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ENHANCEMENT OF SOYBEAN (*GLYCINE MAX* L.) GROWTH BY BIO-FERTILIZERS OF *NOSTOC MUSCORUM* AND *NOSTOC RIVULARE*

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Abstract

In the present study the nitrogenase activity of *Nostoc muscorum* and *Nostoc rivulare* was evaluated *in vitro*; the test showed that *Nostoc muscorum* and *Nostoc rivulare* have the ability to fix nitrogen. In a pot experiment under field conditions, the results of the present study showed that inoculation of the soybean plant with *Nostoc muscorum* and *Nostoc rivulare*, either alone or in combination with N-fertilizer at 50 and 100 kg N/ha, caused a significant increase in the growth of these plants, as reflected in plant height, leaf area, weight of plant as well as the legume weight of soybeans. The combination of biofertilization and N-fertilization, especially at 100 kg N/ha, had more effect on both the growth of soybeans and nitrogenase activity compared to biofertilization alone. *Nostoc muscorum* and *Nostoc rivulare* are a promising biofertilizers for achieving an efficient association between N_2 fixing cyanobacteria and soybeans; and thus enhancement of the growth.

Key words: Cyanobacteria, Soybean, Biofertilization, Ammonium sulphate.

Introduction

The sandy soils in Saudi Arabia have 2 major problems in respect to agriculture: low fertility and inadequate water retention. Nitrogen is a vital element for animal and plant growth, since it is an essential part of many of the chemical compounds, such as proteins and nucleic acids, which are the basis of all life forms. It spite of its abundance (it constitutes 78% of the earth's atmosphere) it is one of the most limiting factors for crop growth. The reserves of nitrogen present in the soil and available for use by plants are continually depleted by such processes as microbial de-nitrification, soil erosion, leaching, chemical volatilization and, perhaps most important, the removal of nitrogen containing crop residues from land. The nitrogen reserve of agricultural soils must therefore be replenished periodically in order to maintain an adequate level for crop production. This replacement of soil nitrogen is generally accomplished by the addition of chemically fixed nitrogen in the form of commercial inorganic fertilizers or by the activity of biological nitrogen fixation systems (Schoot, 1990). Cyanobacteria are friendly biofertilizers for rice based collecting systems, being the major components of wetland rice ecosystems. They are, in addition, easily available and serve as inexpensive sources of natural biofertilizers (Omar, 2000; Ladha & Reddy, 2003; Vaishampayan *et al*., 2001; Ahamd *et al*., 2014). The association between cyanobacteria and plants can profit crop plants by improving the physical and chemical properties of the soil because cyanobacteria have ability to produce the biologically fixed nitrogen, growth promoting substances and different types of secondary metabolites and significant amounts of organic matter which are been solubilized otherwise insoluble phosphates (Goyal, 1993). These processes are called nitrogen fixation, and may be either chemical or biological. The increasing international emphasis on environmentally sustainable development through the use of renewable resources is likely to focus attention on the potential role of biological nitrogen fixation in supplying nitrogen for agriculture (Dixon & Wheeler, 1986; Peoples, *et al*., 1995). This expanded interest in ecology has drawn attention to the fact that BNF is ecologically safe and that its greater exploitation could reduce the use of fossil fuels and be useful in reforestation and the restoration of misused lands to productivity (Sprent & Sprent, 1990; Burris, 1994). Several plants form a special symbiosis with the photosynthesizing cyanobacteria, such as the herb Gunnera with *Nostoc* (Bergman *et al.*, 1992) and the tiny water fern, Azolla, with the filamentous *Anabaena* (Braun-Howland & Nierzwicki-Bauer, 1990). Cyanobacteria are normally located in the intercellular spaces of the tissues except in the case of Gunnera, where bacteria are present inside the cells of the shoot apex (Bergman *et al.*, 1992). Sholkamy *et al.* (2012) reported a potential role of *Nostoc muscorum* and *Nostoc rivulare* as biofertilizers for the enhancement of maize growth under different doses of n-fertilizer; this present study extends this work by aiming to isolate *Nostoc* spp. and to evaluate their role in the enhancement of soybean growth in sandy soil.

