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Azophos Inoculation on Drought Management Under Irrigated, Semidry and Rainfed Rice

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Abstract

Rice is the most diversified crop grown in India over 42 million ha. The cultivated area under rainfed condition in India was increased from 438.63 lakh ha to 445.8 lakh ha. This is due to the failure of monsoon. So the study was focused on water stress management with biofertilizers. The role of biofertilizer viz., Azospirillum, Phosphobacteria and Azophos application in the root morphogenesis of rice varieties viz., ADT 16, ASD 16, CO 43 and PMK 2 was studied under in vitro conditions in Jensen's seedling agar tubes. Among the four varieties tested CO 43 responded better inoculation than the other varieties and hence selected for further studies. The cement tank experiment was conducted to know the results of biofertilizer inoculation in CO -43 rice seedlings with 100 and 50% moisture level. The results suggested that there was no much difference in the root growth and survival of Azophos in the rhizosphere of inoculated CO 43 rice plants grown under flooded and saturated moisture. Field experiments were conducted at Agricultural Research Station, Paramakudi to study the performance of Azophos with application of NPK fertilizer in PMK 3 rice in rainfed conditions. The results of Azophos inoculation on rice under water stress situations imposed by rainfed field conditions was onpar with 100% RDF of fertilizer application.

Keywords: Azophos; Jensen seedling agar; cement tank and field experiment

Introduction

Azospirillum: Biological Nitrogen fixation and gibberellins secretion

Conversion of N into NH3 by Azospirillum is the main response to promote plant growth. Rhizosphere having Azospirillum reduced the necessity of synthetic N fertilization in the crop field (Bashan and Holguin). The presence or dose of O2, NO3 and MO3 can decide the N2 fixing ability and denitrification process in Azospirillum lipoferum. Microaerophilic condition favours the biological N fixation by Azospirillum. Azospirillum brasilense in microaerophilic condition leads to high respiration rate with the help of cytochrome c oxidase. In such a condtion it needs high energy by efficient oxidative complex. Azospirillum, Azotobacter, Rhizobium species have variety of strategies to deal or overcome this apparent O2 Paradox. Azospirillum have the specific environmental adaptation which provides O2 tolerance and N2 fixation (Marchal and vanderleyden, 2000). The mutant developed with Azospirillum brasilense for extra production of carotenoid leads to resist the oxygen concentration (Hartmann and Hurek).

Azospirillum species produced the amino acids purine, pyrimidine bases, Organic compounds by which it can convert NH4 with the help of glutamine synthetase and glutamate synthase (Bani; Webstly and his group).

Azospirillum synthesis the hormone viz., Indole -3 –acetic acid (IAA), Indole lactic acid and Indole 3 butyric acid (IBA), Indole 3 ethanol, Indole -3 – methanol, gibberellins, abscisic acid (Crozier and his group ; Tien and his group). Azospirillum cultures convert the tryptophan to IAA (Reynders and Vlassak).

Root associated Azospirillum brasilense secretes the IAA, cytokinin on the surface leads to changes in root morphology. Zimmer and Bothe also reported higher production of IAA in logarithmic phase by the Azospirillum brasilense and Azospirillum lipoferum cultures. Zimmer conducted experiments to know the expression genes of tryptophan viz., try G, try d and try C in Azospirillum brasilense sp7. Prinsen used radio labeled precursors like indole acetamide pathway, tryptophan dependent pathway and tryptophan independent pathway involvement in IAA production by Azospirillum. Omay and his group reported that concentration of IAA was low during logarithmic growth phase and increased during idiophase. Baca and his group observed the production of IAA due to the presence of tryptophan under axenic conditions. Azospirillum produced gibrelic acid and Gibrelic acid 3 (Rademacher). Purushothaman and Chitrakumar reported that Azospirillum secrete phytohormone under salt stress conditions. Gibberellin producing strain Azospirillum strain was cultured using glucosyl ester (or) gibberellins Azospirillum and found that both conjugate were hydrolyzed. The laboratory study revealed that plant growth promotion induced by application / inoculation of Azospirillum (Piccoli). A. lipoferum produced gibberellic acid even under drought condition. The reaction percentage was 5%. Water potential increased the production of gibberellins within cell also increased (Piccoli and his group). Azospirillum brasilense inoculation in wheat dose not positively increases the root length and root hair formation. But tryptophan addition leads to increased root morphology (Dobberiner).

