

Effect of Bio-fertilization Along with Different Levels of Nitrogen Fertilizer Application on the Growth and Grain Yield of Hassawi Rice (*Oryza sativa* L.)

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Abstract

A field experiment was conducted at the Agricultural and Veterinary Training and Research Station of King Faisal University, Al-Hassa, Kingdom of Saudi Arabia during 2000 and 2001 seasons to study the influence of bio-fertilization (Control, cyanobacteria, *Azospirillum* and *Azotobacter* isolated from Hassawi rice fields in Al-Hassa) and different levels of nitrogen fertilizers (90, 135 and 180 kgN/ha) on growth, grain yield and its components of Hassawi rice (*Oryza sativa* L.). A split plot design with five replicates was used. Nitrogen fertilizer levels represented the main plots and bio-fertilization represented the sub-plots.

The combination of bio-fertilization and nitrogen fertilization at a level of 180 kgN/ha exceeded all other treatments in flag leaf area, plant height, number of spikes/m², number of filled grains/spike, 1000-grain weight and m² grain yield in both seasons. Increasing nitrogen fertilizer rates up to 135 kgN/ha, produced maximum values of flag leaf area, plant height, number of spikes/m², number of filled grains/spike, 1000-grain weight and grain yield in both seasons.

The study recommends the use of bio-fertilization (cyanobacteria + *Azospirillum* + *Azotobacter*) along with nitrogen fertilization at a rate of 180 kg N/ha for Hassawi rice production in Al-Hassa area.

Introduction:

Rice (*Oryza sativa* L.) is the staple diet of over 40% of the world's population making it the most important food crop currently produced (Yadav et al., 2000). Amongst many others, the nutritional requirements of the crop are considered to be the most important factor affecting yield and they go far beyond the natural capacity of any soil type (Ahlawat et al., 1998). Hence, big amounts of chemical fertilizers must be added to the soil where rice is grown. Excessive application of fertilizer nitrogen can result in a high soil nitrate concentration after crop harvest (Jokela and Randall, 1989; Roth and Fox, 1992 and Gordon et al., 1993). This situation can lead

to an increase in the level of nitrate contamination of potable water, because nitrate remaining in the soil profile may leach to groundwater (Singh et al., 1995).

Some bacteria that are associated with the roots of crop plants can induce beneficial effects on their hosts and often are collectively referred to as PGPR (Plant Growth Promoting Rhizobacteria) (Vermeiren et al., 1999). The biological fixation of nitrogen produced by these organisms can constitute a significant and ecologically favorable contribution to soil fertility (Vlassak et al., 1992). However, the low efficiency of use of fixed nitrogen by the plant in the formation of grain protein could be a limitation (Vlassak et al., 1992). Therefore, more attention must be given to the use of bio-fertilization. Several authors have studied the effect of bio-fertilization on rice growth and development (Singh et al., 1988; Yanni, 1992; Hammad, 1994 and Nayak et al., 1986) and concluded that inoculation of *Azospirillum* 10 days after transplanting of rice significantly increased flag leaf area, plant height, spike weight and number of grains/spike. Similar conclusions were reported by Singh et al. (1992) and Wang (1986) who observed that *Azolla* biomass in the soil increased rice stem height by 7.5 cm and the number of spikes/hill by 2.0 over the control.

It is well known that nitrogen fertilization plays a significant role in improving rice yield. A high rate of nitrogen application increases leaf area development, improves leaf area duration after flowering and increases the overall crop assimilation and thus contributing to increased seed yield (Surendra et al., 1984). Hussein and Radwan, (2001), Bassal et al., (1996) and Kreem (1993) found that increasing nitrogen application rates increased number of tillers/hill, number of filled grains/spike, 1000 grain weight and grain yield. El-Kalla et al. (1988) concluded that increasing nitrogen application rates up to 75 kgN/ha increased plant height, flag leaf area, 1000-grain weight, spike weight and grain yield. Abd El-Rahman et al. (1992) indicated that the application of 144 kgN/ha produced higher spike length, 1000-grain weight, number of grains/spike and grain yield.