Material and Methods

Isolation, purification and identification of *Nostoc*: One ml of dilute soil suspension was transferred to each sterile Petri dish. Melted but cooled nitrogen free BG11 medium was then poured into the Petri dish (Rippka & Herdman, 1992) and this was supplemented with agar for solidification (1%), at a kept at a light intensity of 3000 lux.

Three plates were prepared for each sample; the cultures from each plate were incubated at 24°C ±1 for 15 days according to the method described by Abdel-Hafez *et al.* (2000). Developing cultures were identified according to Desikachary (1959). Pure isolates were maintained on N_2 containing BG11 medium for further studies. After 15 days, cyanobacterial cultures were harvested by centrifugation (8000 rpm for 10 min), suspended in sterile H_2O (4g/ 250 ml), and used as an inoculum at an amount of 10^6 heterocysts per seed of wheat plant (El-Shahed, 2005).

***In vitro* evaluation of nitrogenase activity of two *Nostoc* isolates:** The nitrogenase activity of two of the cyanobacterial isolates was evaluated in the 15 days' old culture using the acetylene reduction technique, which was described by Hardy *et al.* (1973). For this evaluation, a Gas Chromatograph, ATIUNICAM 610-GLC (UK) equipped with a glass column filled with activated alumina, was used.

Pot experiments of soybean: Surface sterilized soybean seeds (*Glycine max* L.) were left to germinate on wet sterile filter paper in Petri dishes for three days in the dark. Six grains were transplanted into a pot containing 2 kg of a mixture of sand and clay soil in a ratio of 2:1 respectively. The pots were then inoculated with *N. muscorum* or *N. rivulare* (at 10^6 heterocysts per seedling). One week later, a dose of Ammonium sulphate (at either 100 or 50 kg N/ ha) was added on three periods (i.e. the 100 kg N/ha dose was added in three periods, each of 33.3 kg N/ha). The pots were divided into nine groups with three replicates for each treatment, as illustrated in Figs. (8a, b, c): 1). Seedlings of the first group were not inoculated with *N. rivulare* or *N. muscorum*, and received no additional dose of ammonium sulphate. 2) Seedlings of the second group were also not inoculated with *N. rivulare* or *N. muscorum*, but were treated with half a dose of ammonium sulphate (50 kg N / ha). 3) Seedlings of the third group were not inoculated with *N. rivulare* or *N. muscorum*, but were treated with a full dose of ammonium sulphate (100 kg N/ha). 4) Seedlings of the fourth group were inoculated with *N. rivulare*, but received no ammonium sulphate. 5) Seedlings of the fifth group were inoculated with *N. rivulare*, and were also treated with a half dose of ammonium sulphate. 6) Seedlings of the sixth group were inoculated with *N. rivulare* and a full dose of ammonium sulphate. 7) Seedlings of the seventh group were inoculated with *N. muscorum*, but no ammonium sulphate. 8) Seedlings of the eighth group were inoculated with *N. muscorum* and a half dose of ammonium sulphate. 9) Seedlings of the ninth group were inoculated with *N. muscorum* and a full dose of ammonium sulphate. At the beginning of the second week after sowing, plants were thinned down to two plants per pot. The experiment was performed in a wire proof greenhouse maintained at 26 ± 5°C under natural daylight. Pots were irrigated with water as needed according to field capacity. A month after cultivation, soybean plants were harvested and growth parameters and nitrogenase activity were determined.

Determination of plant growth: The length of the tested plants were measured (cm) and they were then weighed in order to find their fresh weight (mg) and then dried in an oven at 105°C to a constant mass for further analysis. Leaf area was determined according to Norman & Campbell (1994). Chlorophyll a, Chlorophyll b and carotenoids were determined using the spectrophotometric method recommended by Metzner *et al.* (1965). The versene (disodium dihydrogen ethylenediamine tetraacetic acid) titration method (Schwarzenbeck & Biederman, 1948) was employed for the determination of Ca^{+2} and Mg^{+2} concentrations. Sodium and potassium were determined in the samples photometrically by flame photometry according to the method of Golterman *et al.* (1978).