Among the Azospirillum sps, A. brasilense cd produced the maximum level of IAA (380 µ mol/lit) (Radwan and Elkhawas). Change in pH affect the IAA production (Ona). A. lipoferum USA 5b and A. brasilense cd help for elongation of the root sheaths in GA deficient dwarf rice mutants when supplied with gibberelin A20 glucosyl ester. Azospirillum inoculation in wheat leads to earlier flowering, increase in shoot and root weight, total N content, plant height, leaf length, 1000 grain weight (Kapulnik). Inoculation of Azospirillum maize leads to 200 per cent increase in plant dry weight and N (157%) (Hegazi).

Azospirillum inoculation in wheat increases the nitrogen nutrition, total nitrogen content and grain yield (Wlassak and Dreesen). Rice inoculated with Azospirillum increases early tillering and enhanced reproductive growth (Watanabe and Lin). Sorghum inoculated with Azospirillum increased plant dry weight and nitrogen uptake (25%) (Picovskya). Mustard inoculated with Azospirillum increased grain yield and nitrogen uptake (Saha). Rice inoculated with Azospirillum lipoferum increased PO_4^- ion and NH_4^+ ion uptake under hydroponic conditions (Murty and ladha). Maize inoculated with Azospirillum species significantly increased grain yield (Salamone). Azospirillum inoculation in maize increased grain yield from 6700 to 7300 Kg ha-1 in maize (Salamone and Dobereiner). Rice inoculated with A. lipoferum increases soil nitrogen to the 35% and 9% increase in grain yield (Ghosh and Puste).

Azospirillum involved in biosynthesis of amino acid indole acetic acid, cytokinins, gibberellins, favouring root growth and thereby help the plant root for nutrient and water uptake (Bashan y, de –Bashan 2010, Mehnaz, S and Vejan).

Phosphate solubilizing microorganisms

The soil phosphorus is not in freely available for plant root uptake only 1/10 of this is available to plants due to low solubility and chemical fixation in soil. Phosphate solubilizing microorganisms help to solubilize the chemically fixed phosphorus which leads to decrease the synthetic fertilizers (Gaur and Gaind).

Bacillus, Proteus, Serratia, Pseudomonas, Micrococcus and Streptomyces were isolated from the rye and wheat rhizosphere (Molla). Bacillus licheniform, B. mycoidess and B. megaterium were isolated from paddy root soil (Watanabe and Hayano). Azotobacter chroococcus species were isolated from rhizosphere soil of oil seed crops (Narula) at 37°C and 42°C. The bacterium Pseudomonas striata solubilized maximum amount of Mussorrie rock phosphate (5.26 mg P_2O_5/50 ml medium) and tricalcium phosphate (35.9 mg P_2O_5/50ml medium) (Gaur and Arora). The conversion of unavailable P (tricalcium phosphate) (35.9 mg P_2O_5/50 ml medium) to available form by organic acid especially hydroxyl-acids promoted the solution of mineral phosphates possibly owing to their higher chelating properties (Gaur and Arora). Rice, wheat, soyabean crops supplied with Bacillus polymyxa, Pseudomonas striata and Aspergillus awamorri on the yield and P uptake has been recorded. Pseudomonas striata inoculation gave maximum yield 17.86 q ha-1 (Kundu and gaur, 1980). Conversion of unavailable form of P (tricalcium phosphate) by phosphobacteria and this phosphobacteria can be isolated using Hydroxy apatite medium. Phosphate solubilizing bacteria and fungi viz., Bacillus species, Aspergillus flavus, A. carbonoum, A. fumigates and A. wentii from Mussoorie rock phosphate deposits was isolated by Gaur. These were found to solubilize organic phosphates, besides inorganic phosphate solubilization

Effect of PSB inoculation on plant growth

PSB a broad spectrum biofertilizer responded positively in soil with high organic matter content and low available phosphorus. The positive inoculation effect has been realized in cereals, millets and other crop plants. Plantation soils are rich in organic matter due to continuous accumulation of plant debris in soil having slow decomposition rate and poor availability of P. The phosphobacterial application to several crops was found to augment growth and yield (Gaur). Although there are several reports on cereal and millets, investigations on the ability of phosphobacteria on plantation crop is very much limited.