Several research groups, have compared the effects of biological and chemical fertilization in the flooded rice system (Mathar et al., 1981, Khadr et al., 1985 and Jeyaraman and Purushothaman, 1988) and reported that

bio-fertilization could provide a better alternative for the extensive use of nitrogen fertilizers in rice production.

This study was initiated to assess the effect of bio-fertilization of rice crop in the presence of different nitrogen fertilizer levels on plant growth and grain yield.

Materials and Methods

A field trail commencing in 2000-2001 was continued for two consecutive years, i. e. up to 2001-2002 on a Typic Torrispammments soil of the research farm of the Agricultural and Veterinary Training and Research Station of King Faisal University, Al-Hassa, located at 25° 40' N latitude, 49° 46' E longitude and 137 m above sea level, in the Eastern Province of the Kingdom of Saudi Arabia. The climate of the area is arid subtropical, with dry hot summers and cold winters. The average annual rainfall is 66.6 mm, and nearly 95% of the total rainfall is received during the winter season. The average monthly minimum and maximum temperatures fluctuate from 6.7 to 9.6 and 17.6 to 25.7°C in January (the coldest month), and from 20.2 to 23.2 and 38.1 to 42.5°C in July (the hottest month) respectively.

The soil of the experimental site was a sandy loam (15% clay, 18.5% silt, 64.5% sand) with EC = 3.5 dS/m, pH = 6.7 and CaCO₃ = 8.7. The experiment design was split plot with four replicates. The main plots (20 × 50 m) included 3 treatments of nitrogen (90, 135 and 180 kgN/ha) in the form of urea (46% N) while The sub-plots (10 × 50 m) included the 2 bio-fertilization treatments namely: no bio-fertilization "control" and bio-fertilization (a combination of cyanobacteria, *Azospirillum* and *Azotobacter*). The cyanobacteria, *Azospirillum* and *Azotobacter* bacteria were isolated from rice fields in Al-Hassa area.

The experimental field was well prepared and calcium superphosphate (15.5% P₂O₅) at a rate of 100 kg P₂O₅/ha was added on the dry soil before ploughing. 45 days old seedlings were transplanted at a rate of 4 - 5 seedlings/hill. There were 25 hills/m², and 2 - 3 cm of standing water. Other cultural practices of growing rice were adopted as recommended except the factors under study.

Azotobacter was inoculated at the rate of 3 kgDW/plot a week after transplanting. *Azospirillum* and cyanobacteria were added before transplanting by inoculation of the bacterial suspension with soft soil before irrigation. The nitrogen fertilizer was applied in two equal splits, one half was added two weeks after transplanting and the other half was added 30 days later.

At maturity an area of 25 × 20 cm was harvested randomly from each plot for determination of flag leaf area and plant height. Flag leaf area was measured using a leaf area meter (Licor, Model 3100, LICOR Ltd. Lincoln, NE). Ten plants from guard rows were randomly selected from each plot to determine the number of spikes/m², spike length (cm), number of filled grains/spike, 1000-grain weight (g). In addition, the sun-dried biomass of the crop in one square meter of each plot was threshed manually, and the grain yield was recorded and then converted to tons/ha.

The data were statistically analyzed using analysis of variance according to the procedures outlined by Cochran and Cox (1957). The new Least Significant Differences (N-L.S.D.) was used to compare treatment means (Steel and Torrie, 1996)

Results and Discussion

Bio-fertilization had significant effects on growth, yield and yield components in both seasons (Table 1). Bio-fertilization (the combination of cyanobacteria, *Azospirillum* and *Azotobacter*) produced maximum values of flag leaf area, plant height, number of tillers/m² and number of filled grains/spike, 1000-grain weight and grain yield in both seasons. The beneficial effect of bio-fertilization in increasing rice yield and its components may be attributed to an additional amount of nitrogen made available by biological fixation of nitrogen by organisms of the rhizosphere. This nitrogen have helped in improving growth and hence increased leaf area that intercept light and increased photosynthetic rates resulting in the accumulation of more dry matter by the crop (Rekhi et al., 2000). The results substantiate the findings of Nayak et al. (1986) and Wang (1986) as well as Abou-Zeid et al. (1996). It appears that any deficiency in fertilizer – derived N in the soil was corrected by biological nitrogen fixation, resulting in a stable response across all nitrogen application rates.

