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Field Evaluation of Biofertilizer Potential of Bacillus Polymyxa Isolated from Rhizosphere of Brinjal (*Solanum melongena* L.)

Jenifer Lolita C.[1], Shivashankrappa L. H.[2], Keshamma E.[3], Kavitha K. R.[4]

[1]Associate Professor, Department of Botany, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India

[2]Associate Professor, Department of Sericulture, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India

[3]Associate Professor, Department of Biochemistry, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India

[4]Associate Professor, Department of Botany & PG Studies, Nrupathunga University, Bengaluru, Karnataka, India

Abstract: The main aim of the current study to evaluate the potential of Bacillus polymyxa isolated from Brinjal (Solanum melongena L.) as a biofertilizer. The Brinjal (Solanum melongena L.) cultivar Arka keshav were used in this study. The isolated bacteria from rhizosphere of brinjal identified as Bacillus polyrnyxa was used as the inoculant. The seeds of brinjal were obtained from Division of Vegetable crops. IIHR, Hesaraghatta. Bangalore. Results depicted that the plants, which received half dose of nitrogen and phosphorus showed the maximum percent increase in the shoot, dry weight (59%) over the uninoculated controls, which received the same level of fertilizers. The maximum percent increase in shoot fresh weight (51%) and photosynthetic efficiency (88%) was observed at the half level of nitrogen and phosphorus in plants treated with Bacillus polymyxa as compared with uninoculated controls at the same fertilizer level. The inoculated plants that received half of nitrogen and half dose of phosphorus showed an increase of 56% of copper, 56% of calcium and 58% of manganese content in the plants as compared with the uninoculated controls at the same fertilizer levels. The phosphorus content of the treated plots in the rhizosphere soil during the period of the experiment in which brinjal cv. Arka Keshav was cultivated showed significantly higher values of available phosphorus content than the control plots. In conclusion, the bacterial isolate from brinjal (Solanum melongena L.) plants identified as Bacillus polymyxa when used as inoculum in field trials showed significant improvement and beneficial in growth parameters of treated plants over the uninoculated controls at all levels of fertilizer application.

Keyword: Field trial, Solanum melongena L. Bacillus polymyxa, Azospirullum

1. INTRODUCTION

Beneficial associative microorganisms isolated from many crop plants have been effectively used to fertilize the same crops [1]. Many such application studies are related to cereals, legumes and vegetables. Barea and Brown (1974) reported that inoculation with Azotobacter paspali significantly improved the growth of tomato and lettuce. Plant root association with Azospirillum has been reported to improve plant productivity [2,3]. Legumes that responded well to this type of fertilizer treatment were winged bean, soybean, cow pea [4], and Peas [5]. Vegetables such as Yam, sweet potatoes, beet root, potatoes, tomato, sugar beet and cabbage showed significant improvement when treated with associative bacteria isolated from the stem of the same crop [6].

Other crops which recorded significant improvement when given this treatment were mustard, sugarcane, Kallar grass, green house bell pepper, lupine, lodge pine, banana, jute, rye and coconut palm [7]. Cereals such as rice, wheat, pearl millet, oats, zea mays, corn, Barley and sorghum showed significant improvement in plant biomass and grain yield after inoculation with Azospirillum. Different levels of fertilizers were applied along with the isolated associative microorganisms to supplement bio fertilization [8]. Siripin (1987) studied the effect of using 14 species of associative nitrogen fixing bacteria on sugarcane in an inoculation experiment using four levels of nitrogen. He reported significant increase in plant biomass, total plant nitrogen in treated plants over that of uninoculated controls [9]. Murali and

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Purushothaman, (1988), isolated a number of efficient nitrogen fixing bacteria identified to be Azospirillum from the roots of upland rice. These were successfully used as biofertilizer in a field experiment using different fertilizer levels [10]. The treated rice plants showed significant increase in grain and straw yield. Grain yield was highest in Azospirillum treated plants, which received 75-Kg hectare^{-1}nitrogen than the plants, which received 100 kg fertilizer N/hectare.