Statistical analysis: The triplicate sets of data for the various parameters evaluated were subjected to ANOVA (Analysis of variance) in accordance with the experimental design (i.e. a completely randomized design) using the SPSS11 statistical package in order to quantify and evaluate L.S.D values, which were calculated at a P level ≤ 0.05 (Steel & Torrie, 1960).

Results

The results showed that both of the cyanobacterial isolates tested have the ability to fix nitrogen. As shown in Figs. (1, 2, 3, 4, 5, 6 and 8) shoot length, root length, leaf area, plant weight and the number of legumes increased significantly with the addition of combined nitrogen compared to plants not fertilised with nitrogen. The results also indicated that both half and full doses of N-fertilizer, together with *N. muscorum* and *N. rivulare* inoculation, significantly increased soybean growth as well as legume number and legume weight compared to inoculation with *N. muscorum* or *N. rivulare* alone. *N. muscorum* was more effective in enhancing the growth of the soybean plants compared to inoculation with *N. rivulare*. The results in Fig. 7 show that the addition of a half or full dose of combined nitrogen caused an increase in pigment content, with biofertilization also improving from pigment content**.** The results also indicated that the growth of soybean plants inoculated with *N. muscorum* together with a half dose or full dose of combined nitrogen significantly increased compared with the growth of soybean plants inoculated with *N. rivulare* at half dose or full dose of combined nitrogen. Overall, therefore, it was evident that *N. muscorum* and *N. rivulare* demonstrated a nitrogen fixing capacity on soybean roots. Furthermore, in comparison to non-fertilised plants, this nitrogen fixing activity was enhanced by the addition of N-fertilizer. Pot experiments, in which large pots containing non-sterilized sandy soil were used to test the ability of cyanobacteria to improve the growth of tested plants under field conditions. Results of the present study showed that inoculation of soybean plant with *N rivulare* and *N. muscorum* caused a significantly increase in growth of this plant, represented in plant height, leaf area, weight of plant as well as legume weight of soybean, pigment and minerals content.

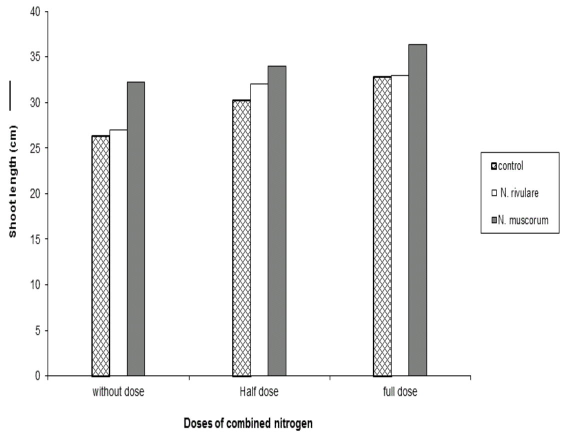

Fig. 1. Effect of *N. rivulare* and *N. muscorum* inoculation on the shoot length of the soybean plant with N-fertilization at half and full dose. L.S.D (5%) = 2.86

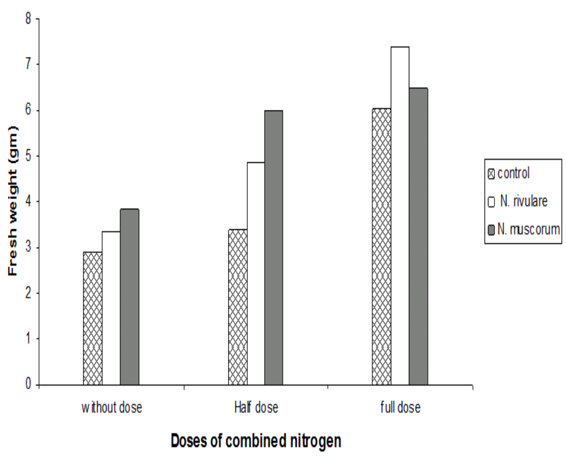

Fig. 2. Effect of *N. rivulare* and *N. muscorum* inoculation on fresh weight of the soybean plant with N-fertilization at half and full dose. L.S.D (5%) = 0.39