Table (1)
Flag leaf area, plant height, number of spikes/m² and spike length as affected by bio-fertilization and nitrogen fertilizers applied at different levels in 2000 and 2001 seasons

Treatment	Flag leaf area (cm ²)		Plant height (cm)		Spikes/m ²		Spike length (cm)	
	2000	2001	2000	2001	2000	2001	2000	2001
Bio-fertilization (BF)								
Without BF(Control)	18.6	20.3	60.7	64.2	356	368	18.8	19.1
With BF	23.7	25.7	79.3	86.7	414	416	21.0	20.7
N-LSD (0.05)	1.3	2.5	13.4	9.7	12.4	39.2	1.2	0.9
Nitrogen fertilizer (kg/ha)								
90	19.0	21.2	65.9	72.2	368	375	18.0	18.2
135	21.6	23.4	71.6	74.8	388	396	20.5	19.8
180	22.9	24.4	72.5	79.4	399	405	21.2	21.7
N-LSD (0.05)	0.3	0.4	0.8	1.4	10.0	8.0	1.2	1.3

The data presented in Tables 1 and 2 show that flag leaf area, plant height, number of spikes/m², spike length, tillers/m², 1000-grain weight, number of filled grains/spike and grain yield/ha were significantly affected by added nitrogen fertilizer levels in both seasons. Higher values for all traits were obtained with 180 kgN/ha (Tables 1 and 2). The yield was significantly superior to 135 kgN/ha. The lowest yield was found under 90 kgN/ha in both seasons. Singh et al., (1983) observed a gradual increase in the yield of rice as the levels of nitrogen fertilizer increased to 120 kgN/ha. Addition of 180 kgN/ha significantly recorded maximum values for all growth attributes, yield and yield components. The increase in grain yield due to increases in nitrogen fertilizer levels may be attributed to increases in spikes/m², grains/spike and 1000-grain weight. The results confirm those obtained by Bhattacharyya and Singh (1992), Prasad et al. (1992) and Kalita and Sarmah (1992).

Tabl (2)

Number of filled grains/spike, tillers/m², 1000-grain weight and grain yield as affected by bio-fertilization and nitrogen fertilizer applied at different levels in 2000 and 2001 seasons

Treatment	No. of filled grains/ spike		Tillers/m ²		1000-grain weight (gm)		Grain yield (ton/ha)	
	2000	2001	2000	2001	2000	2001	2000	2001
Bio-fertilization								
Without BF(Control)	70.57	74.3	397	404	19.5	19.9	17.2	19.7
With BF	80.7	82.2	434	450	22.0	23.1	26.3	26.7
N-LSD (0.05)	4.21	3.34	23.6	20.2	1.9	2.3	4.96	7.22
Nitrogen fertilizer (kg/ha)								
90	72.2	75.8	405	412	20.4	21.1	18.3	20.1
135	76.7	78.3	414	431	20.9	21.5	22.9	23.7
180	78.9	80.6	428	438	21.0	21.9	24.1	25.8
N-LSD (0.05)	1.42	1.91	12.3	4.3	0.4	0.3	1.74	1.31

In general, the correlations of the characters were of the same relative order each year (Table 3). Correlations in 2000 were generally lower than in 2001 because of rank growth of plant characters, which tended to decrease values of grain characters.

