Vol.4, No.12, 715-720 (2013)
http://dx.doi.org/10.4236/as.2013.412097

Agricultural Sciences

Response of rice cultivation to biofertilizers in Campeche, Mexico

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Received 27 August 2013; revised 3 November 2013; accepted 22 November 2013

ABSTRACT

The activity of various biofertilizers on rice production (Sabanero A95) was evaluated in Palizada, Campeche, Mexico, in the wet season of 2009 (year one) and 2011 (year two). On year one, arbuscular mycorrhiza INIFAP® (*Rhizophagus intraradices*), *Azospirillum brasilense* plus arbuscular micorrhiza *Rhizophagus* sp., and chemical fertilizer (92, −92, −60 kg·ha^{-1}) were evaluated, while on year two marine algae extracts, a consortium of growth promoting bacteria (*Pseudomonas* spp.) and a control (not fertilized) were evaluated. The results showed that there were no significant differences on grain yield among treatments during the year one. The average grain yield was 2,800 kg·ha^{-1}. As for the year two, the highest grain yield was observed on plots fertilized with chemical fertilizer (3333 kg·ha^{-1}), followed by plots treated with mycorrhiza INIFAP® (3000 kg·ha^{-1}). The economic analysis for rice production in both years showed that the use of arbuscular mycorrhiza decreases the cost of production by 18.5% and 16.3%, which suggests that microbial inoculants might be good substitutes of chemical fertilizers in rice production.

Keywords: Rice; Wet Season; Validation; Biofertilizers

1. INTRODUCTION

Rice is one of the main crops in the state of Campeche. The economic cost for rice production in the wet season ranges from $700 to $864 USD. Typically rice crop requires high amounts of nitrogen, phosphorus and potassium, which are supplied by the chemical fertilization. High cost for chemical fertilizers has caused 25% increase in total costs of rice crop production in tropical Mexico, where the efficiency of plant fertilization is low. The efficiency of fertilizer use for nitrogen is lower than 50%, for phosphorus lower than 10% and for potassium 40%. This low efficiency of fertilizer use is also associated with other losses by immobilization, volatilization, denitrification, leaching, and clay adsorption [1-3]. The availability and efficiency of these mineral elements depend on the organic matter content and the biological activity of soils. In this context, the biofertilizers, products based on microorganisms, are an alternative for plant nutrition as these microorganisms enhance nutriaments availability by biological activity, which reduces the amount of chemical fertilization applied [4]. In addition to the aforementioned, low quality of seeds is also a major problem that increases the cost of crop production. All these factors diminish the crop productivity and cause economic losses to farmers. Rice grain yield in wet season ranges from 2.8 to 3.2 tons per hectare. Chemical fertilizer, in this case, is used with no knowledge of optimum dose or crop requirement.

Several works for applied research, validation and technology transfer on the use of biofertilizers have been carried out in corn, sorghum, wheat and rice. In this regard, Torres [5] observed 30% reduction in the use of nitrogenous fertilizers when rhizobacteria such as *Azotobacter choococum* and *Azospirillum amazonense* were used. Similarly, phosphorous fertilization decreased 50% when the P-solubilizing fungus *Penicillium jantbinellum* were used. In tropical countries, such as Colombia, the use of biofertilizers has increased rice crop production [5-7]. The use of this biological product has been due to the big market for such products and the high quality of these biofertilizers. The present research has been carried out to evaluate the effects of various biofertilizers made in Mexico on rice crop production in the state of Campeche, Mexico.

2. MATERIALS AND METHODS

2.1. Rice Crop Management

The study was carried out in Palizada, Campeche, located in 17˚57′27″N, 91˚45′10″W, during 2009 and 2011. Soil analysis was made as follows: pH in 1:2 (soil-water); organic matter was measured with potassium dichromate; electric conductivity was measured in a saturated soil; inorganic nitrogen (NO_3-N) was analyzed by the method of salicylic acid; the available P was determined by Olsen method; and available K was measured by atomic absorption, results are shown in **Table 1**.

Rice sowing was carried out directly in single row under wet season conditions. Rice variety used (Sabanero A95) has narrow-long grain, developed specifically for wet seasons. The amount of seeds used was 80 kg·ha^{-1}; in 2009 (year one) each treatment was set in 650 m^2 plots. Total experimental area was 1950 m^2; and 2011 (year two) was the 6720 m^2, each treatment was set in 1092 m^2 plot.

