation on vegetative growth and pigment content of bhendi cv. *Arka anamika* [*Abelmoschus esculentus* (L.) Moench]. A total number of 35 endophytic bacteria were isolated from bhendi from three localities (Annamalai University, Karaikal and Putthur). They were biochemically characterized and nitrogen fixing ability was tested. Based upon their nitrogen fixation ability and IAA production, two superior strains *AORU5 -Azospirillum brasilense and PORU3 -Pseudomonas fluorescens* were selected from the roots of bhendi collected from Annamalai University and its performance was tested in bhendi. Among the treatments given, T_7- 75% Chemical fertilizer + *Azospirillum brasilense* + *Pseudomonas fluorescens* was found superior in all parameters observed compared with other treated plates than control.

Introduction

Although the term "endophytes" is most commonly associated with fungi, there is a significant amount of literature pertaining to bacteria as endophytes as well. For more than 50 years, bacteria have been observed to exist inside plants as beneficial without causing apparent disease symptoms (Tervet and Hollis, 1948; Hollis, 1951). Various reports indicate that such bacteria exist in a variety of tissue types within numerous plant species, suggesting ubiquitous existence in most if not all higher plants.

The main objectives of integrated plant nutrient management (IPNM) are to maintain and possibly enhance soil fertility through a balanced use of chemical fertilizers combined with organic and biological sources to improve efficiency of plant nutrients, increase crop productivity and minimizing losses to the environment. Nutrient management is one of the prime considerations for getting higher yield of any crop. Among the major cultivating vegetable crop in India, especially in Tamilnadu, bhendi occupies unique space in nutrient diet. It 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*. Bhendi is mainly cultivated for its "pods". 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 tons (70% of the total world production) of bhendi 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. Bhendi is a good source of vitamins, minerals, calories and amino acid found in seeds. Keeping the important of the above nutritional facts and widespread of bhendi and the lack of sufficient information and commercialization of cost effective eco-friendly alternative agricultural methods to farmers in agriculture fields, the present experiment was undertaken to isolate the native microbes (Bacteria) of bhendi which reside as endophytes and apply its own as inoculants by seed inoculation to observe the vegetative growth and pigment contents in bhendi.

Material and Methods

Collection of seeds

Certified seeds of Bhendi var. *Arka anamika* was received from Department of Agriculture, Karaikal, Pondicherry.

Inoculants preparation

The isolates of *Azospirillum brasilense* and *Pseudomonas fluorescens* which were identified by biochemical tests and molecular characterization by sequencing 16srRNA 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 (14×10^5) were mixed along with seeds, shade dried and immediately sown in proplates. There were three replicates maintained for each treatment.

Treatment details of pot experiment

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

Nursery in proplates

Seeds of 7gms (125 seeds approx.) per treatment was thoroughly mixed with 1.5ml of prepared microbial cultures. Then they were shade dried and immediately sown in proplates (96 cups in each plate, only one seed in each cup) containing coco peat as substrate and their chemical constituents are given in Table 1. Irrigation and other needs were done as when required.

Parameters studied in nursery bed

Initial parameters like, germination percentage of seeds, days taken to germinate, plant height via. Root and shoot length, number of leaves in seedlings and pigment contents were observed in plates after 15 and 30 days from DOS.

Results and Discussion

There is significant increase in each treatment in combination with inorganic fertilizers. Seed inoculation with endophytic bacterial isolates significantly enhanced vegetative growth and pigment content of bhendi. However, the rate of enhancement varied with isolates, i.e. whether it applied as sole or in combined inoculation. Germination percentage was observed maximum in T_7 - 100% chemical fertilizer + *Azospirillum* + *Pseudomonas*. T_7 recorded maximum value of root length (1.16cm at 15^{th} and 3.22cm at 30^{th} day), shoot length (3.50cm at 15^{th} and 5.10cm 30^{th} day) and number of leaves per plant (2.41 at 4.27 15^{th} day and at 30^{th} day) followed by T_6, T_3, T_2, T_5 and T_4 (Table 2). Similar trend was observed in pigment content of leaves in brinjal (Table 3). Chlorophyll content was observed maximum in combined inoculation with 75% chemical fertilizer - T_7 thanT_6 followed by T_2, T_3, T_4, T_5 and T_1. Similar results were found by Thirumaran *et al*., 2009 and Rahman, 2012.