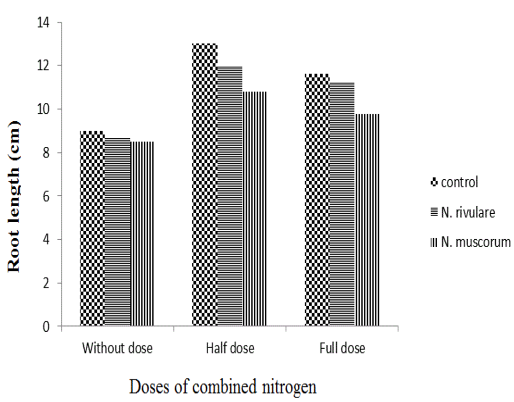

Fig. 3. Effect of *N. rivulare* and *N. muscorum* inoculation on the root length of the soybean plant with N-fertilization at half and full dose. L.S.D (5%) = 2.13.

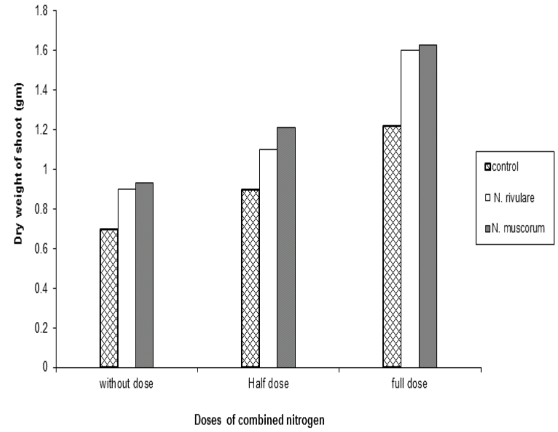

Fig. 4. Effect of *N. rivulare* and *N. muscorum* inoculation on the dry weight of the soybean plant with N-fertilization at half and full dose. L.S.D (5%) = 0.033.

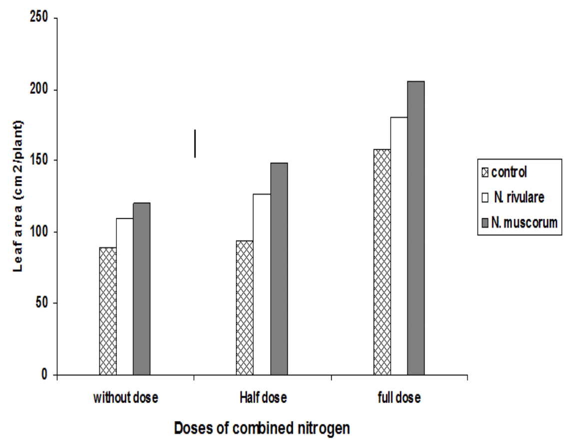

Fig. 5. Effect of *N. rivulare* and *N. muscorum* inoculation on the leaf area of the soybean plant with N-fertilization at half and full dose. L.S.D (5%) = 40.

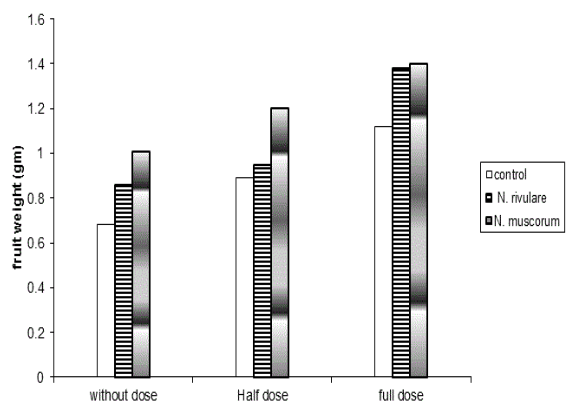

Fig. 6. Effect of *N. rivulare* and *N. muscorum* inoculation on the fresh weight of soybean legume with N-fertilization at half and full dose. L.S.D (5%) = 0.04

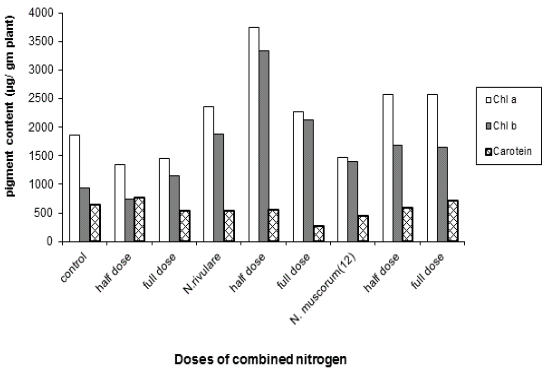

Fig. 7. Effect of *N. rivulare* and *N. muscorum* inoculation on the pigment content of the soybean plant with N-fertilization at half and full dose.