Combined application of Azospirillum and musooriephos had beneficial effect not only to improve the micronutrients (Zn, Mn, Cu and Fe) contents of leaves, but also enhanced the number of berries per plant, weight of the ripe berry resulting in 45 per cent increased yield of coffee over control (Anonymous). Nageswari reported that application of phosphobacteria through soil slurry and the combination of both significantly increased the per cent rooting, root length and number of roots per cutting in cinnamon.

The phosphate solubilizing activity occurs in the solution form by microorganism help to reduce the cost of chemical fertilizer manufacturing and mobilization of nutrient in soil. Phosphate solubilizing microorganism helps for N fixation, siderophore production phytohormone production besides P solubilization (Vassilev). The Co- reaction between more than 1 microbes leads to low input biotechnology, help sustainable environmentally their by improve agricultural and natural ecosystem (Barea)

Materials and methods

Azophos a mixed inoculant

A mixed biofertilizer inoculum has been developed consisting of Azospirilluum and phosphobacteria. This mixed biofertilizer inoculum has been named as 'Azophos'. The following procedure has been adopted to develop this mixed inoculum. The compatibility test for Azospirillum and phosphobacteria was conducted by cross streak assay method described by Aneja. Both the organisms were able to grow together in on the surface of the medium in the Petri dish without inhibiting one another. This confirms the compatibility of both organisms. Azospirillum and phosphobacteria inoculum were mass multiplied in nitrogen free malate broth and nutrient broth respectively. The well grown broth cultures were mixed separately in rice husk ash, which is used as a carrier material for preparing mixed inoculum. Rice husk ash is the rice mill waste having a pH of 7.5 to 8.0, 20 – 39%, organic matter content and 260% moisture holding capacity. The carrier material was finely powdered, sieved and autoclaved at 15 psi for
20 minutes. The Azospirillum and phosphobacterial broths were mixed in equal quantities with the carrier material to prepare Azophos inoculum.

Studies on the inoculation of Azophos on root morphogenesis of drought tolerant rice

The effect of inoculation of Azospirillum and phosphobacteria either individually or as mixed inoculant Azophos on the root morphogenesis of four different rice varieties viz., CO 43, PMK 2, ASD 16 and ADT 36 was studied under in vitro conditions using seedling agar tubes. The seeds of four rice varieties viz., CO 43, PMK 2, ASD 16 and ADT 36 were rinsed with 70% ethanol for 1 minute and 0.1% HgCl2 for 2-3 minutes, followed by two or three washing with sterile distilled water for complete removel of chemical sterilizing agents. The washed seeds were placed on the sterile whatman No. 1 filter paper moisterned with Jensen's nutrient solution in a Petri plate for pregermintion. After three days, the pregerminated seeds were placed on the germination tubes containing Jensen's seedling agar medium inoculated with 0.1 ml of Azospirillum or phosphobacteria or Azophos broths in separate tubes with three replication along with suitable uninoculcated control. This was kept for incubation upto 15 days. After 15 days, the seedlings were carefully removed from the seedling agar tube with the help of sterile forceps in the laminar air flow champer and the root portions were washed with sterile distilled water. The observations on root length, shoot length, root volume, number of main roots / plant and number of lateral roots / main root were recorded.

Studies on the effect of inoculation of Azophos on the growth of CO 43 rice under pot culture with flooded and saturated moisture conditions

A pot culture experiment was conducted to study the effect of inoculation of Azophos, Azospirillum and phosphobacteria on the CO 43 rice and the survival of Azospirillum and phosphobacteria in the rhizosphere of CO 43 rice under flooded and saturated moisture conditions. The experiments were conducted in 1 Sq. meter cement tanks in a randomized block design with 5 replications.

The treatments are as follows

- T1 – Control
- T2 – Azospirillum (75: 50:50)
- T3 – Phosphobacteria (100: 25: 50)
- T4 – Azophos (75: 25: 50)
- T5 – Recommended dose of NPK (100: 50: 50)

To maintain the flooded conditions, 5 cm depth of water above soil level was stagnated throughout the experimental period. The saturated moisture conditions were maintained by keeping the soil completely moist without excess stagnant water. The fertilizer N was applied as basal and two top dressing (50% basal, remaining 50% in 2 splits of 25% each as top dressings at the time of active tillering and panicle initiation stage) P and K fertilizer were applied basally.

