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Effects of Nitrogen Fixing Bacteria, *Azotobacter* spp. and *Azospirillum* spp. on the Growth of Rice *Oryza sativa* L.

Kyaw Myo Naing[1] and Thant Zin[2]

Abstract

In order to evaluate the effect of *Azotobacter* and *Azospirillum* inoculants on the growth of rice, pot experiment with four treatments (*Azotobacter* sugarcane 1-T_1, *Azotobacter* maize 2- T_2, *Azospirillum* sugarcane 3-T_3 and *Azospirillum* maize 2 -T_4) and one control (without bacteria) each with six replicates was carried out in Zoology Department, University of Mandalay during March to November 2011. The results indicated that the shoot length of treatment increased over control with a range of 8.04 to 33.14% and the root length of treatments increased over control with a range of 2.13 to 132.89%. The fresh shoot weight and fresh root weight of treatments increased over control with a range of 0 to 1160% and 0 to 462.92% respectively. The dry shoot and root weights of treatments at maturity stage increased significantly ($p<0.01$) over control with a range of 53.31 to 109.28% and 163.89 to 280.56% respectively. In this study, panicle length and total seed numbers per panicle of treatments increased significantly ($p<0.01$) over control with a range of 18.75 to 35.02% and 96.55 to 114.83% respectively. *Azotobacter* sugarcane 1 is found to be the most effective species for the growth of rice and all tested bacteria could be useful as biofertilizers to increase the productivity of crops.

Key words: *Azotobacte*, *Azospirillum*, nitrogen fixing bacteria, rice

Introduction

Rice (*Oryza sativa* L.) is the staple food for half of the world's population especially in oriental countries (Kannan and Ponmurugan, 2010). In the next three decades, the world will need to feed the extra billion people. Nitrogen is the major nutrient limiting rice production. Rice requires 1 kg of nitrogen to produce 15-20 kg of grain. Increased future demand for rice will entail increased application of fertilizer N (Ladha and Reddy, 2003). Finding an alternative for such a nutrient has become important. Soil microorganisms like *Azotobacter* and *Azospirillum* are free living N_2 fixing bacteria which can successfully grow in the rhizosphere zone of crops and fix 10-20 kg N ha^{-1} cropping season (Yasari *et al.*, 2008).

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Various kinds of cereals were tested by using a member of nitrogen fixing bacteria viz., *Azotobacter*, *Nitrosomonas* and *Azospirillum* to increase yield under controlled conditions (Kannan and Ponmurugan, 2010).

Several authors have shown the beneficial effects of *Azotobacter chroococcum* on vegetative growth and yield of maize (Mishra *et al*., 1995; Pandey *et al*., 1998; Radwan, 1998), as well as the positive effect of inoculation with this bacterium on wheat (Elshanshoury, 1995; Pati *et al*., 1995; Fares, 1997) (cited in Aquilanti *et al*., 2004).

In numerous studies, *Azospirillum* inoculations have been reported to reduce the use of chemical fertilizers in particular nitrogen by 20% to 50% (Attitalla *et al*., 2010). Inoculation of plants with *Azospirillum* strains alters root morphology, increases numerous plant shoot growth parameters, and eventually increases the yield of many cereal crops (Patriquin *et al*., 1983), vegetables, and other agricultural plants (Sala *et al*., 1985; Crossman and Hill, 1987; Bashan *et al*., 1989) (cited in Bashan *et al*., 1990).

Inoculation of rice with *Azospirillum* strains (10^7 CFU ml^{-1}) can be increased in plant growth and grain yield of rice (Zaw Lwin Oo, 2010).

Based on the above information, it was realized that the isolated bacteria should be investigated to determine their effects on the growth of plants. This paper aimed to determine the effects of bacteria on the growth of rice.

Materials and Methods

Experimental Site and Study Period

Pot experiment was conducted in the net house of Zoology Department, University of Mandalay during March to November 2011 (Plate 1.A).

Source of Bacteria

Bacteria were isolated from the root of sugarcane and maize. Among them, the best growth species; *Azotobacter* sugarcane 1 (T_1), *Azotobacter* maize 2 (T_2), *Azospirillum* sugarcane 3 (T_3) and *Azospirillum* maize 2 (T_4) were selected and used to inoculate the paddy seed.