Treatments evaluated in 2009 were: 1) 3 kg·ha^{-1} arbuscular mycorrhiza INIFAP® (*Rhizophagus intraradices*, General Teran Experimental Station, INIFAP, General Teran, Nuevo Leon, Mexico) with 100 kg·ha^{-1} urea; 2) 350 g·ha^{-1} microbial inoculants PGPB [*Azospirillum brasilense* (Bio-Radix®) Ultraquimia, S. A., Morelos, Mexico] plus 1 kg·ha^{-1} arbuscular mycorrhiza [*Rhizophagus* sp. (Spectrum Mico®) Ultraquimia, S. A., Morelos, Mexico] with 100 kg·ha^{-1} urea; and 3) chemical fertilization, 92 kg·ha^{-1} P and 60 kg·ha^{-1} K, applied previous to the sowing and 92 kg·ha^{-1} N, applied after emerging plants (35 and 70 days after emerging). In 2011 three additional treatments were included: 4) 1 L·ha^{-1} Marine algae extracts (ALGAENZIMS^{MR} Palaubioquim, Saltillo, Mexico) with 100 kg·ha^{-1} urea; 5) a consortium of PGPB [*Pseudomonas* spp. (Bacteriano 2709®), Bajio Experimental Station, INIFAP, Celaya, Guanajuato, Mexico] with 100 kg·ha^{-1} urea; 6) and a control (no fertilization and no biofertilizer). All microbial inoculants were applied to seeds before sowing.

2.2. Evaluated Variables

All phenological variables were recorded, including days to flowering, days to grain harvest, plant growth, pest and disease presence, number of vain and full grain, number of grain per panicle, weight of grain, grain yield (14% humidity in grain), dry biomass, and dry weight of root. In addition, protein content (%) and lipid content (%) was determined, according to standard evaluation of rice published by Centro Internacional de Agricultura Tropical (CIAT) [8]. Data were taken randomly in five samples. Precipitation was also measured daily during the experiment.

Table 1. Soil characteristics determined previous to the experiment stablishment.

pH	M.O. (%)	NO_3-N (mg·L^{-1})	P (mg·kg^{-1})	K (mg·kg^{-1})	Texture
5.6	3.62	0.10	5.94	174	Clay

2.3. Statistical Analyses

The effects of treatments on variables in each year were determined by analysis of variance. The experiments were set in a randomized block design. Mean comparison was carried out by Duncan method. In 2011, correlation by Person method was also performed. All statistical analyses were carried out in SAS version 9.2 [9].

3. RESULTS AND DISCUSSION

Results of the year one (2009) showed that the variable days to flowering had an average value of 87 days in all treatments. There were no significant differences among treatments (**Table 2**), however, visual recording there was a difference of 3 days in flowering among treatments. There were no significant differences on grain yield (2800 kg·ha^{-1}) among treatments. The economic analysis of the rice production showed that there was a decreased of 18.5% in the economic cost of production when the biofertilizer mycorrhiza-INIFAP® was used. The economic cost of rice production was $650 USD/ha and 910 USD/ha when Mycorrhiza-INIFAP® and chemical fertilizer, respectively, were used.

It is worth pointing out that in the last years the rice production area in Mexico has decreased due to the high costs of chemical pesticides and fertilizers. Likewise, other constraints are the low quality seeds and bad rainy seasons. With regards to the efficiency of chemical fertilizers in the tropics, Baligar and Bennett [10] found that there are big losses of minerals from soils, for example, nitrogen loss is about 50%, phosphorous 10% and potassium 40%. These losses are associated to immobilization, volatilization, denitrification, leaching, surface runoff, and clay adsorption.

Results from year two (2011) showed that there were significant differences among treatments on dry biomass

Table 2. Mean of grain yield, economic cost/ha and cost/benefit ratio for rice crop production with microbial inoculants used. Palizada, Campeche, 2009.