Azophos, Azospirillum and phosphobacteria were inoculated as seedling root dipping and soil application. The plant and soil samples were collected at periodical intervals viz., 30, 60 and 90 DAT and the observations on root length, root volume, number of main roots / plant, number of lateral roots / main root and proline contents were estimated

Studies on the effect of inoculation of Azophos on PMK 3 rice under rainfed conditions.

The field experiment was conducted at Agricultural Research Station, Paramakudi during the October 2016 to February 2018 to study the effect of inoculation of Azophos on PMK 3 rice under rainfed conditions. The experiments were laid out in a randomized block design with 8 treatments and three replications. The treatment details are as follows.

T1	-	Control
T2	-	Azospirillum (25: 25 : 25)
T3	-	Phosphobacteria (50 : 12.5 : 25)
T4	-	Azophos (25 : 12.5 : 50)
T5	-	Azospirillum (37.5 : 25 : 25)
T6	-	Phosphobacteria (50 : 18.7 : 25)
T7	-	Azophos (37.5: 18.7 : 25)
T8	-	Recommended dose of NPK (50 : 25 : 25)

Azophos, Azospirillum and phosphobacteria were applied through soil application. Fertilizer N was applied as two top

dressings of 50% each at the time of active tillering and panicle initiation stages. The size of the experimental plots was
8 x 5m and the seeds (60 kg /ha) were broadcasted manually. No irrigation was given and the crop was raised purely based on monsoon rainfalls. Plant and soil sample were collected at periodical intervals viz., 30, 60, 90 DAS. The biometrical observations on root length, root volume, number of main roots / plant, number of lateral roots / main roots and proline content were recorded

Results and Discussion

Effect of inoculation of Azophos on plant root system under invivo condition

The effect of Azophos inoculation on the number of main roots / plant and the number of lateral roots / main root of the four different rice varieties viz., CO 43, PMK 2, ASD 16 and ADT 36 was assessed to study the root morphogenesis in response to inoculation and the results are furnished in Table 1 & 2. Among the treatment maximum number of root length (10.5cm) and root volume (0.5 cc) were observed in the treatment Azophos than other on 15 days after inoculation (15 DAI). All the treatments recorded significantly higher number of main roots / plant and lateral roots / main root than uninoculated control. The maximum number of main roots (16 / plant) and lateral roots (25 / main root) were observed in CO 43 rice seedling inoculated with Azophos and phosphobacteria respectively. However, the number of lateral roots per main root due to Azophos and phosphobacteria inoculation was found to be on par with each other. It was also observed that the interaction effect for number of main roots / plant was found to be significant in CO 43 and PMK 2 varieties, whereas it was non significant in ASD 16 and ADT 36.

Table1: Effect of Azophos inoculation on root length and root volume of different rice varieties grown in Jensen's seedling agar tubes.

Treatments	Root length * (cm)				Root volume * (cc)			
	CO 43	PMK 2	ASD 16	ADT 36	CO 43	PMK 2	ASD 16	ADT 36
T1 – Control	8.0	5.3	4.0	4.0	0.20	0.15	0.10	0.10
T2 - Azospirillum	10.0	7.5	6.5	4.6	0.30	0.20	0.20	0.20
T3- Phosphobacteria	9.3	7.0	5.0	4.7	0.30	0.30	0.30	0.25
T4 - Azophos	10.5	7.8	6.1	5.6	0.50	0.50	0.40	0.30 -
SEd	T	V	T x V	T	V	T x V		
	0.18	0.18	0.35	0.06	0.54	0.12		
CD (P: 0.05)	0.36	0.36	0.72	0.12	1.2	0.25		

Table2: Effect of Azophos inoculation on the number of main roots and lateral roots of different rice varieties grown in Jensen's seedling agar tubes

Treatments	Main roots * (No / plant)				Lateral roots * (No / main root)			
	CO 43	PMK 2	ASD 16	ADT 36	CO 43	PMK 2	ASD 16	ADT 36
T1 – Control	7.0	5.5	5.0	4.0	8.1	7.0	7.0	6.0
T2 - Azospirillum	14.0	11.0	11.0	10.0	21.8	18.8	11.4	8.3
T3 - Phosphobacteria	12.0	10.0	7.0	7.0	25.0	20.0	19.8	15.4
T4 - Azophos	16.0	13.0	11.0	10.0	24.2	20.0	19.5	13.9
SEd	T	V	T x V	T	V	T x V		
	0.36	0.36	0.78	0.78	0.78	1.52		
CD (P: 0.05)	0.74	0.74	1.48	1.58	1.58	3.16		