Preparation of Soil for Pot Experiment

The soil for pot experiment was collected from the field site near Tada-U Town, Mandalay Region. For soil analysis, soil was collected at a depth of 15 cm with V-shaped method and Zig-zag pattern. These were dried at room temperature and transported to the Laboratory of Soil Science Section, Department of Agricultural Research, Yezin. Sterilization of soils for pot experiment were made by autoclaving (121°C, 1.05 kgcm^{-2}, 30 minutes) and then placed into oven for one night and again sterilized by 180°C for one hour. After sterilization, 5 kg of soil was placed into each of sterilized earthen pot and sealed with plastic bag before sowing (Plate 1.A).

Source of Paddy Seeds and Sterilization of Seeds

Paddy seeds (*Oryza sativa* L.) were obtained from Seed Division, Department of Agriculture Service, Mandalay Region. The seeds were surface sterilized by treatment with 0.1% $HgCl_2$ for 2 min which were then washed 5 times with sterile distilled water.

Pot Experiment

Before sowing, pots with 5 kg of sterile soil were watered and stirred to mix ingredients of soil thoroughly. The following four inocula (bacteria) and one control with six replicates were imposed: *Azotobacter* sugarcane 1 (T_1), *Azotobacter* maize 2 (T_2), *Azospirillum* sugarcane 3 (T_3) and *Azospirillum* maize 2 (T_4) and control (C) without bacteria. Both inoculated and un-inoculated (control) seeds were then sown in the pots (30 seeds per pot) of net house under natural condition. Pots were arranged in complete randomized design and placed 23 cm apart from each other (Plate 1.B). Thinning was done after 7 days of sowing and 15 seedlings were kept. Inoculations of each diluted bacterial suspension (10^6 CFU ml^{-1}) and sterile diluted nutrient broth (one ml per seedlings) to pots were made from 10th day onwards at 10 day intervals until harvesting period. One liter of filtered tap water was used to water each pot two times a day. No chemical fertilizer was applied during the experiment.

Parameters of Growth Employed

A single seedling was selected at random from each pot on the 10th, 20th, 30th, 40th, 50th, 60th, 70th, and 80th days of sowing and necessary measurements were taken. At maturity, the crops were harvested and fresh and dry weights of shoot and root were recorded. The dry weights were determined after placing the plants into the oven at 80ºC for 48 h. The length of panicles, the numbers of seed per panicle and 1000 grains weight were also recorded.

Statistical Analysis

The measured data were subjected to analysis of variance and means of sample were compared by least significance difference (LSD) using SPSS (Statistical Package for Social Science) software version 17.0.

Results

The experimental soil was texturally made of silty clay and measured 8.86 of pH, 61 ppm of available N, 9 ppm of available P, 147 ppm of available K, 165% organic matter and 0.81% moisture.

Inoculation with *Azotobacter* and *Azospirillum* species showed noticeable effects on the growth of paddy plants. Mean values of shoot length, root length, shoot weight, root weight, panicle length, seed numbers per panicle and 1000 grains weight are presented in Tables 1, 2,3,4,5 and 6 respectively.

The percentage of treated plant height increase over control ranged from minimum 8.04% at 10 DAS (days after sowing) to maximum 33.14% at 30 DAS. Significant effects of inoculation on plant height were observed at 30 DAS to 80 DAS ($p<0.05$) (Table 1). The percent increase of root length of treated plants over control ranged from minimum 2.13% at 20 DAS to maximum 132.89% at 80 DAS. Significant effects were observed at 40 DAS, 50 DAS and 80 DAS ($p<0.05$) (Table 2). The percent increase of fresh shoot weight of inoculated plants over control ranged from 0% at 10 DAS to 1160% at 50 DAS. Shoot weights were significantly different ($p<0.01$) between control and inoculated plants at 20 DAS to 80 DAS, but not significant at 10 DAS (Table 3). The percent of fresh root weight increase over control ranged from 0% to 266.67%. Root weights were not significantly different between control and inoculated plants at 10 DAS, 30 DAS and 40 DAS. But significant effects were observed at 20 DAS, 50 DAS, 60 DAS and 80 DAS ($p<0.05$) (Table 4).