Treatments	Yield ($kg \cdot ha^{-1}$)	Cost of Crop/ha	Cost-Benefit B/C
Mycorrhizal INIFAP®	2800 a*	8240	1.18
Bio-Radix®/Spectrum Mico®	2800 a	9277	1.05
92N-92P-60K	2800 a	11524	0.85

*Equal values do not differ Duncan *s* ($p < 0.05$).

of roots, as well as in plant height, grain weight and grain yield (**Table 3**). Plots treated with chemical fertilizers and mycorrhiza-INIFAP® showed the highest grain yield, 3300 and 3000 $kg \cdot ha^{-1}$, respectively. It is important to note that high coefficient of variation was observed in number of vain grain, dry biomass of foliage and dry weight of roots, while low coefficient of variation was observed in grain weight, plant height and grain yield. The lowest grain yield was observed in plots treated with PGPB Bacteriano 2709®.

The present work points out the importance of various formulated products of microbial inoculants, such as arbuscular mycorrhizal fungus, plant growth promoting bacteria, as well as algae. The use of these products was compared with the use of chemical fertilizers recommended for rice crop production in Palizada, Campeche. In general, the highest grain yield were observed in plots treated with the chemical fertilizers, however, when only 50% of chemical fertilizer was used in combination with mycorrhiza, grain yield decreased only nine percent. It is worth noting that the cost of using 50% of chemical fertilizer (250 kg-NPK) is much higher than the economic value of grain yield diffence (333 kg) between plots treated with chemical fertilizer and those treated with mycorrhiza. Consequently, the cost/benefit ratio was lower in plots treated with chemical fertilizer.

With regards to the high coefficient of variation observed in the analysis of variance of some variables, such as number of vain grain (NGV), dry biomass of foliage (PSBA) and dry biomass of root (PSBR) (**Table 3**), it is worth mentioning that this result was expected as the erratic rainy season might have affected the plants, particularly for number of vain grain (NGV) where the range of data observed was very wide.

Previous studies have reported increases in use of biofertilizers, particularly there is a handful of literature on the use of plant growth promoting bacteria [5-11], however, in the present work, the use of the bacterial inoculant PGPB Bacteriano 2709® showed no effect on any of the evaluated variables. Grain yield, in this case, was as low as that observed in plots of the control (no fertilization) (**Table 3**).

It is interesting to note that the variable dry biomass of foliage (PSBA) did not show significant differences in the analysis of variance, but there was clear grouping of some treatments (**Table 4**). As for the most important variable, grain yield, there were four groups: the grain yield from plots treated with chemical fertilizer show the highest values, followed by those of plots treated with mycorrhiza-INIFAP®. Pearson correlation analysis showed that the grain yield is positively correlated with plant height, panicle length and dry biomass of foliage, but negatively correlated with number of vain grain. Plant height showed positive correlation with panicle lenght, weight of grain and grain yield. Number of grain per panicle showed positive correlation with number of

Table 3. Effects of biofertilizer on agronomic variables of rice in Palizada, Campeche, 2011.

Evaluated traits	Mean square	Values of F	Significance	C.V.	Media	Minimum	Maximum
AP	229.047619	6.06	**	5.13	119.74	103.0	138.0
LP	7.0971429	1.69	NS	8.49	24.11	21.0	30.0
LR	26.4571429	0.91	NS	24.16	22.28	15.0	40.0
NGLL	762.780952	0.93	NS	22.13	129.54	86.0	208.0
NGV	58.3238095	0.63	NS	56.21	17.08	3.0	44.0
NGP	1027.561905	1.08	NS	21.03	146.63	104.0	241.0
PMG	12.74691905	43349.8	**	0.069	24.65	22.1	27.41
PSBA	488.853531	1.34	NS	36.09	47.70	25.70	107.35
PSBR	2056.17422	2.60	*	44.96	62.54	12.62	137.46
REND	1389039.048	34.74	**	7.98	2504.0	1490	3657.0

AP = height of plant, LP = panicle length, LR = length of root, NGLL = number of filled grains, NGV = number of vains grains, NGP = number of grains per plant, PMG = thousand grain weight, PSBA = aerial biomass dry weight, PSBR = dry weight of root biomass, C.V. = coefficient of variation. *,** significant at $p < 0.05$ and 0.01 respectively.

full grain. All these correlations were highly significant. There were also positive and negative correlations that were not significant among variables (**Table 5**).