Effect of Azophos inoculation on the root morphology of CO 43 rice seedlings under pot culture conditions

Number of main roots / plant, number of lateral roots / main root

The maximum number of main roots / plant (16.0) and number of lateral roots / main root (27.6) of CO 43 rice seedlings were recorded on 28 DAT due to inoculation with Azospirillum, phosphobacteria and Azophos respectively (Table 3).

Table3: Effect of Azophos inoculation on the number of main roots and lateral roots of rice (var. CO 43) under pot culture conditions

Treat	Main r	Lateral

ments	oots*/plant	roots*/main root										
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT						
	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
T1 – Unfertilized uninoculated control	104.0	95.0	102.3	100.0	70.0	65.0	109.0	104.0	80.0	75.0	60.0	55.0
T2 – Azospirillum + NPK (75:50:50)	225.0	213.0	125.0	115.0	90.0	85.0	136.0	130.0	100.0	93.0	85.0	80.0
T3 - Phosphobacteria + NPK (100:25:50)	210.0	208.0	109.0	105.0	80.0	75.0	144.0	123.0	110.0	98.0	90.0	85.0
T4 – Azophos + NPK (75:25:50)	215.0	210.0	116.0	105.0	85.0	80.0	120.0	115.0	105.0	96.0	88.0	82.0
T5 – Recommended dose of NPK (100:50:50)	265.0	255.0	127.0	125.0	95.0	90.0	127.0	119.0	120.0	115.0	95.0	90.0
T	SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd	CD
	1.04	2.20	0.40	0.63	0.36	0.75	0.11	0.38	0.12	0.26	0.15	0.31
M	0.66	1.39	0.19	0.40	0.22	0.47	0.18	0.23	0.08	0.16	0.09	0.19
TxM	1.48	3.11	0.43	0.89	0.50	1.06	0.25	0.53	0.17	0.36	0.21	0.43

M1 - Flooded; M2 - Saturated

Root length and root volume

The root length, shoot length and root volume of the inoculated CO 43 rice plants were significantly higher than uninoculated control (Table 4). The above parameter was found to increase with increase in the age of the seedlings. The maximum shoot length (15.0 cm) and root volume (0.60 cc) were recorded in Azophos inoculated CO 43 rice seedlings on 28 DAT, whereas the maximum root length (8.43 cm) was registered in Azospirillum inoculated seedlings on 28 DAT. In all the treatments, increase in root volume was observed till 14 DAT and not much change was observed thereafter.

Table4: Effect of Azophos inoculation on the root length and root volume of rice (var. CO 43) under pot culture conditions

Treatment	**Root length* (cm)**	**Root volume* (cc)**										
	30 DAT	**60 DAT**	**90 DAT**	**30 DAT**	**60 DAT**	**90 DAT**						
	M1	**M2**	**M1**	**M2**	**M1**	**M2**	**M1**	**M2**	**M1**	**M2**	**M1**	**M2**
T1 – unfertilized uninoculated control	5.0	5.0	10.0	8.0	9.0	8.0	0.9	0.9	2.5	2.0	2.1	2.0
T2 – Azospirillum + NPK (75:50:50)	11.5	9.0	17.0	15.0	17.2	16.6	1.5	1.3	3.5	3.0	3.2	3.1
T3 - Phosphobacteria + NPK (100:25:50)	8.0	8.0	11.5	11.0	11.0	11.0	3.0	2.8	4.2	4.0	3.8	3.5
T4 – Azophos + NPK (75:25:50)	9.0	9.0	13.0	10.0	16.0	15.8	2.0	2.1	4.0	3.5	3.3	3.4
T5 – Recommended dose of NPK (100:50:50)	10.0	9.0	14.0	11.0	16.2	15.2	3.3	2.8	5.0	4.5	4.5	4.2

T	SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd	CD
	0.18	0.38	0.16	0.35	0.38	0.80	0.03	0.07	0.03	0.06	0.05	0.11
M	0.11	0.24	0.10	0.22	0.24	0.51	0.02	0.04	0.02	0.04	0.03	0.07
T x M	0.25	0.53	0.23	0.49	0.54	1.10	0.05	0.10	0.04	0.08	0.08	0.16