The results of correlation between plant characters, grain yield/ha and its attributes presented in Table 3 are based on the average of the two years, two bio-fertilization treatments and three nitrogen fertilizer levels. Three indirect yield components (plant height, flag leaf area and number of tillers/m²) were highly correlated to yield of rice plants. Flag leaf area was the only indirect yield component correlated to 1000-grain weight. The indirect yield components were integrated in growth and increases in growth were responsible for increases in yield and two of its components i.e. spikes/m² and grain/spike.

The direct yield components (spikes/m², grains/spike and 1000-grain weight) were not significantly intercorrelated but they were all highly significantly correlated with rice yield. These results are in good accordance with those reported by Ibrahim (1995).

Conclusions:

Bio-fertilization combined with different levels of nitrogen fertilizer application significantly affected the growth and yield components of Hassawi rice. Bio-fertilization plus the application of nitrogen fertilizer at a rate of 180 kgN/ha increased grain yield significantly in both years and so it can be recommended for Hassawi rice in Al-Hassa region

Table (3)
Correlation of plant characters, grain yield/ha and its attributes
(Data over treatments and seasons)

Characters	1	2	3	4	5	6	7	8
1- Grain yield/ha	-	0.65*	0.76**	0.81**	0.85**	0.87**	0.88**	0.85**
2- Plant height	-	-	0.83**	0.72*	0.68*	0.83**	0.80**	0.23
3- Flag leaf area	-	-	-	0.66*	0.82**	0.86**	0.88**	0.87**
4- Number of tillers/m ²	-	-	-	-	0.88**	- 0.76**	0.70**	0.17
5- Number of spikes/m ²	-	-	-	-	-	-0.42	0.39	-0.58
6- Spikes length	-	-	-	-	-	-	0.81**	0.85**
7- No. of filled grains/spike	-	-	-	-	-	-	-	-0.37
8- 1000-grain weight	-	-	-	-	-	-	-	-

* Significant at (0.05)

** significant at (

References:

1. Abd El-Rahman, A. A. M., M. A. Shata and M. A. Nour. 1992. Effect of some cultural practices on broadcast seeded rice productivity under saline condition. Proceeding 5th Con. of Agronomy, Zagazig J. Agric. Res. 1: 87 – 95.

2. Abou-Zeid, S. T., A. Abdel-Monem and Y. A. Abd El-Aal. 1996. Interaction between Azolla and N fertilizers under flooded systems green house study. *J. Agric. Sci. Mansoura Univ.* 21: 1203 – 1209.
3. Ahlawat, I. P. S., M. Ali, R. L. Yadav, J. D. V. K. Rao, T. J. Rego and R. P. Singh, 1998. Biological nitrogen fixation and residual effect of summer and rainy season grain legume in rice and wheat cropping systems of the Indo-Gangetic Plain. In: Rao, J. D. V. K., Johansen, C., Rego, T. J. (Eds.), *Residual Effects of Legumes in Rice and Wheat Cropping Systems of the Indo-Gangetic Plain*. Oxford/IBH Publishing Co., New Delhi, India. pp. 31-54.
4. Bassal, S. A. A., A. M. Abd El-All, I. O. E. Metwally and K. E. El-Habbak. 1996. Growth and yield of rice in relation to winter preceding crops and N fertilizer levels. *J. Agric. Sci. Mansoura Univ.* 21: 79 – 88.
5. Bhattacharyya, H. C. and K. N. Singh. 1992. Response of direct-seeded rice (*Oriza sativa*) to levels and time of nitrogen application. *Indian J. Agron.* 37: 681 – 685.
6. Cochran, W. G. and G. M. Cox. 1957. *Experimental Designs*. Wiley, New York.
7. El-Kalla, S. E., M. S. Balal, El-Kassaby, A. N. Attia and I. O. El-Sayed. 1988. Response of rice cutlivar (IR 50) to nitrogen and zinc sulphate application. *J. Agric. Sci. Mansoura Univ.* 629 – 634.
8. Gordon, W. B., D. A. Whitney and R. J. Raney. 1993. Nitrogen management in furrow irrigated, ridge-tilled corn. *J. Pord. Agric.* 6: 213 – 217.
9. Hammad, S. A. 1994. Evaluation of Azolla and ammonium sulfate as a source of nitrogen of rice production. *J. Agric. Mansoura Univ.* 19: 375 – 385.
10. Hussein, H. F. and S. M. A. Radwan. 2001. Effect of biofertilization with different levels of nitrogen and phosphorus on wheat and associated weeds under weed control treatments. *Pakistan Journal of Biological Sciences.* 4: 435 – 441.
11. Ibrahim, E. L. 1995. Effect of dates and methods of sowing on grown and yield of rice. Ph.D. Thesis, fac.of Agric. Mansoura Univ.
12. Jeyaraman., S. and Purushothaman. 1988. Biofertilizer efficiency in lowland rice. *International Rice Research News Letter.* 13: 24 – 26.
13. Jokela, W. E. and G. W. Randall. 1989. Corn yield and residual soil nitrate as affected by time and rate of nitrogen application. *Agron. Progress Rep.* 398.
14. Kalita, M. C. and N. N. Sarmah. 1992. Effect of nitrogen level and mulch on yield and yield attribution characters of summer rice (*Oraza sativa*) under the rainfed condition. *Indian J. Agron.* 37: 690 – 693.