When used mycorrhiza for rice production, it is expected to have a decrease in grain yield as the chemical fertilizer is applied in lower amounts, however, economic cost of rice crop production decreases 18.5%, compared to that of rice production treated with only chemical fertilization. Then, the recommendation to the farmers of using mycorrhizal when cultivating rice is feasible. In this context, Arévalo [4] reported that the availability and efficiency of NPK depends on the content of organic matter and the biological activity of soils, which highlights the use of biofertilizers as an alternative for plant nutrition. San Juan and Moreno [12] mention that it is important that the use and commercialization of microbial inoculants, the public and private sector have to be involved in research and development of this kind of technology.

Analyses of protein and lipids in rice grains showed significant differences. The highest protein content was observed in grain of plants treated with Bio-Radix®/ Spectrum Mico®, followed by those treated with ALGAENZIMS^{MR}, and Mycorrhizal INIFAP®. The highest contents of lipids were observed in grain from plants treated with Bacteriano 2709® and those treated with ALGAENZIMS^{MR} as well as the control plants (**Table 6**). It is worth noting that grains from plants treated with *Azospirillum brasilense* plus arbuscular mycorrhiza showed high protein content and low lipid content. These results highlight the importance of increasing content of protein in grain with the use of this microbial inoculants. Results also showed that grains from plants treated with mycorrhiza had the highest content of protein (8.42%) and the lowest content of lipid (2.47%) (**Table 6**).

In year one of study (2009), the precipitation was 1153 mm (**Figure 1**), while in year two (2011) it was 1368.5 mm. In year two we observed plant losses due to the high

Table 4. Mean comparison (Duncan, $p = 0.05$) of evaluated traits in rice crop production in Palizada, Campeche, 2011.

Treatment	Yield	AP	LP	LR	NGLL	NGV	NGP	PMG	PSBA	PSBR
Mycorrhizal INIFAP®	3000.0 b	120.8 ab	23.4 ab	24.6 a	124.0 a	13.8 a	137.8 a	24.2 d	63.5 a	62.4 ab
Bio-Radix®/Spectrum Mico®	2222.0 d	126.6 a	25.0 ab	24.0 a	130.8 a	18.0 a	148.8 a	22.1 g	44.0 ab	59.3 ab
ALGAENZIMS^{MR}	2639.0 c	113.2 bc	23.8ab	21.2 a	118.2 a	18.6 a	136.8 a	23.9 d	51.0 ab	93.1 a
Bacteriano 2709®	1778.0 d	117.8 ab	23.2ab	20.4 a	142.2 a	22.6 a	164.8 a	24.8 b	33.1 b	46.8 b
92N-92P-60K	3333.0 a	126.0 a	26.2 a	20.6 a	132.6 a	2.8 a	145.4 a	24.7 c	47.9 ab	36.8 b
Absolute Control	2333.0 d	124.8 a	24.2 b	25.4 a	118.2 a	15.0 a	127.4 a	25.4 a	46.6 ab	76.8 ab
Mean	2551.0 d	121.53	24.3	22.7	126.7	16.8	143.5	24.19	47.7	62.54

AP = height of plant, LP = panicle length, LR = length of root, NGLL = number of filled grains, NGV = number of vain grains, NGP = number of grains per plant, PMG = thousand grain weight, Yield = grain yield kg/ha, PSBA = aerial biomass dry weight, PSBR = dry weight of root biomass, Values within a column followed by equal letters do not differ significantly ($p < 0.05$).

Table 5. Pearson correlation analysis between agronomic variables of rice, Palizada, Campeche. P.V. 2011.

	AP	LP	LR	NGLL	NGV	NGP	PMG	YIELD	PSBA	PSBR
AP	1.00000	**0.4585****	0.18836	0.09622	0.11961	0.0474	**0.4244****	**0.3025***	0.07628	0.07045
LP	----------	1.0000	−0.015	−0.077	−0.273	−0.1583	−0.2063	**0.3115***	−0.023	−0.0799
LR	----------	----------	1.0000	0.0243	−0.087	−0.0064	−0.1718	0.1638	0.1134	−0.079
NGLL	----------	----------	----------	1.0000	0.15802	**0.9473****	0.1530	−0.0778	−0.11991	0.26698
NGV	----------	----------	----------	----------	1.0000	**0.4659****	0.01994	**−0.3189***	−0.252	−0.186
NGP	----------	----------	----------	----------	-----------	1.0000	0.1436	−0.1732	−0.20051	−0.14684
PMG	----------	----------	----------	----------	-----------	----------	1.0000	0.1136	−0.03526	−0.03526
REND	----------	----------	----------	----------	-----------	----------	-----------	1.0000	**0.39626***	0.01869
PSBA	----------	----------	----------	----------	-----------	----------	-----------	-----------	1.0000	0.26374
PSBR	----------	----------	----------	----------	-----------	----------	-----------	-----------	-----------	1.0000