Furthermore, Gopalaswamy and Vidyasekaran (1988) studied the response of Azospirillum inoculation on different varieties of rice using different levels of fertilization and reported that treated plants produced higher number of productive tillers, and had increased straw and grain yield [11]. Rajagopalan and Rangaswamy, (1988) studied the effect of Azospirillum inoculation on rice yield in both Kharif and Rabi season. They used farm yard manure (FYM) mixed with Azospirillum and different levels of nitrogen in their experiment. They found significant yield improvement during the rabi season [12]. Balasubramanian, (1987) observed enhanced tillering by combining fertilizer nitrogen at different levels along with Azospirillum inoculation. They reported significant increase in grain and straw yield at all levels of nitrogen application. The mean grain yield at 50% nitrogen fertilizer with Azospirillum treatment equally yield with 100% nitrogen fe1tilizer. Significant increase was recorded at all levels [13]. With this background, in the present study field trial we aimed to evaluate the potential of Bacillus polymyxa isolated from brinjal as a biofertilizer.

2. MATERIALS AND METHODS

Brinjal (Solanum melongena L.) cultivar Arka keshav were used in this study. The isolated bacteria identified as Bacillus polyrnyxa was used as the inoculant. Inorganic fertilizers such as urea, superphosphate, and muriate of potash were used in reduced doses. The seeds of brinjal were obtained from Division of Vegetable crops. IIHR, Hesaraghatta. Bangalore.

Brinjal (Solanum melongena L.) cv Arka keshav seedlings were raised in nursery beds measuring l.0m x 1.5m. One of the nursery beds received 500ml of 48 hr broth of Bacillus polymyxa mixed with FYM. The other nursery bed received 250ml of 48-hour culture of Bacillus polymyxa, and 250 ml of 48 hr culture of Azospirillum, both having a population of 10^9/mL. The bacterial inoculants were mixed with farm yard manure (FYM) and applied. The third bed served as the control, which received 500 mL of heat, killed bacterial culture broth. Seeds of Brinjal (Solanum melongena L.) were sown in rows 5 cms apart above the inoculum in the field soil at a depth of 2 cms. Starter cultures of Bacillus polymyxa were subcultured in Burk's media [1], and multiplied using TYMB Media (pH 7.0) [14].

All seedlings were given a drenching of respective inoculum before transplantation. One month old seedlings were transplanted to the experimental plots measuring 2m x 2m in rows with a spacing of 80 cms between rows and 40 cms between plants. The soil had a pH of 6.3 having an organic nitrogen content of 3.03 ppm, 0.04 ppm phosphorus and 0.67% of organic carbon. Nitrogen and phosphorus were applied in different levels viz., NrPf. NrPH, NHPf. and NHPH ('f' means full dosage and 'h' means half dosage). The plots were topped with FYM.

Recommended fertilizer dosage for Brinjal i.e., 170N: 8OP: 60K was applied in the form of urea, superphosphates and muriate of potash respectively. Each treatment and its respective controls were in triplicates. The plants were irrigated regularly and plant samples were collected to record the biometric observations and physiological parameters recorded were shoot fresh weight, plant height, photosynthetic efficiency, leaf surface area, mineral content and yield. Nitrogen and phosphorus content of the soil were analyzed periodically.

The average maximum and minimum temperature during the period of the experiment were 27.57°C and 14.66°C. The average relative humidity was 73.96%. The average rainfall was 1340mm. Measurements of leaf surface area, photosynthetic rate, yield, mineral content of the plant, dry weight and yield were recorded periodically. Nitrogen and phosphorus content of the soil were analysed periodically. The field trials were performed in a completely randomised design with three replications for each level of fertilizer application. The results were analysed statistically by F-test.

Photosynthetic efficiency was measured in percentage using photosynthetic efficiency analyzer (Hansa Tek) which measures the Dark reaction of photosynthesis. The leaf area was measured using a Skyla leaf area analyzer. Measurements were made in the fully expanded intact leaf developed at the 3rd internode. The leaf area was expressed in cm^2. The yield was collected periodically and the data was expressed in kg/plot.