M1 - Flooded; M2 - Saturated

Effect of Azophos inoculation on the root growth of rice under rainfed field conditions

A field experiment was conducted at Agricultural Research Station, Paramakudi to study the influence of Azophos inoculation in comparison with individual inoculation of Azospirillum and phosphobacteria on the root length, root volume, shoot length, number of main roots / plant, number of lateral roots / main root, proline content, survival of Azospirillum and phosphobacteria in the rhizosphere of PMK 3 rice under rainfed field conditions. Observations were recorded at three different intervals viz., 30, 60 and 90 DAS

Root length and root volume

Inoculation of Azospirillum and phosphobacteria either individually or as a mixed inoculant both in the presence of NPK fertilizers significantly influenced the root length and root volume of PMK 3 rice over uninoculated control under rainfed field conditions (Table 5). Among the different treatments, inoculation of Azophos with NPK (25:12.5:25) registered the maximum root length (26.3 cm) of PMK 3 rice on 60 DAS, whereas the maximum root volume (14.5 cc) was observed in PMK rice inoculated with phosphobacteria + NPK (50:12.5:25) on 60 DAS.

Table5: Effect of Azophos inoculation on the root length and root volume of rice of rice (var. CO 43) under rainfed field conditions

Treatments	Root length*(cm)			Root volume*(cc)		
	30 DAs	60 DAS	120 DAS	30 DAs	60 DAS	90 DAS
T1 – Unfertilized uninoculated control	8.0	10.0	9.0	4.0	5.	2.0
T2 – Azospirillum + NPK (75:50:50)	11.0	12.0	10.0	5.0	8.0	4.0
T3 – Phosphobacteria + NPK (100:25:50)	10.5	11.0	9.0	9.2	10.0	8.0
T4 – Azophos + NPK (75:25:50)	13.0	26.3	12.0	6.0	11.0	9.0
T5 – Azospirillum + NPK (87.5:50:50)	11.0	15.0	10.0	6.0	8.8	7.0
T6 – Phosphobacteria+ NPK (100:37.5:50)	12.0	12.0	11.0	12.0	14.5	12.0
T7 – Azophos+ NPK (87.5:37.5:50)	14.0	15.0	12.0	7.0	8.0	7.0
T8 – NPK (100:50:50)	15.0	21.9	14.0	9.0	9.0	8.0
SEd	0.70	18.0	0.14	0.34	0.17	0.12
CD (P: 0.05)	1.50	0.83	0.31	0.73	0.36	0.24

Number of main roots / plant and number of lateral roots / main root

The maximum number of main roots / plant (159.0) and the maximum number of lateral roots / main root (96.0) of PMK 3 rice were recorded on 30 DAS due to Azospirillum inoculation with NPK (25:25:25) and phosphobacteria + NPK (50:12.5:25) inoculation respectively (Table 6). The number of main roots / plant and number of lateral roots / main root decreased with increase in the age of the plants.

Table6: Effect of Azophos inoculation on the number of main roots and lateral roots of rice (var. CO 43) under rainfed field conditions

Treatments	Main roots * /plant			Lateral roots * /plant		
	30 DAs	60 DAS	90 DAS	30 DAs	60 DAS	90 DAS
T1 – Unfertilized uninoculated control	114	98	70	35	10	8
T2 – Azospirillum + NPK	151	112	80	55	15	12

(75:50: 50)						
T3 – Phosphobacteria + NPK (100:25:50)	147	115	75	96	20	15
T4 – Azophos + NPK (75:25: 50)	133	120	78	66	16	13
T5 – Azospirillum + NPK (87.5:50:50)	159	125	85	56	20	15
T6 – Phosphobacteria+ NPK (100:37.5:50)	146	130	75	56	24	18
T7 – Azophos+ NPK (87.5:37.5:50)	132	130	80	40	22	16
T8 – NPK (100:50:50)	140	133	65	50	18	10
SEd	0.29	0.29	0.29	0.60	0.41	0.28
CD (P: 0.05)	0.70	0.70	0.70	1.30	0.89	0.59

Total proline content of rhizosphere soil of rice

The total proline content of PMK 3 rice increased upto 60 DAS and decreased thereafter. The maximum proline content (930 µg / g) of PMK 3 rice were recorded due to the application of recommended dose of NPK fertilizer and Azospirillum inoculation with NPK (25:25:25) respectively on 60 DAS (Table 6). In the inoculated plants, the total proline content was significantly enhanced compared to the uninoculated control (Table 7).