At maturity stage, the effects of inoculation were significant ($p<0.01$) over control in all plant growth and yield parameters (Plate 1.C) (Table 5). The percent of panicle length increase over control ranged from 18.75% to 35.02%. Significant ($p<0.01$) effect of *Azotobacter* and *Azospirillum* inoculation on panicle length were observed in this work. The longest panicle length was observed in *Azotobacter* maize 2 (T_2) inoculated plant (22.17 cm) (Plate 1.D). The percent of total and fertile seed numbers per panicle increase significantly ($p<0.01$) over control ranged from 96.55% to 144.83% and 142.86% to 209.52% respectively. The weights of 1000 grains were not significantly different between control and treatments. But the 1000 grain weights of inoculated plants (a range of 22.802 g to 23.05g) were higher than the control (21.83g) (Table 6).

Table 1. Mean shoot length of paddy plant at 10th, 20th, 30th, 40th, 50th, 60th, 70th and 80th days after sowing in control and treatment (n = 6)

Treatment	Shoot length (Mean ± SD (cm))							
	10 DAS	20 DAS	30 DAS	40 DAS	50 DAS	60 DAS	70 DAS	80 DAS
Control	13.69[a] ±4.86	29.52[a] ±5.86	39.53[a] ±3.71	42.80[a] ±1.33	61.73[a] ±8.56	65.68[a] ±2.17	67.32[a] ±8.72	68.42[a] ±13.75
T_1	16.73[a] ±3.97	34.62[a] ±5.44	52.63[c] ±5.28	55.47[b] ±5.66	77.83[c] ±5.10	78.50[b] ±7.53	80.08[b] ±6.09	83.28[b] ±5.38
T_2	15.95[a] ±4.66	34.2[a] ±3.10	45.13[a] ±4.03	51.70[b] ±5.33	69.67[b] ±11.27	75.68[b] ±5.77	78.88[b] ±4.97	87.17[b] ±10.75
T_3	15.00[a] ±4.09	34.72[a] ±3.18	43.78[a] ±6.19	51.60[b] ±7.09	70.08[b] ±4.85	78.17[b] ±7.22	79.40[b] ±8.96	81.97[b] ±4.36
T_4	16.57[a] ±4.14	36.62[a] ±2.27	47.12[b] ±4.62	52.53[b] ±6.49	74.70[b] ±2.94	77.58[b] ±5.18	77.60[b] ±7.55	82.48[b] ±6.88

Table 2. Mean root length of paddy plant at 10th, 20th, 30th, 40th, 50th, 60th, 70th and 80th days after sowing in control and treatment (n = 6)

Treatment	Root length (Mean ± SD (cm))							
	10 DAS	20 DAS	30 DAS	40 DAS	50 DAS	60 DAS	70 DAS	80 DAS
Control	2.5^{a} ±0.63	3.7^{a} ±1.29	4.83^{a} ±1.08	5.05^{a} ±0.54	7.67^{a} ±1.70	7.73^{a} ±1.79	7.90^{a} ±0.39	8.33^{a} ±5.42
T_1	3.17^{a} ±0.82	3.98^{a} ±0.95	6.05^{a} ±0.93	8.37^{b} ±4.37	12.18^{b} ±7.61	12.88^{b} ±4.46	14.62^{b} ±3.66	15.58^{b} ±8.84
T_2	3.73^{b} ±1.32	4.58^{a} ±2.56	5.08^{a} ±1.07	7.22^{b} ±1.18	8.88^{b} ±2.59	12.57^{b} ±3.89	14.58^{b} ±8.79	16.12^{b} ±4.29
T_3	3.25^{a} ±0.52	3.83^{a} ±1.20	5.23^{a} ±3.50	7.08^{b} ±1.30	11.22^{b} ±4.87	13.62^{b} ±3.98	14.12^{b} ±3.08	19.40^{b} ±6.77
T_4	3.33^{a} ±0.88	3.92^{a} ±1.77	6.43^{a} ±2.56	9.80^{b} ±1.94	10.33^{b} ±2.52	12.60^{b} ±3.72	14.20^{b} ±2.88	15.28^{b} ±2.61

Means followed by a common letter in the same column are not significantly different at 5% level by LSD