To determine the plant nitrogen, Kjeldhal procedure was followed. Leaf samples were collected from the field and air dried in a hot air oven at 60 °C. The air dried samples were ground and sieved. 100 mg of the dried plant material was digested in Kjeldhal digestion flasks with 5ml of concentrated sulphuric acid. Digestion tablets were added to aid the digestion. 1% v/v hydrogen peroxide was added at the end of the digestion. The volume in the cooled digested flasks was

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made up to 100 mL. 25 mL of the diluted digest along with 15 mL of 40% sodium hydroxide was distilled. The distillate was titrated against 0.0l N hydrochloric acid [15].

Plant phosphorus, potassium and other microelements were determined by the method described by Jackson (1973). 100 mg of the plant material, which was dried, ground and sieved and digested using 10 ml of diacid mixture. The volume was made up to 100 ml and filtered and the plant mineral content was analyzed using an atomic spectrophotometer (Perkin Elmen 5000 Atomic absorption spectrometer). The amount of plant potassium was analysed using a flame photometer. The phosphorus, calcium, magnesium, sulphur and potassium content were expressed in percentage. The amount of the other elements was expressed in parts per million [15].

Nitrogen content of the soil was analysed by the total carbon method of Walkley and Black described by Jackson (1973). One gram of finely powdered and sieved soil was taken in a 500ml flask. 10ml of l N potassium dichromate and 20ml of concentrated sulphuric acid was added and the flask was placed in a shaker for 30 minutes for digestion. The digest was made up to 200ml and left to stand for 30 minutes. The excess chromic acid left over unreduced in the digest was determined by titration with standard ferrous sulphate using diphenylamine as an indicator. The appearance of apple green colour indicated the end of the reaction. The amount of nitrogen in the soil was indirectly calculated by the amount of total organic compound present in the soil [15].

The soil phosphorus content was estimated by the Bray method. Ammonium molybdate and stannous chloride were added to the soil filtrate and the developed colour was read at 690nm. The phosphorus content was calculated using a standard graph. Dilutions of potassium hydrogen phosphate in the range of 0.0 to 2.0 mg/L was used to prepare the standard graph with OD on the X axis and concentration on the Y axis [15].

Nitrogenase activity m rhizoplane and phylloplane was detected by the acetylene reduction assay following the method of Turner and Gibson (1980) [16]. Two months old brinjal plants (treated and control) were taken from the field. The roots and leaves were separated and washed in running water followed by washing with sterile distilled water and cut into 1 inch bits. The Nitrogenase activity in these plant parts was estimated by acetylene reduction technique using a gas chromatograph (Hewlett Packard series 5000). The time period between the harvest of the plants and Nitrogenase assay was 25 mins. The plant materials were kept in 250 mL flasks, which was made airtight. 1 mL of 10% acetylene was injected into the flask after drawing out the required volume of air. 1 mL of the gas phase was analysed after 1 hr of incubation at 37 °C.

Field experiments were conducted to test the response of brinjal to inoculation with Bacillus polyrnyxa. Brinjal (Solanum melongena L.) cvs Arka Keshav were treated with Bacillus polymyxa during the Kharif season. Interaction of Bacillus polymyxa with Azospirillum was also tested.

3. RESULTS

Growth parameters recorded in this field trial revealed that the brinjal (Solanum melongena L.) cv. Arka keshav plants which received the combined treatment of Bacillus polymyxa and Azopirillum showed significant increase in shoot dry weight, plant height, photosynthetic efficiency and yield, over the uninoculated controls at all fertilizer levels (Tables 1 & 2).

The plants, which received half dose of nitrogen and phosphorus showed the maximum percent increase in the shoot, dry weight (59%) over the uninoculated controls, which received the same level of fertilizers. The plants treated with Bacillus polymyxa showed a significantly higher plant height than the uninoculated controls at all fertilizer levels.

The maximum percent increase in shoot fresh weight (51%) and photosynthetic efficiency (88%) was observed at the half level of nitrogen and phosphorus in plants treated with Bacillus polymyxa as compared with uninoculated controls at the same fertilizer level. Plants, which received full doze of nitrogen and phosphorus and treated with Bacillus polymyxa, had a percent increase of 39% in shoot dry weight over the uninoculated control at the same fertilizer level.

Table. 1. Growth parameters of Brinjal (Solanum melongena L.) cv. Arka keshav plants fertilized with bacterial inoculants in a field trial at three fertilizer levels.