15. Khadr, M. S., M. N. Baker and A. M. El-Sayed. 1985. Evaluation of some local isolates of BGA as nitrogen sources for rice. *Agric. Res.* 63: 209 – 213.
16. Kreem, M. K. E. 1993. Effect of different nitrogen rates and compnitrification inhibitor on growth and yield of rice. *J. Agric. Res. Tanta Univ.* 19: 525 – 536.
17. Mathar, S., S. Krishnamoorthy and P. Anavaram. 1981. Azolla influenced on rice. *International Rice Research News Letter*, 5: 23.
18. Nayak, D. K., J. K. Ladha and J. Watanabe. 1986. The fate of marker *Azospirillum lipoferum* inoculated into rice and its effect on growth, yield and N₂ fixing plants studied by acetylene reduction N¹⁵ feeding and N¹⁵ dilution technique. *Biology and fertility of soils.* 2: 7 – 14.
19. Prasad, U. K., T. N. Prasad and A. Kumar. 1992. Response of direct-seeded rice (*Oriza sativa*) to levels of nitrogen and irrigation in calcareous soil. *Indian J. Agron.* 37: 686 – 689.
20. Rekhi, R. S., D. K. Benbi and B. Singh. 2000. Effect of fertilizers an organic manure on crop yields and soil properties in rice-wheat cropping system. In: Abrol, I. P., Bronson, K. F., Duxbury, J. M., Gupta, R. K. (Eds.), *Long-term Soil Fertility Experiments in Rice-Wheat Cropping Systems. Rice-Wheat Consortium Paper Series 6. Rice-Wheat Consortium for the Indo-Gangetic Plains, New Delhi, India.* pp. 1-6.
21. Roth, G. W. and R. H. Fox. 1992. Corn hybrid interaction with soil nitrogen level and water regime. *J. Pord. Agric.* 5: 137 – 142.
22. Singh, A. L., P. K. Singh and P. L. Singh. 1988. Comparative studies on the use of green manuring and Azolla and Blue-Green Algae biofertilizer to rice. *J. of Agric. Sci.* 2: 337 – 348.
23. Singh, A. L., P. K. Singh, V. P. Pandey, A. K. Dubey and D. C. Veemd. 1992. Effect of Blue-Green Algae inoculation on yield of paddy rice in different districts of U. P. *News Agriculturist.* 3: 189 – 192.
24. Singh, B., Y. Singh and G. S. Sekhon. 1995. Fertilizer N use efficiency and nitrate pollution of groundwater in developing countries. *J. Contam. Hydrol.* 20: 167-184.
25. Singh, T. N., G. Singh and H. P. Singh. 1983. Nitrogen fertilization spreading to maximize upland rice yields. *International Rice Research Institute Newsletter* 8: 27.
26. Steel, G. D. and J. H. Torrie. 1996. *Principles and procedure of statistics with special references to the biological sciences.* McGraw-Hill Book. Co., Inc., New York. P. 481.
27. Surendra Singh, R. Prasad and V. Ishwanran. 1984. New nitrogen carries for rice. *Madras. Agric. J.* 71: 371 – 374.