AP = height of plant, LP = panicle length, LR = length of root, NGLL = number of filled grains, NGV = number of vains grains, NGP = number of grains per plant, PMG = thousand grain weight, Yield = grain yield kg/ha, PSBA = aerial biomass dry weight, PSBR = dry weight biomass root. *,**significant at $p < 0.05$ and 0.01 respectively.

Table 6. Results of certain characteristics of the grain of rice to six composite samples obtained during the 2011 cycle.

Evaluated traits	Analysis of Soil				Analysis Bromatologic	
	NO_3-N (mg·L^{-1})	P (mg·L^{-1})	K (mg·L^{-1})	M.O. (%)	Crude protein (%)	Fat (%)
Mycorrhizal INIFAP®	0.10	5.87	93.64	3.62	8.42	2.47
Bio-Radix®/Spectrum Mico®	0.10	6.03	111.24	3.60	8.63	3.10
ALGAENZIMS^{MR}	0.10	5.15	109.49	3.59	8.52	3.15
Bacteriano 2709®	0.11	6.12	125.86	5.43	6.81	3.79
92N-92P-60K	0.10	5.17	174.16	3.62	8.20	3.08
Absolute Control	0.10	5.94	132.63	3.62	7.49	3.44

N = nitrogen, P = phosphorus, K = potassium, M.O. = organic matter.

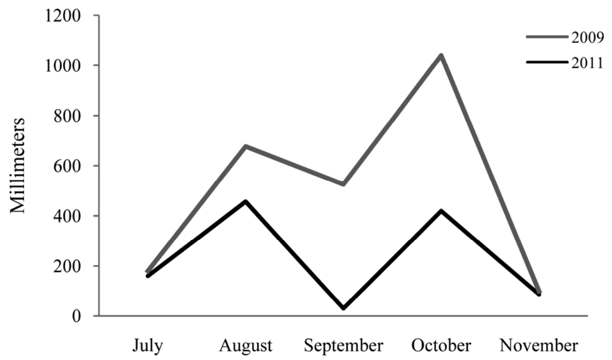

Figure 1. Annual precipitation collected during the 2009-2011 rainy season.

amount of water in the field. Soil analysis showed that there was poor in nitrogen content (**Table 1**) in plots treated with biofertilizers as well as those where no microbial inoculants were used. This might have been due to the runoff of organic matter due by the heavy rainy season (**Figure 1**). The significant positive correlation between grain yield and plant height might be explained by the fact that higher plants can have better exposure to solar radiation and consequently better photosynthesis rate that allows more grains yield in plants.

Economic Analysis

Data of rice production showed that the cost ranged from $550 using ALGAENZIMS^{MR}, to 840 USD using full chemical fertilization in year two (2011). The highest cost/benefit ratio was observed in the control plots (1.17), followed by plots in which mycorrhiza-INIFAP® (1.16) was used. In contrast, the lowest cost/benefit ratio was observed when using PGPB Bacteriano 2709® and *Azospirillum brasilense* plus arbuscular mycorrhiza (Bio-Radix®/Spectrum Mico®), while values were 0.69 and 0.87, respectively. The use of Mycorrhiza-INIFAP® decreased the economic cost of rice production by 18.5 and 16.3% in year one and year two of evaluation, respectively.

4. CONCLUSIONS

The use of chemical fertilizer for rice production showed 9% increase in grain yield compared to treatments where biofertilizers were used. The analysis of economic cost of production showed a reduction of 17.4% in the cost when mycorrhiza-INIFAP® as biofertilizer was used. The highest economic benefit was obtained using mycorrhizal fungi, which showed a ratio cost-benefit of 1.16. The uses of biofertilizers are feasible alternatives for rice cultivation, and are of particular importance if with the use of mycorrhizal-INIFAP®.

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