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Treatment	Shoot fresh weight (kgs)	% variation over control	Shoot dry weight (gms)	% variation over control	Root dry weight (gms)	% variation over control
Control N_0P_0	0.721		52.33		3.86	
Control Nh P,	0.864		78.33		14.61	
Control Nr Ph	1.650		153.33		20.83	
B.p. treated N_0P_0	0.900	24	78.113	39	6.13	58
B.p. treated N,P,	1.308	51	124.66	59	22.81	56
B.p. treated N,P,	I.952	18	167.66	09	27.43	31
B.p & *Azo.* Treated N_0P_0	0.910	26	80.66	54	6.76	75
B.p & *Azo.* Treated NhPh	1.217	40	118.33	51	19.36	32
B.p & *Azo.* treated N,P,	1.697	02	193.66	26	23.76	14
'F' test	*				*	
CD at 5%	0.09		0.06		3.16	
SEM	1.351		50.591		1.077	

* significant   * * highly significant

B.p. *Bacillus polymyxa*   *Azo.* *Azospinllum*

N_0P_0 zero doze of nitrogen and phosphorus   Nh P, half doze of nitrogen and phosphorus

N,P, full doze of nitrogen and phosphorus   Fertilizer dosages as recommended for *solanum melongena* L. cv. *Arka keshav* plants

Table. 2. Growth parameters of Brinjal (Solanum melongena L.) cv. Arka keshav plants fertilized with bacterial inoculants in a field trial at three fertilizer levels

Treatment	Plant height (cms)	% variation over control	Photosynthetic efficiency(%)	% variation over control	Yield (kg/plot)	% variation over control
Control N_0P_0	23.46		0.153		3.667	
Control Nh P,	34.26		0.303		3.686	
Control Nr Ph	40.63		0.360		5.303	
Bp treated N_0P_0	35.83	52	0.271	77	5.953	62
Bp treated N, Ph	50.83	48	0.570	88	7.300	98
B.p treated N,P,	71.63	76	0.563	56	9.698	82
B.p & A.::o.treated N_0P_0	33.73	46	0.266	73	4.860	82
Bp & A.:-o.treated N,P,	52.90	54	0.476	57	6.266	69
B.p & Aw.treated NrP,	69.50	71	0.596	65	8.900	67
"F' test	*		*		*	
CD at 5%	I'6.19		0.708		1.878	
SEM	5.529		3.021		1.141	

* significant   * * highly significant

B.p. *Bacillus polymyxa*   Azo. *Azospiril!um*

N_0P_0 zero doze of nitrogen and phosphorus   N, P, half doze of nitrogen and phosphorus

N, P, full doze of nitrogen and phosphorus   Fertilizer dosages as recommended for *solanum melongena* L.cv. *Arka keshav* plants

The inoculated plants that received half of nitrogen and half dose of phosphorus showed an increase of 56% of copper, 56% of calcium and 58% of manganese content in the plants as compared with the uninoculated controls at the same fertilizer levels. The plants that received half dose of nitrogen and phosphorus and treated with Bacillus polymyxa had increase of 52% in the phosphorus content as compared with the uninoculated controls in the same fertilizer level. The plants that received a combined inoculation of Bacillus polymyxa and Azospirillum and given full doze of nitrogen and half dose of phosphorus showed the maximum percent increase of 76% in potassium content as compared with the uninoculated controls which were given the same fertilizer level. (Table-3) The phosphorus content of the treated plots in the rhizosphere soil during the period of the experiment in which brinjal cv Arka Keshav was cultivated showed significantly higher values of available phosphorus content than the control plots (Figure 1). The plots treated with full doze of nitrogen and phosphorus showed 33% variation over the uninoculated controls (Figure 1 and 2).