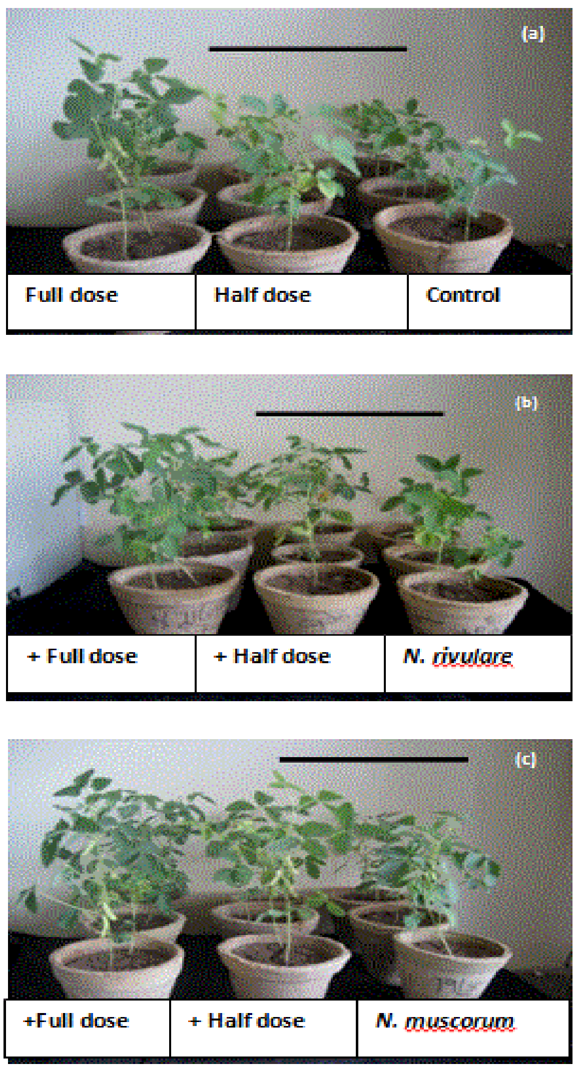

Fig. 8. Pot experiment: a) Effect of different doses of combined nitrogen on growth of the soybean plant. Bar = 12.1cm; b) Effect of inoculation with *N. rivulare* on soybean growth at different doses of combined nitrogen. Bar = 12.1 cm; c) Effect of inoculation with *N.* muscorum at different doses of combined nitrogen. Bar = 12.1 cm