DAS = Days after sowing

Control = Dilute nutrient broth without inoculum

T_1 = *Azotobacter* sugarcane 1

T_2 = *Azotobacter* maize 2

T_3 = *Azospirillum* sugarcane 3

T_4 = *Azospirillum* maize 2

Table 3. Mean shoot weight of paddy plant at 10th, 20th, 30th, 40th, 50th, 60th, 70th and 80th days after sowing in control and treatment (n = 6)

Treatment	Shoot weight (Mean ± SD (g))							
	10 DAS	20 DAS	30 DAS	40 DAS	50 DAS	60 DAS	70 DAS	80 DAS
Control	0.02^{a} ±0.01	0.04^{a} ±0.01	0.08^{a} ±0.030	0.14^{a} ±0.06	0.20^{a} ±0.05	1.45^{a} ±0.26	1.47^{a} ±0.15	2.09^{a} ±1.21
T_1	0.02^{a} ±0.01	0.09^{c} ±0.04	0.40^{c} ±0.13	0.42^{b} ±0.21	2.52^{b} ±0.67	3.10^{b} ±1.13	3.70^{b} ±0.85	4.13^{b} ±0.95
T_2	0.02^{a} ±0.01	0.07^{b} ±0.01	0.23^{b} ±0.07	0.29^{a} ±0.08	1.87^{b} ±0.48	2.43^{b} ±0.39	3.40^{b} ±1.29	4.97^{b} ±1.35
T_3	0.02^{a} ±0.004	0.07^{a} ±0.014	0.22^{b} ±0.08	0.31^{b} ±0.11	1.90^{b} ±0.61	3.06^{b} ±0.75	3.46^{b} ±1.00	3.60^{a} ±1.56
T_4	0.02^{a} ±0.007	0.08^{b} ±0.03	0.29^{b} ±0.03	0.45^{b} ±0.08	1.93^{b} ±0.37	2.69^{b} ±0.43	3.54^{b} ±1.12	3.79^{b} ±1.57

Table 4. Mean root weight of paddy plant at 10th, 20th, 30th, 40th, 50th, 60th, 70th, 80th days after sowing in control and treatment (n = 6)

Treatment	Root weight (Mean ± SD (g))							
	10 DAS	20 DAS	30 DAS	40 DAS	50 DAS	60 DAS	70 DAS	80 DAS
Control	0.02[a] ±0.002	0.02[a] ±0.01	0.02[a] ±0.015	0.03[a] ±0.01	0.04[a] ±0.01	0.05[a] ±0.02	0.06[a] ±0.01	0.10[a] ±0.06
T_1	0.02[a] ±0.01	0.03[a] ±0.01	0.04[a] ±0.02	0.06[b] ±0.04	0.10[b] ±0.05	0.10[b] ±0.03	0.22[b] ±0.02	0.28[b] ±0.09
T_2	0.03[a] ±0.002	0.03[a] ±0.01	0.03[a] ±0.01	0.07[b] ±0.05	0.08[b] ±0.02	0.10[b] ±0.05	0.12[a] ±0.04	0.30[b] ±0.02
T_3	0.02[a] ±0.01	0.03[a] ±0.002	0.03[a] ±0.01	0.04[a] ±0.02	0.07[a] ±0.03	0.09[b] ±0.03	0.13[a] ±0.03	0.26[b] ±0.03
T_4	0.02[a] ±0.01	0.03[a] ±0.005	0.04[a] ±0.02	0.05[a] ±0.03	0.07[a] ±0.01	0.10[b] ±0.03	0.12[a] ±0.05	0.25[b] ±0.05

Means followed by a common letter in the same column are not significantly different at 5% level by LSD

DAS = Days after sowing T_1 = *Azotobacter* sugarcane 1 T_3 =*Azospirillum* sugarcane 3

Control = Dilute nutrient broth without inoculum T_2 = *Azotobacter* maize 2 T_4 =*Azospirillum* maize 2

Table 5. Mean shoot and root lengths, fresh and dry weights of shoot and root of paddy plant at maturity stage (n=6)