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Table. 3. Leaf surface area of Brinjal (Solanum melongena L.) cv. Arka keshav plants fertilized with three fertilizer levels and bacterial inoculants in a field trial

. Treatment	First leaf cm²	% variation over control	Second leaf cm²	% variation over control	Third leaf cm²	% variation over control	Fourth leaf cm¹	% variation over control
Control $N_o P_o$	46.22		57.8		39.86		28.00	
Control Nh Ph	45.42		50.06		44.28		41.91	
Control Nr Pr	54.54		57.66		53.6		45.32	
B.p. treated $N_o P_o$	71.66	55	77.10	33	67.44	69	45.68	63
B.p. treated NhPh	65.92	45	85.25	64	73.87	70	57.82	37
B.p. treated NrPr	73.38	33	75.90	31	85.36	59	77.64	71
B.p & Azo.treated $N_o P_o$	73.82	59	67.56	16	53.87	35	43.78	56
B.p.& Azo. treated NhP.	88.98	95	87.08	73	72.51	67	46.58	11
B.p. & Azo.treated NrPr	64.86	18	114.76	99	82.94	54	70.46	54
Aw.treated $N_o P_o$	50.66	08	64.00	JO	51.1&	28	44.79	59
Azo. treated N.Pb	62.21	36	74.34	48	68.86	59	50.86	21
Aw.treated NrPr	56.65	03	87.66	52	74.32	38	56.56	24
'F' test	*		*		*		*	
CD at 5%	45.94		23.77		16.13		31.16	
SEM	9.79		144.44		9.31		8.13	

* significant * * highly significant

B.p. *Bacillus polymyxa* Azo. *Az.ospirillum*

$N_o P_o$. zero doze of nitrogen and phosphorus NrPr full doze of nitrogen and'Phosphorus

N,Pb half doze of nitrogen and phosphorus Fertilizer dosages as recommended for *solanum melongena* L. cv. *Arka keshav* plants

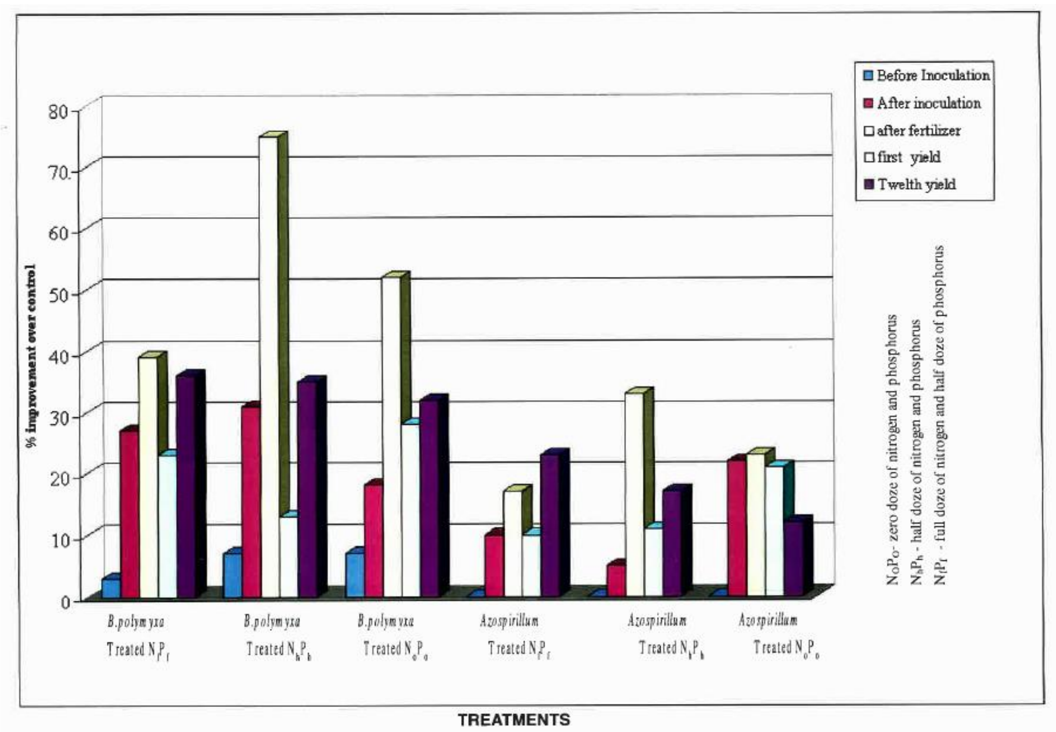

FIGURE 1: Soil nitrogen (ppm) at different intervals in a field trial with brinjal (Solanum melongena L.) cv. Arka keshav plants fertilized with bacterial inoculants and inorganic fertilizers