Discussion

Results of the present study showed that shoot length, root length, leaf area, plant weight and the number of legumes increased significantly with the addition of combined nitrogen compared to plants not fertilised with nitrogen. These results are in accordance with the recent findings of Nanjappan *et al.* (2007), Sholkamy *et al.* (2012) and Al-Noaim & Hamad (2004) who established that cyanobacteria improved the growth, respectively, of wheat, maize and rice grown in pot experiments. Additionally, the stimulatory effect of tested cyanobacteria on the growth of the tested plants agrees with the earlier findings of Obreht *et al.* (1993) and Rai *et al.* (2000) who established that strains of *Nostoc* and *Anabaena* positively affect plant growth by making a high contribution to N_2 fixing activity in liquid and soil cultures. Growth stimulation by cyanobacteria isolates could be attributed to production of auxins / bioactive molecules (Sergeeva *et al.*, 2002), production of secondary metabolites linked to the bio-control of bacterial and fungal diseases (Biondi *et al.*, 2004) or improvements to soil structure through secretion of mucilage and polysaccharides assisting soil aggregation (Gantar *et al.*, 1993; Aziz & Hesham, 2003 and 2004; Nisha *et al.*, 2007). These results also showed that inoculation of soybean plants with *N. muscorum* isolate had a stronger positive effect on the growth of these plants than inoculation with *N. rivulare*. The second main finding of our study was that the combination of biofertilization (*N. rivulare* and *N. muscorum)* and N-fertilization (at half and full doses) significantly increased the growth of soybean plants, more than those of control non-inoculated and fertilized plants. These results are in accordance with previous observations by Al-Noim & Hamad (2004); Rekhi *et al.* (2000) who established that the combination of biofertilization and N-fertilization at 180 Kg/ha significantly increased the growth of rice as measured by leaf area, plant height and grain yield. The results also substantiate the findings of Nayak *et al.* (1986); Wang (1986), and accord with the observations of Sholkamy *et al.* (2012) who established that biofertilization by *Nostoc muscorum* or *Nostoc rivulare* significantly increased shoot length and leaf area of maize, either alone or in combination with N-fertilizer at 50 and 100 kg N/ha. The combination of biofertilization and N-fertilization, especially at 100 kg N/ha had more effect on the growth of maize and on nitrogenase activity compared to biofertilization alone. Similarly, Hussein & Radwan (2001); Bassal *et al.* (1996); Kreem (1993) established that increasing nitrogen application rates increased the number of tillers/ hill, number of filled grains/ spike, 1000 grain weight and grain yield of wheat plants. Similar conclusions were reported by Singh *et al.* (1992); Wang (1986) who observed that *Azolla* biomass in the soil increases rice stem height and the number of spikes/ hill over the control. The stimulatory effect of the addition of N-fertilizer on nitrogen fixing activity is due to its stimulation of root growth, as well as the stimulation of the growth of the cyanobacterial population in soil cultivated with soybean. Similar conclusions were reported by Jha *et al.* (2001); Jha & Prasad (2007) who established that chemical fertilizers at recommended doses caused increases in cyanobacterial population and nitrogenase activity in paddy soil. The results of the current study showed that N-fertilization at half and full doses increased cyanobacterial abundance (MPN/ g soil) in soil cultivated with soybean plants. Similar conclusions had been reported by Nanjappan *et al.* (2007) showing that biofertilization by cyanobacteria promoted wheat plant growth, and also that the application of N-fertilizer at the recommended level stimulated the growth of the diazotrophic cyanobacterial population in tested soil. Similar conclusions were reported by Roger & Reynaud (1977) who observed higher N_2 fixing cyanobacteria biomass in fertilized pots than unfertilized pots.

Conclusion

It can be concluded that *Nostoc muscorum* and *Nostoc rivulare* could be used as renewable biofertilizers for improvement of soybean growth and decreasing use of N-fertilizers

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References

Abdel-Hafez, S.I.I., A.M. Moharram and M.A. Abdel-Sater. 2000. Monthes variations in the mycobiota of Wheat fields in El karga Oasis Western desert, Egypt. *Bull. Faculty of Sci. Assuit Uni.*, 29(2-D): 195-211.

Ahamd, M., Z.A. Zahir, S.M. Nadeem, F. Nazli, M. Jamil and M.U. Jamshaid. 2014. Physiological response of mung bean to Rhizobium and Pseudomonas based biofertilizers under salinity stress. *Pak. J. Agri. Sci*., 51: 557-564.

Al-Noaim, A.A. and H.S. Hamad. 2004. Effect of Bio-fertilization along with different levels of nitrogen fertilizer application on the growth and grain yield of hassawi rice (*Oryza saiva* L.). *Sci. J. of King Faisal Uni.*, pp. 215-225.

Aziz, M.A. and M.A. Hashem. 2003. Role of cyanobacteria in improving fertility of saline soil. *Pak. J. Bio. Sci.*, 6(20): 1751-1752.

Aziz, M.A. and M.A. Hashem. 2004. Role of cyanobacteria on yield of rice in saline soil. *Pak. J. Bio. Sci.*, 7: 309-311.

Bassal, S.A., A.M. Abd El-All, E.O.I. Metwally and E.K. El-Habbak. 1996. Growth and yield of rice in relation to winter preceding crops and N-fertilizer levels. *J. Agri. Sci.*, 21: 79-88.

Bergman, B., C. Johansson and E. Sosderbasck. 1992. The Nostoc- Gunnera symbiosis. *New Phytolo.*, 122: 379-400.

