2 *Egypt. J. Agron.* **Vol. 38,** No. 1, pp. 23- 54 (2016)

The Interaction Effect of Biofertilization and Nitrogen Fertilization Level on Growth and Yield Attributes of Rice

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THIS INVESTIGATION was carried out in the Experimental Farm, Ghazala Village, Fac. of Agric., Zagazig Univ., El-Sharkia Governorate, Egypt, during two successive seasons (2014 and 2015) to find out the effect of biofertilization with Cyanobacteria (*Anabaena oryzae*, *Anabaena* extract and the *Anabaena* + *Anabaena* extract) under four nitrogen levels (0, 25, 50 and 75 kg N/ fad[*]) on growth and yield of rice (Giza 178). The obtained results could be summarized as follows: biofertilization was effective to increase significantly grain, straw and total yields/ fad particularly when *Anabaena* extract was tried through rice foliage at 10 and 25 days after transplanting. Mineral N fertilization failed to increase the rice grain yield/ fad and almost all yield attributes at harvest where the check N treatment recorded at par averages as those recorded due to addition of 50 kg N/ fad where the further increase in N level was followed by significant decrease in this respect. However, plant height and physiological attributes at heading were significantly increased due to the increase of N level to 50 kg N/ fad. The bio. X mineral fertilization interaction affected grain yield/ fad and almost all yield attributes where the response equations predicted maximum grain yield/ fad of 4.26 ton due to a predicted addition of 54.1kg N/ fad. According to these results, a complementary effect could be traced between the foliar application of *Anabaena* and the 50 kg N/ fad level to maximize the grain, straw and total yields/ fad. An eutrophication effect could had been taking place when *Anabaena* was soil added due to over growth by the algae particularly, during rice grain filling where a possible toxic effect and nutrient imbalance could have had played a role in ceiling the favourable effect of biofertilization observed up to heading particularly on plant height and greeness of leaves which was expressed in their content from chlorophyll and carotniods.

Keywords: Rice, Biofertilization, *Anabaena*, Eutrophication, Nitrogen fertilizer.

Rice (*Oryzae sativa* L.) is an important cereal crop which ranks the second after wheat. In Egypt, the cultivated area of rice reached 1.67 millions fad., in 2013 season, which produced 6.75 millions ton (FAOSTAT, 2013). Several studies have been carried out to find the effect of biofertilization on growth , yield and yield attributes which showed significant increase in growth and grain yield of rice. The use of mineral N fertilizers in rice fields is expensive, disturbs the

[*] fad = 4200 m^2 and ha = 2.4 fad

equilibrium of agro-ecosystem, and causes pollution to the environment. Hence, the use of biofertilizers might play important role in minimizing these problems. Cyanobacteria, particularly *Anabaena* was reported to enhance rice plant growth directly and/or indirectly. The direct effects include the production of a number of plant growth- promoting biologically active substances including phytohormones, such as auxin , gibberellins and cytokinins. The indirect promotion effects on plant growth occurs when cyanobacteria prevent or counter deleterious effects of one or more of phytopathogenic microorganisms (Prasanna *et al*., 2010). Also, Whitton (2000) reported that rice significantly responded to cyanobacterial inoculants, because the rice field submerged conditions provide an ideal environment for the luxurious growth of blue–green algae. Abd El-All *et al*. (2013) reported that, yield components, in term of rice grain and straw yields and weight of 1000 grains were significantly increased by using biofertilization encompassing *Azolla*- cyanobacteria- bacteria. Sathish & Bhaskara (2012) mentioned that, *Azolla*, cyanobacteria and bacteria have been used to successfully increase rice yield. Cyanobacteria, as natural biofertilizer, play an important role in maintenance and build-up of soil fertility, consequently increase rice growth, yield and yield attributes (Hossain *et al*., 2001; Song *et al*., 2005; Paudel *et al*., 2012; Farah *et al*., 2014 and Neni *et al*., 2014). Moreover, Pereira *et al.* (2009), found, significant increase in photosynthetic pigments as a result of biofertilizer treatments which may be due to the role of nitrogen in the increase of photosynthetic activity of the chloroplast.

Mineral fertilization with nitrogen was also reported to increase growth and grain yield of rice. Uddin *et al*. (2013), Abd El-Rahman (2002) and Sharief *et al*. (2006), reported that, the increase of nitrogen levels to 30, 40 and 45 kg N/ fad, respectively significantly increased growth, yield and yield attributes. Also, Metwally *et al*. (2011), mentioned that, panicle weight, number of panicles/ hill, number of filled grains/ panicle, as well as, grain yield were increased due to the increase of N level up to 83 kg N/ fad but, the harvest index was decreased. Moreover, Abou-Khalifa (2012) reported that, maximum rice growth, yield and yield attributes of rice were recorded with the highest level of 91.7 kg N/ fad.

Therefore the present study seeks answers for the following two questions: 1. Is it possible to biofertilizer rice crop without any mineral fertilization. 2. Does biofertilization help in minimizing the use of mineral fertilization. Accordingly, the biofertilization treatments included inoculating rice field through soil application with *Anabaena* , full foliar spraying of rice foliage twice with *Anabaena* extract or biofertilization partly through soil application and foliar spray of *Anabaena* extract. To test the effect of these biofertilization treatments a check without biofertilization and four mineral N levels were tried. The effect of these two factors and their interaction were traced on some growth and physiological attributes at rice heading and grain yield and its attributes at harvest.