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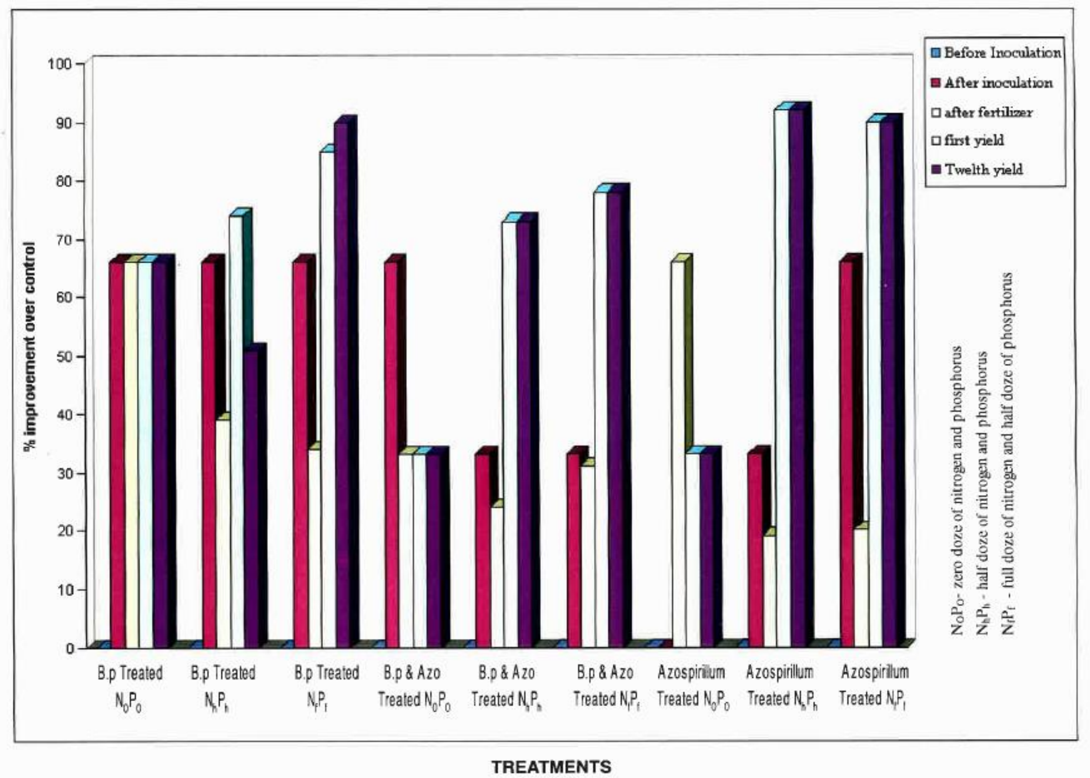

FIGURE 2: Available soil phosphorus (mg/gm) at different intervals in a field with brinjal (Solanum melongena L.) cv. Arka keshav plants fertilized with bacterial inoculants and inorganic fertilizers

4. DISCUSSION

The bacteria isolated from brinjal (Solanum melongena L.) plants identified as Bacillus polymyxa when used as inoculum in field trials showed significant improvement m growth parameters of treated plants over the uninoculated controls at all levels of fertilizer application. The isolate when multiplied and reinoculated showed competency in establishing themselves, fixing nitrogen and solubilizing phosphorus in the rhizosphere soil in field condition. Inoculation of brinjal both singly or in combination with Azospirillum, significantly enhanced the uptake of mineral and improved plant growth parameters. In a similar study Sukhada (1988) reported growth promotion under field conditions by bacteria isolated from the endorhizosphere of vegetables like tomato, okra and chillies. In okra and chillies these bacteria improved plant growth by increasing phosphate solubilization and releasing growth-promoting substances [17]. Subba Rao (1983) summarized the beneficial effects of inoculation of seeds of crop plants with Azotobacter, particularly Azotobacter chroococcum and Azospirillum brazilience. They recorded an increase of 2 to 62 % depending upon the host plant, method of inoculation, locality and agroclimatic conditions. Azospirillum inoculation of sorghum resulted in a yield increase of 17 to 105% in different regions. Pearl millet inoculation with sorghum gave a yield increase of 70% in some regions. These results demonstrated that nitrogen fixing and phosphate solubilizing rhizobacterial isolates could be effectively used to increase yield and save on the use of inorganic nitrogen and phosphatic fertilizers [1].