Biondi, N., R. Paccardi, C.M. Margheri, L. Rodofi, D.G. Smith and R.M. Tredici. 2004. Evaluation of *Nostoc* strain ATCC 53789 as a potential source of natural pesticides. *Appli. and Envir. Microbiol.*, pp. 3313-3320.

Braun-Howland, E.B. and S.A. Nierzwicki-Bauer. 1990. *Azolla-Anabaena* symbiosis: biochemistry, physiology, ultrastructure and molecular biology. In: *CRC Handbook of symbiotic cyanobacteria*. (Ed.): A.N. Rai, Boca Raton, FL, USA: CRC Press Inc., pp. 65-117.

Burris, R.H. 1994. Biological nitrogen fixation-past and future. In: *Nitrogen fixation with non-legumes.* (Eds.): N.A. Hegazi, M. Fayez and M. Monib, The American University in Cairo Press. Cairo, Egypt.

Desikachary, T.V. 1959. Cyanophyta. I.C.A.R. Monographs on algae. Indian council of agriculture research publications, New Delhi, pp. 686.

Dixon, R.O.D. and C.T. Wheeler. 1986. Nitrogen fixation in plants. Blackie, Glasgow, United kingdom.

El-Shahed, A.M. 2005. Interaction of 2,4-D with a nitrogen fixing *Nostoc rivulare* Kutzing associated with wheat plants. *Bull. Faculty of Sci., Assiut Uni.*, 447-466.

Gantar, M., N.W. Kerby and P. Rowell. 1993. Colonization of wheat (*Triticum vulgare* L.) by N2-fixing cyanobacteria: III. The role of a hormogonia promoting factor. *New Phytolo.*, 124: 505-513.

Golterman, H.L., R.S. Clymo and M.A.M. Ohnstad. 1978. Methods for physical and chemical analysis of fresh waters. Blackwell Scientific publications, Oxford, pp.153.

Goyal, S.K. 1993. Algal biofertilizer for vital soil and free nitrogen. *Ind. Nat. Sci. Acad.*, 59(4):295-301.

Hardy, R.W.F., B.C. Burns and R.D. Holsten. 1973. Application of the acetylene-ethylene assay for measurement of nitrogen fixation. *J. Soil Biol. & Biochem.*, 5: 47-81.

Hussein, S.A. and A.M.S. Radwan. 2001. Effect of biofertilization with different levels of nitrogen and phosphorus on wheat and associated weeds under weed control treatments. *Pak. J. Bio. Sci.*, 4: 435-441.

Jha, M.N. and Prasad, A.N. 2007. Efficacy of New inexpensive cyanobacterial biofertilizer including its shelf-life. *World J. Microbiol. and biotech.*, 22: 73-79.

Jha, M.N., A.N. Prasad, S.G. Sharma and R.C. Bharati. 2001. Effects of fertilization rate and crop rotation on diazotrophic cyanobacteria in paddy field. *World J. Microbiol. and biotechnol.*, 17: 463-468.

Kreem, M.K.E. 1993. Effect of different nitrogen rates and comp nitrification inhibitor on growth and yield of rice. *J. Agri. Res.*, 19: 525-536.

Ladha, J.K. and P.M. Reddy. 2003. Nitrogen fixation in rice systems: state of knowledge and future prospects. *Plant Soil*, 252: 151-167.

Metzner, H., H. Rau and H. Senger. 1965. Untersuchungen Zur synchronisierbar keiteinzelner pigment mangel-mutanten von *Chlorella*. *Planta*, 65: 186-194.

Nanjappan, K., P. Radha, N. Lata and D.K. Brahama. 2007. Evaluation the potential of plant growth promoting cyanobacterial as inoculants for wheat. *Eur. J. Soil Biol.,* 43: 23-30.

Nayak, D.K., K.J. Ladha and J. Watanable 1986. The fate of marker *Azospirilum lipoferum* inoculated into rice and its effect on growth, yield and N_2 fixing plants studied by acetylene reduction N^{15} feeding and N^{15} dilution technique. *Biol. and Fert. Soils*, 2: 7-14.