Table.1 Chemical analysis of the experimental cocopeat

S.No	Parameters	
1	pH	6.15
2	EC(mmhos/cm/25^{o}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

Table.1 Effect of efficient isolates of *Azospirillum* and *Pseudomonas* on vegetative parameters at 15th and 30th day of bhendi in proplates

Treatments	Germination % of seeds	RootLength(cm)		ShootLength(cm)		No. ofLeaves/plant	
		15^{th}day	30^{th}day	15^{th}day	30^{th}day	15^{th}day	30^{th}day
T_1	89.5	1.05±0.0404	2.20±0.0212	2.36±0.0353	3.62±0.0212	2.09±0.0282	3.48±0.0282
T_2	90.8	1.14±0.0450	3.10±0.0282	3.16±0.0494	4.54±0.0353	2.18±0.0424	3.65±0.0424
T_3	88.2	1.09±0.0351	2.87±0.4030	3.10±0.0282	4.28±0.0353	2.20±0.0424	4.23±0.0424
T_4	86.4	1.11±0.0450	3.13±0.0565	2.88±0.0141	4.32±0.0212	2.25±0.0424	3.72±0.0282
T_5	87.6	1.07±0.0655	2.98±0.0424	3.02±0.0707	4.17±0.0212	2.19±0.0707	3.60±0.0141
T_6	88.6	1.16±0.0305	3.14±0.0424	3.46±0.0282	5.06±0.0494	2.38±0.0353	4.22±0.0424
T_7	92.8	1.16±0.0503	3.22±0.0494	3.50±0.0212	5.10±0.0141	2.41±0.0353	4.27±0.0424

Values are mean ± S.D of three samples

Table.2 Effect of efficient isolates of *Azospirillum* and *Pseudomonas* on leaf chlorophyll content of bhendi in proplates

Treatments	Chlorophyll 'a'		Chlorophyll 'b'		Total Chlorophyll (a+b)	
	15 days	30days	15 days	30days	15 days	30days
T_1	0.22±0.0503	0.58±0.0416	0.18±0.0556	0.26±0.0404	0.40±0.0416	0.84±0.0404
T_2	0.30±0.0568	0.74±0.0152	0.28±0.0702	0.69±0.0351	0.58±0.0702	1.43±0.0351
T_3	0.44±0.0602	0.77±0.0416	0.22±0.0602	0.70±0.0351	0.66±0.0069	1.47±0.0404
T_4	0.28±0.0503	0.69±0.0450	0.20±0.0378	0.58±0.0416	0.48±0.0926	1.27±0.0758
T_5	0.32±0.0503	0.66±0.3512	0.26±0.0550	0.62±0.360	0.58±0.0611	1.28±0.0458
T_6	0.47±0.0776	1.12±0.0251	0.32±0.0503	1.09±0.0360	0.79±0.0665	2.21±0.0351
T_7	0.51±0.0802	1.14±0.0450	0.34±0.0737	1.12±0.0305	0.85±0.0407	2.26±0.0435

Values are mean ± S.D of three samples

From the above findings and results, it was clear that the microbial inoculants and the process of nitrogen fixation of that have greater contribution in soil fertility and also in vegetative growth and pigment contents of bhendi in proplates. Consequently combined inoculation of both the microbes effectively influences the growth of bhendi in all observed parameters. Our results clearly demonstrate remarkably different bacteria colonize unique habitat and as efficient colonizers-'endophytes', not only in bhendi but also in others as native. From this experiment and findings, it gives an idea to isolate the native colonizers of this kind of microbes as inoculants in field application to develop organic/bio fertilizers.

References

AI- Tindall, H.D., Rice, R.P. 1983. Fruit and vegetable production in warm climates. The Macmillan press Ltd, Nigeria. 85 Pp.

Tervet, I.W., Hollis, J.P. 1948. Bacteria in the storage organs of healthy plants. *Phytopathology*, 38: 960–967.

Hollis, J.P. 1951. Bacteria in healthy potato tissue. *Phytopathology*, 41: 350–366.

Thirumaran, G., Arumugam, M., Arumugam, R., Anantharaman, P. 2009. Effect of seaweed liquid fertilizer on growth and pigment concentration of *Abelmoschus esculentus* (l) medikus. *American-Eurasian J. Agronomy*, 2 (2): 57–66.

Rahman, MA., Ferdousi Akter, 2012. Effect of NPK fertilizers on growth, yield and yield attributes of okra (*Abelmoschus esculentus* (L.) MOENCH.). *Bangladesh J. Bot.*, 41(2): 131–134.