28. Vermeiren, H., A. Willems, G. Schoofs, R. De Mot, V. Keijers, W. Hai and J. Vanderleyden. 1999. The rice inoculant strain *Alcaligenes faecalis* A 15 is nitrogen-fixing *Pseudomonas stutzeri*. *Appl. Microbiol.* 22: 215 – 224.
29. Vlassak, K., L. Van Holm, L. Duchateau, J. Vanderleyden and R. De Mot. 1992. Isolation and characterization of fluorescent *Pseudomonas* associated with the roots of the rice and banana grown in Sri Lanka. *Plant and Soil.* 145: 51 – 63.
30. Wang, Z. H. 1986. Rice yield increased effects of *Azolla* aculture in winter and spring. *Zhejiang Agriculture.* 4: 155 – 157.
31. Yadav, R. L., B. S. Dwivedi, K. Prasad, O. K. Tomar, N. J. Shupali and P. S. Pandey. 2000. Yield trends, and changes in soil organic-C and available NPK in a long-term rice-wheat system under integrated use of manures and fertilizers. *Field Crops Res.* 68: 219-246.
32. Yanni, Y. G. 1992. The effect of Cyanobacteria and *Azolla* on the performance of rice under different levels of fertilizer nitrogen biotechnology. 8: 132 – 136

(*Oryza sativa* L.)

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كلية العلوم الزراعية و الأغذية – جامعة الملك فيصل

الأحساء – المملكة العربية السعودية

الملخص :

أجريت تجربة حقلية بمحطة التدريب والبحوث الزراعية والبيطرية التابعة لجامعة الملك فيصل بالأحساء لموسمي ٢٠٠٠ و ٢٠٠١م لدراسة تأثير التسميد الحيوي (بدون تسميد حيوي وتسميد حيوي بخليط من السيانونيكتيريا والأزوسبريللم والأزوتوباكتر) وثلاثة معدلات مختلفة من النيتروجين (٩٠ ، ١٣٥ ، ١٨٠ كجم نيتروجين/هكتار) على إنتاجية محصول الأرز الحساوي. صممت التجربة على نظام القطع المنشقة بأربعة مكررات. مثلت معدلات النيتروجين القطع الرئيسية ومعاملات التسميد الحيوي القطع المنشقة.

أشارت النتائج إلى أن التسميد الحيوي أدى إلى زيادة معنوية في طول النبات ومساحة الورقة العلم وعدد الخلف في المتر المربع وعدد السنابل في المتر المربع وعدد الحبوب الممتلئة بالسنبلة ووزن الألف حبة ومحصول الحبوب (طن/هكتار). كما أدت زيادة معدلات التسميد النيتروجيني إلى ١٨٠ كجم/هكتار إلى زيادة معنوية في طول النبات ومساحة الورقة العلم وعدد الخلف في المتر المربع وعدد السنابل في المتر المربع وعدد الحبوب الممتلئة بالسنبلة ووزن الألف حبة ومحصول الحبوب (طن/هكتار). كان هناك ارتباط معنوي موجب بين محصول الحبوب للهكتار وكل من صفات المحصول غير المباشرة (طول النبات ، مساحة الورقة العلم وعدد الخلف في المتر المربع) والمباشرة (عدد السنابل في المتر المربع ، عدد الحبوب الممتلئة في السنبلة ووزن الألف حبة).

لذلك توصي هذه الدراسة باستخدام ١٨٠ كجم نيتروجين/هكتار مع التسميد الحيوي المتمثل في خليط من السيانونيكتيريا والأزوسبريللم والأزوتوباكتر.