Treatments	Mean length ± SD (cm)		Mean weight ± SD (g)			
	Shoot	Root	Fresh shoot	Fresh root	Dry shoot	Dry root
Control	70.67^{a} ±13.06	8.42^{a} ±1.02	2.38^{a} ±1.00	0.089^{a} ±0.031	0.711^{a} ±0.14	0.036^{a} ±0.004
T_1	104.75^{b} ±14.19	16.97^{b} ±6.82	6.92^{b} ±1.26	0.437^{b} ±0.077	1.488^{c} ±0.33	0.137^{b} ±0.060
T_2	95.33^{b} ±12.57	16.83^{b} ±3.67	6.34^{b} ±1.31	0.394^{b} ±0.108	1.201^{b} ±0.09	0.099^{b} ±0.033
T_3	89.13^{b} ±4.88	19.63^{b} ±0.95	4.95^{b} ±1.83	0.501^{b} ±0.073	1.090^{b} ±0.07	0.095^{b} ±0.034
T_4	91.70^{b} ±14.54	16.17^{b} ±2.58	5.47^{b} ±1.47	0.410^{b} ±0.082	1.212^{b} ±0.35	0.102^{b} ±0.041

Table 6. Mean panicle length, seed numbers per panicle and 1000 grains weight

Treatments	Panicle length mean±SD(cm)	Total seed numbers/panicle mean±SD	Fertile seed numbers/panicle mean±SD	Sterile seed numbers/panicle mean±SD	1000 grains weight (g)
Control	16.42^{a} ±1.28	29^{a} ±10	21^{a} ±8	8^{a} ±3	21.828^{a}
T_1	21.17^{b} ±1.94	71^{b} ±17	65^{b} ±17	7^{a} ±4	23.048^{a}
T_2	22.17^{b} ±2.40	60^{b} ±14	53^{b} ±15	7^{a} ±3	22.960^{a}
T_3	19.50^{b} ±1.98	57^{b} ±17	51^{b} ±14	6^{a} ±4	23.004^{a}
T_4	21.67^{b} ±2.73	63^{b} ±12	55^{b} ±10	8^{a} ±2	22.804^{a}

Means followed by a common letter in the same column are not significantly different at 5% level by LSD

Control = Dilute nutrient broth without inoculum T_2 = *Azotobacter* maize 2 T_4 =*Azospirillum* maize 2

T_1 = *Azotobacter* sugarcane 1 T_3 = *Azospirillum* sugarcane 3

A. Pots in net house before sowing

B. Pots with paddy seedlings

C. Paddy plant at maturity stage

D. Panicle at maturity stage

Plate 1. Pot experiment in net house and effect of bacteria on the paddy plant at maturity stage

Discussion

The best grown bacteria were selected as inoculums for pot experiment of paddy plant. The bacterial concentration for the inoculums used in this work is 10^6 CFU/ml. This is the optimal concentration for many plant species (Bashan, 1986 and Bashan *et al.*, 1989) (cited in Puente and Bashan, 1993).

The results indicated that the growth of *Azotobacter* spp. and *Azospirillum* spp. treated seedlings excelled over the untreated ones. Effects of bacterial inoculation on plant height at different stages of growth were observed in this study with an increase over control ranged from minimum 8.04% at 10 DAS to maximum 33.14% at 30 DAS. At 20 DAS, *Azospirillum* maize 2 was found to be effective in increasing plant height and caused 24.05% increase over control. At 30 DAS, 40 DAS, 50 DAS, 60 DAS and 70 DAS, *Azotobacter* sugarcane 1 was found to be highly effective in increasing plant height. At 80 DAS, *Azotobacter* maize 2 inoculated plants showed the maximum height (87.17 cm) with 27.40% increase over control. At maturity stage, *Azotobacter* sugarcane 1 inoculated plants showed the maximum height (104.75cm) with 48.22% increase over control. At 20 DAS, the significant effects of treatment on the plant height were observed in *Azospirillum* sugarcane 3 ($p<0.05$) and *Azospirillum* maize 2 ($p<0.01$) inoculated plants. Significant differences of plant height were observed between the treatments and control at 30 DAS to 80 DAS and at maturity stage ($p<0.05$). However, the difference in plant height among four treatments was not significant ($p>0.05$). The same condition was also reported by Gunarto *et al.* (1999) in that the inoculation of indigenous strains of *Azospirillum* to rice led to increase plant height at some growth stages.