Table7: Effect of Azophos on proline content of rice (Var. PMK 3) under rainfed field conditions

Treatments	Proline * (□g /g)		
	30 DAT	60 DAT	90 DAT
T1 – Unfertilized uninoculated control	840	880	464
T2 – Azospirillum + NPK (75:50:50)	885	927	768
T3 – Phosphobacteria + NPK (100:25:50)	860	900	640
T4 – Azophos + NPK (75:25:50)	854	907	736
T5 – Azospirillum + NPK (87.5:50:50)	880	930	480
T6 – Phosphobacteria+ NPK (100:37.5:50)	840	880	512
T7 – Azophos+ NPK (87.5:37.5:50)	860	900	420
T8 – NPK (100:50:50)	840	895	528
SEd	1.15	1.67	0.86
CD (P: 0.05)	2.48	3.57	1.85

Discussion

Studies on the root morphogenesis of selected rice varieties due to Azophos inoculation

The results of the compatibility test of Azospirillum and phosphobacteria showed that compatibility with each other lead to scope for production of mixed inoculum. The mixed inoculum Azophos was prepared by mixing of equal proportions of Azospirillum and phosphobacteria with rice husk ash as carrier material. Bashan and Holguin pointed out the synergistic effect of bacterial mixtures. Belimov observed that bacterial mixture of N2 fixing and P solubilizing bacteria provided a more balance nutrients for the plants. Krishnamurthy found that coinoculation of Azospirillum and phosphobacteria significantly enhanced the seed germination, seedling growth, nutrient uptake and chlorophyll content in cardamom.

The results of the present investigation established that among the four varieties tested CO 43 produced better response to Azophos inoculation than the other varieties. The results indicated that the root length (10.5 cm) and root volume (0.5 cc) were higher in the Azospirillum inoculated plants on 15 DAI. The number of main roots and lateral roots were higher in Azophos (16.0 / plant) and phosphobacteria (25.0 / main root) inoculation on 15 DAI respectively. The shoot length (16.0 cm) was higher in Azospirillum and Azophos inoculation on 15 DAI respectively.

Ramamoorthy showed that seed treatment with Azospirillum increased the amylase activity during germination. The enzyme level was more in vigorous seeds. Secretion of gibberellins by the bacterium may be the reason for this increase and subsequent hydrolysis resulting in enhanced seedling vigour encompassing speed of germination, seedling length and dry weight. The positive influence of Azospirillum and phosphobacteria on the root morphogenesis of the rice varieties observed in the present study may be attributed to the production of the growth promoting substances by Azospirillum and enhanced nutrient by phosphobacteria, which are considered to play important role in the root growth of the plants.

Azophos Inoculation on root morphology of CO 43 rice with two different moisture levels under pot culture conditions

Azospirillum has a well adopted and highly versatile metabolism that allows them to survive in a carbon rich, nitrogen poor rhizosphere, rhizoplane and endorhizosphere environment (Okon). The N2 fixation is the first mechanism suggested to promote the plant growth of plant by Azospirillum. Azospirillum also produced complex compounds called siderophores, which sequester Fe (III) in the rhizosphere, thereby making it unavailable to other microorganisms, which lack the assimilation system of the siderophores (Hartmann).

In the present study, recommended dose of NPK fertilizer applied plants resulted in maximum root length (17.0 cm) and root volume (15.0 cc) on 60 DAT. The maximum shoot length (106.0 cm) and plant dry weight (10.7 g) were observed in the Azospirillum and Azophos inoculated plants on 60 and 90 DAT respectively.

Azospirillum under flooded conditions resulted in maximum number of main roots (225.0 / plant) and phosphobacteria inoculation resulted in maximum number of lateral roots (144 . 0 / main root) on 30 DAT. Only marginal difference in growth was observed in the inoculated plants grown under flooded and saturated moisture level. The plants applied with recommended dose of NPK fertilizer resulted in maximum number of total tillers (11.0 / plant) and productive tillers (6.0 / plant) on 90 DAT.