Nisha, R., A. Kaushik and P.C. Kaushik. 2007. Effect of indigenous cyanobacterial application on structural stability and productivity of an organically poor semi-arid soil. *J. Geoderma*, 138: 49-56.

Norman, J.M. and G.S. Campell. 1994. Canopy structure In: *Plant physiological ecology.* (Eds.): R.W. Peary, J. Ehleringer, H.A. Mooney and P.W. Rundel, Chapman and Hall, London, pp. 301-326.

Obreht, Z., N.W. Kerby, M. Gantar and P. Rowell. 1993. Effect of root-associated N2-fixing cyanobacteria on the growth and nitrogen content of wheat (*Triticum vulgare*) seedlings. *Biol and Fert. Soils*, 15: 68-72.

Omar, H.H. 2000. Nitrogen-fixing abilities of some Cyanobacteria in sandy loam soil and exudates efficiency of rice grain germination. *Egypt. J. Phycol.*, 1: 157-167.

Peoples, M.B., D.F. Herridge and J.K. Ladha. 1995. Biological nitrogen fixation; an efficient source of nitrogen for sustainable agriculture. *Pl. soil*, 174: 3-38.

Rai, A.N., E. Soderback and B. Bergman. 2000. Tinsley Review: cyanobacterium–plant symbioses. *New Phytolo.*, 147: 449-481.

Rekhi, R.S., K.D. Benbi and B. Singh. 2000. Effect of fertilizers an organic manure on crop yields and soil properties in rice- wheat cropping system. In: *Long-term soil fertility experiment in rice-wheat cropping systems.* (Eds.): I.P. Abrol, K.F. Bronson, J.M. Duxbury and R.K. Gupa, Rice-Wheat consortium paper series 6 Rice-Wheat consortium for the Indo-Ganetic plains, New Delhi, India, pp. 1-6.

Rippka, R. and M. Herdman. 1992. Pasteur Culture Collection of Cyanobacterial Strains in Axenic Culture. Catalogue of strains. Institut Pasteur, Paris, France, (1): pp. 103.

Roger, P.A. and P.A. Reynaud. 1977. Algal biomass in rice fields of Senegal; relative importance of N_2 fixing blue green algae. *Rev. Ecol. and Biol.*, 14: 519-530.

Schoot, A.J.M. 1990. Nitrogen cycling and human intervention, In: *Nitrogen fixation: achievement and objectives.* (Eds.): P.M. Gresshoff, L. Roth, E.G. Stacey and W.E. Newton, Chapman and Hall, New York, pp. 55-66.

Schwarzenbech, G. and W. Biederman. 1948. Komplexone. X. Erdalkali-komplex von 0,6-Dioxyzofarbstoffen. *Helvetica Chimica Acta*, 31: 678-687.

Sergeeva, E., A. Liaimer and B. Bergman. 2002. Evidence for production of the phytohormone indole-3-acetic acid by cyanobacteria. *Planta*, 38(2): 215-229.

Sholkamy, E.N., H. El-Komy, A.A. Al-Arfaj, A. Abdel-Megeed and A.A. Mostafa. 2012. Potential role of *Nostoc muscorum* and *Nostoc rivulare* as biofertilizers for the enhancement of maize growth under different doses of n-fertilizer. *Afri. J. Microbiol. Res.*, 6(48): 7435-7448.

Singh, A.L., K.P. Singh, P.V. Pandey, K.A. Dubey and C.D. Veemd. 1992. Effect of blue green algae inoculation on yield of paddy rice in different districts of U. P. *News Agri.*, 3: 189-192.

Sprent, J.I. and P. Sprent. 1990. Nitrogen fixing organisms. Pure and applied aspects. Chapman and Hall, London, United kingdom.

Steel, R.G. and J.H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hall Book Co., New York.

Vaishampayan, A., R.P. Sinha, D.P. Hader, T. Dey, A.K. Gupta, U. Bhan and A.L. Rao. 2001. Cyanobacterial biofertilizers in rice agriculture. *Bot. Rev.*, 67(4): 453-516.

Wang, Z.H. 1986. Rice yield increased effects of *Azolla* a culture in winter and spring. *Zhegiang Agri.*, 4:155-157.

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