In this study, root length of treated increased over control with the range of 2.13% to 132.89%. At 80 DAS and maturity stage, *Azospirillum* sugarcane 3 inoculated plants showed the maximum length (19.40 cm and 19.63 cm) with 132.89% and 133.14% respectively increase over control. Significant effects were observed at 40 DAS to 80 DAS.

Kannan and Ponmurugan (2010) reported that 10^{th}, 20^{th} and 30^{th} days of sowing revealed that *Azospirillum* treated seeds of rice had higher productivity than control. The seedlings from this particular biofertilizer treated seeds had longer shoot and root lengths than the untreated ones. So, the results in this work are in agreement with the previous work.

The percent increase of fresh shoot weight of treated plants over control ranged from 0 to 1160% at 10 DAS and 50 DAS respectively. Significant effects were observed at 20 DAS to 80 DAS and at maturity stage ($p<0.01$). The percent increase over control were very height at 50 DAS in all treated plants and highly significant ($p<0.01$) over the control. At maturity stage, the highest fresh and dry shoot weights were observed in *Azotobacter* sugarcane 1 inoculated plant.

The percent increase of fresh root weight of treated plants over control ranged from 0% to 266.67%. Significant ($p<0.05$) effects were observed at 20 DAS, 50 DAS, 60 DAS, 80 DAS and at maturity stage. At maturity stage, *Azospirillum* sugarcane 3 inoculated plants possessed maximum fresh and dry root weights.

Kannan and Ponmurugan (2010) stated that the fresh weights of root and shoot system of paddy varieties were also found to be increased to a considerable extent in *Azospirillum* treated seedlings.

The percent increase of fresh weight of treated wheat plants over control ranged from 24.71 to 106.22 and 43.89 to 57.23 at pre-flowering stage (30-35 days after sowing) and post-flowering stage (45-50 days after sowing) respectively (Mubassara *et al.*, 2008).

In this work, the percent of fresh weight of treated plants over control ranged from 175% to 400% and 835% to 1160% at 30 DAS and 50 DAS respectively. So, the fresh weights of treated plants were more enhanced than the control and are in agreement with the previous works.

In this study, panicle lengths were significantly ($p<0.01$) increased over control with the percentage of 18.75% to 35.02%. Total and fertile seed numbers per panicle were also significantly ($p<0.01$) increased over control of 96.55% to 144.83% and 142.86% to 209.52% respectively. The highest total and fertile seed numbers per panicle were observed in *Azotobacter* sugarcane 1 inoculated plant and the lowest in uninoculated (control) plant.

So, *Azotobacter* sugarcane 1 is the most effective in all plant growth and yield parameters followed by *Azotobacter* maize 2, *Azospirillum* maize 2 and *Azospirillum* sugarcane 3 respectively.

Zaw Lwin Oo (2010) also stated that the inoculation with selected strains of *Azospirillum* sp. causes significant increase in length of panicle of rice with ranges of 3.95% to 7.01%. Total seeds per panicle were significantly different from control.

A significant positive response with single inoculation of *Azotobacter chroococcum* in all growth parameters of both vegetative and reproductive stages of rice plants can be attributed to the ability to fix atmospheric nitrogen (Prajapati *et al.,* 2008).

The rice plant inoculated with *Azotobacter* spp. and *Azospirillum* spp. showed significant beneficial effect on all the growth parameters and the findings are in agreement with the results of above works.

In this work, 1000 grains weights of inoculated plants were not significantly different from the control. This result is same with the report of Zaw Lwin Oo (2010).

Conclusion

In conclusion, the inoculation of isolated bacteria from rhizosphere of sugarcane and maize, into paddy plants showed the enhancement of the plant growth parameters. So, the isolated bacteria of this work were not plant specific bacteria and can be used as biofertilizers for the growth and yield parameters of commercially important cash crops and other plants. But field experiment will be needed to confirm the effect of these isolated bacteria. Therefore, the increase use of the various biological processes in soil will decisively contribute to make agriculture more productive with less harm to the environment. This fact may be of importance for developing countries where the use of fertilizers is costly. It is hoped for substantial increase in food production in order to eliminate undernourishment and poverty, which is the main goal to be achieved by using biofertilizers.

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