In the present study, the brinjal plants which received half dose of nitrogen and phosphorus confirmed the most percentage growth with inside the shoot, dry weight (59%) over the uninoculated controls, which obtained the equal level of fertilizers. The plants inoculated with Bacillus polymyxa confirmed a extensively better plant peak than the uninoculated controls in any respect of fertilizer levels. The maximum percent increase in shoot fresh weight (51%) and photosynthetic efficiency (88%) was observed at the half level of nitrogen and phosphorus in plants treated with Bacillus polymyxa as compared with uninoculated controls at the same fertilizer level. Plants, which received full dose of nitrogen and phosphorus and inoculated with Bacillus polymyxa, had a percentage growth of 39% in shoot dry weight over the uninoculated controls on the equal fertilizer stage. The half dose of nitrogen and half dose of phosphorus treated with Bacillus polymyxa confirmed the increase of 56% of copper, 56% of calcium and 58% of manganese content material with inside the plant life compared with the uninoculated controls on the equal fertilizer levels. The plant received half

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dose of nitrogen and phosphorus and treated with Bacillus polymyxa had increase in 52% of phosphorus content compared with the uninoculated controls in the same fertilizer level. The plants which received a mixed inoculation of Bacillus polymyxa and Azospirillum and given full doze of nitrogen and half dose of phosphorus showed the maximum increase of 76% in potassium content material compared with the uninoculated controls which have been given the same fertilizer level.

Quimio and Coroza (1988) isolated and evaluated PGPR from the rhizosphere of tomatoes, white potatoes, sorghum, cowpea and corn. In corn they reported 24-43% increase in yield and 12-15% increase in dry weight. In sorghum, they reported an increase of up to 67% in yield and a dry weight increase of about 2-7% [18]. In cowpea a yield increases of 28-42% and a dry weight increase of 13-48% was reported. Treated corn seedlings showed a significantly higher seedling weight, shoot length, root length and root weight. No increase in yield and growth parameters was observed when seeds of corn, rice, soybean and cotton were coated with Rhizobacterium cultures. Zea mays inoculation with Azospirillum by Kapulnik et al., 1983 demonstrated that inoculated plants take up N, P and K from mineral solutions significantly faster than the uninoculated controls. Similarly, dry matter, N, P and K accumulated at greater rates in field-inoculated sorghum and wheat plants [19]. According to Okon et al., (1977) maximum nitrogen fixing activity was recorded during flowering [20]. Lin et al., (1983) obtained similar results when corn seeds was inoculated with Azospirillum. They reported significant increase in growth parameters and yield of treated plants over control [21]. However, studies involving Azotobacter inoculation of seeds of cereals and vegetables in Soviet Union did not produce substantial and reproducible increase of yield for any plant [22]. Studies by Mehrotra and Lehri (1971) showed only small increase in yield. In almost all cases beneficial effects were observed only when nitrogenous fertilizers and FYM were applied [23].

Furthermore, Barea and Brown (1974) were able to obtain significantly high increase in plant growth and nitrogen content of both dicotyledonous and monocotyledonous plants incubated withAzotobacter (Paspalum notatum, Loliwn perenne and centrosema pubescens) [2]. Inoculation with Azatobacter improved the growth of Paspalum notatwn [24,25]. The present study showed that the isolated strain of Bacillus polymyxa are effective inoculants, as they have the ability to establish themselves on the brinjal root surface in significant numbers and stimulate growth and increase yield. The isolated Bacillus polymyxa showed varied response at Rabi season. It was observed that the inoculated plants were also quite resistant to infections. Extent of influence towards increase in yield and other growth parameters could be assessed if all the factors that biofertilization could be considered.

5. CONCLUSION

The beneficial effect of the isolated strain of Bacillus polymyxa used as inoculants for the cultivation of brinjal plants which stimulated growth and increased yield. Since inoculation with B. polymyxa prompted a high-quality impact on plant development, similarly future research needs to be taken to check if any growth promoting hormones had been produced through this bacterial isolate which may be beneficial to the plant environment.

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