The results showed that Azospirillum population was higher (0.45 x 106 cells /g) in Azospirillum inoculated soil and phosphobacteria population was higher (145 x 106 cfu /g) in phosphobacteria inoculated soil on 60 DAT. Recommended dose of NPK fertilizers resulted in maximum proline content (1275µg / g) on 60 DAT. Sarig observed that the inoculation of Azospirillum brasilense increased root number and root lengh of adventitious roots by 33 to 40 per cent over unionoculated control. Girija obtained increased root and shoot elongation due to Azospirillum inoculation.

Watanabe and Lin observed early tillering and enhanced reproductive growth of rice due the Azospirillum inoculation Picovsky indicated that Azospirillum inoculation lead to increased plant dry weight and nitrogen assimilation by 25 per cent in sorghum. Kapulnick found in wheat and sorghum, earlier flowering, increase in shoot and root weight, total N, 1000 grain weight and yield due to Azospirillum inoculation. Similar beneficial effect due to inoculation was observed in the present study also.

Response of PMK 3 rice to Azophos inoculation under rainfed field conditions

Azospirillum inoculation at NaCl concentration upto -1.2 MPa significantly increased chlorophyll, K, Ca, soluble saccharides and protein content as compared with control. Plants growing without NaCl (Hamdia and El-Komy) showed a mechanism similar to alleviating water stress on wheat plant growing under drought conditions (El-Komy). Inoculating Azospirillum brasilense on wheat seedling exposed to severe salt (NaCl) stresses significantly reversed part of the negative effects; both stresses reduced relative elongation rate of shoots. Fresh weight, dry weight, water content and relative water content were higher in shoots from inoculated plants than in stressed controls (Creus). Turgor pressure at low water potential was higher in inoculated seedlings in two wheat cultivars under osmotic stress. This could result from better water uptake as a response to inoculation that, in turn, is reflected by faster shoot growth in inoculated seedlings exposed to these stresses.

The present investigation revealed that inoculation of Azophos resulted in maximum root length (21.9 cm), root volume (14.5 cc), shoot length (83.0 cm), total chlorophyll (1.13 mg /g) and maximum proline (768.0 µg / g) content on 60 DAS. Whereas, the maximum plant dry weight (38.0g) on 90 DAS and maximum grain (3.63 t / ha) and straw (6.78 t / ha) yield were also observed in the Azophos inoculated plants.

The Azospirillum and phosphobacteria inoculation lead to higher population of Azospirillum (2.4 x 106 cells /g) and phosphobacteria (72 x 106 cfu /g) on 60 DAS respectively. The Azospirillum inoculation resulted in maximum number of main roots (159.8 / plant), lateral roots (96.0 / main root) on 30 DAS.

Mixed inoculant, Azophos performed similar to that of the combined inoculation of Azospirillum + PSB and recorded 8-22.0% increase in grain yield over control and recommended dose of N + P (75%) levels in ASD 18 and ADT 36. Sadasivan and Neyra noted that Azospirillum has the capacity to produce non motile, highly refractive, encapsulated cyst and flocs containing abundant poly ß hydroxyl butyrate under stress conditions that showed increased resistance to desiccation and UV radiation. Azospirillum spp. can accumulate compatible solute such as glycine betaine, glutamate, proline and trehalose to allow adaptation to fluctuation in soil salinity / osmotalarity. Addition of this osmoprotectant to bacterial culture under saline stress usually increased cellular growth and nitrogenase activity (Choi and Gal; Tripathi and Mishra). Tripathi and Mishra studied the inhibition of Azospirillum lipoferum by salinity induced glycine betaine transport system.

The results of this study clearly indicated that mixed / individual inoculation of Azospirillum and phosphobacteria produced better response in PMK 3 rice under semidry and rainfed field conditions. Azospirillum inoculation resulted, maximum root length, main root and proline content which facilitate the growth of rice in drought situation. The phosphobacteria inoculation resulted in maximum root volume, lateral roots, and grain yield, which lead to plant can with stand drought situation. Inoculation of Azophos resulted in combined effect of Azospirillum and phosphobacteria on all the above parameters. Azophos application at 5 packets / ha for seed treatment and 10 packets / ha for soil application could be helpful in the water stress tolerance to rice especially under semidry and rainfed conditions.

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