Materials and Methods

The present study was conducted in the Agric. Experiment Station (Ghazala Village), Fac. Agric., Zagazig Univ., Egypt, during two successive seasons (2014 and 2015) to find out the response of rice to three biofertilization treatments (*Anabaena oryzae*, *Anabaena oryzae* extract and foliar spray of the anabaena extract) compared with a check without biofertilization under four nitrogen levels (0, 25, 50 and 75 kg N/ fad) on growth, physiological attributes, yield and it's attributes of rice (Giza 178). Biofertilization treatments were applied as follows: *Anabaena* soil application at 10 days from transplanting (40 DAS), foliar spray of the *Anabaena* extract at 10 and 25 days from transplanting (40 and 55 DAS) and the combination of the two former treatments. Cyanobacteria (*Anabaena oryzae*) inoculuin was provided by Microbiology Department, Fac. Agric., Zagazig University. Nitrogen fertilizer was applied as urea (46.5% N) in three splits; *i.e.*, ⅓ was applied in nursery (20 DAS), ⅓ was applied at 10 days after transplanting (40 DAS) and ⅓ was added at panicle initiation (55 DAS). The preceding crop was Egyptian clover (one cut variety). A split plot design of four replications was used, where the biofertilization treatments were allocated in the main plots whereas N fertilization levels were allocated in the sub plots (12 m^2). Phosphorus at a level of 15.5 kg P_2O_5/fad, as ordinary superphosphate (15.5% P_2O_5) was band placed at the time of seeding. Rice seedlings were transplanted at 30 DAS in hills 20 x 20 cm apart, seeding was on 3^{rd} of June in the two seasons.

At heading, flag leaf area (cm^2) was measured according to Yoshida *et al*. (1976). The photosynthetic pigments (chlorophyll a, b and carotenoids) were extracted from fresh leaf sample by pure acetone according to Fadeel' s method (Fadeel, 1962). The pigments were filtered, the optical density of the filtrate was determined spectro-photo-chemically using the wave length 662, 644, 440.5 nm for Chl. a, Chl. b and carotenoids, respectively. The pigments (as mg/g fresh weight) were calculated using the formula adapted by Wettestein (1957) as follows:

Chl. a = (9.784 x E 662) – (0.99 x E 644): in mg / liter.

Chl. b = (21.426 x E644) – (4.65 x E 662): in mg / liter.

Carot. = (4.695 x E 440.5) – (0.268 x chl.a+chl.b): in mg/ liter.

where: E is the reading of the optical density at given wave length. The concentrations of pigments were then expressed in mg/g fresh weight of leaves. Carbohydrate fractions were determined in the dried samples of shoots and grains of all treatments photometrically according to Bernfeld (1955) and Miller (1959) methods with some modifications. One gram of 3 , 5 dinitro salicylic acid was dissolved in 20 ml of 2N NaOH , then 50 ml distilled water and 30 gm of Rochelle salt were added and the mixture was shaken well until dissolving the salt , then the volume was made up to 100 ml with distilled water .One tenth gram of dry shoot and grains of wheat powder with 20 ml of 6 N HCl were taken in a carbohydrate tube, then the samples were heated for 6 h in a boiling water bath then filtered using whatman paper No.1. Twenty ml of 6 N NaOH were added to the filtrate for neutralization, then made up to 100 ml with distilled water. Five ml from the filtrate were added to 2 ml of color reagent in a test tube, shaked well and heated exactly for 10 min in a boiling water bath then cooled under running tap water. The color intensities were measured colorimetrically at 550 nm using spectronic-20 spectrophotometer. From the filtrate of the above mentioned reducing sugars, 10 ml were taken in a carbohydrate tube, 5 ml 6 N HCl were added and incubated for 2 h in water bath. After incubation, 5 ml of 6 N NaOH were added for neutralization and 2 ml of the color reagent were mixed as previously mentioned in case of total carbohydrate. Chemical determination of nitrogen, phosphorous and potassium, concentrations in the different plant organs (shoots and grains) were carried out using the acid digest of the dried wheat organs according to Piper (1947). Total nitrogen concentration in shoots and grains for wheat were determined colorimetrically using Nesslar solution according to the method described by Naguib (1963). Total phosphorus concentration in shoot and grains for wheat were determined colorimetrically by the hydroquinone method as described by Snell & Snell (1954) calculated as percentage (%). The potassium concentration in shoots and grains for wheat were determined using a Carlzeiss Flamphotometer with acetylene burn as described by Brown & Lilleland (1946) calculated as percentage (%).

At harvest (end of August in the two seasons), the number of panicle per m^2 and the following yield attributes were recorded on ten panicles, *i.e.*, panicle length, filled grains no./ panicle, unfilled grains no./ panicle, panicle grain weight and 1000 grain weight. Also, the following final yield traits were recorded from a central area of 2 m^2 per plot: grain yield at grain moisture content of 14 %, total yield, straw yield (ton/fad) and harvest index (%). Soil samples were collected from the experimental sites at the depth of 0 - 30 cm before sowing to determine soil physical and chemical properties.

Data were statistically analyzed according to Gomez & Gomez (1984) by using MSTAT-C (1989) where statistical program Version 2.1 was used for analysis of variance (ANOVA). A combined analysis was undertaken for the data of the two seasons after testing the homogenity of the experimental errors. Duncan Multiple range test was used to compare statistical significant differences (Duncan, 1955). In interaction Tables, capital and small letters were used to denote significant differences among rows and columns means, respectively. Interaction Tables are provided with response equations to compare the response of rice yield and its attributes to the increase of N level at the different biofertilization treatments. The predicated maximum trait average ($\hat{Y}_{max}$) which could have been obtained due to the addition of the predicted maximum N level (X_{max}) are also included. The response equations were calculated according to Snedecor & Cochran (1967) using the orthogonal polynomial Tables. The significancy of the linear and quadratic components of each of these equations was tested, then the response could be described as linear (first order) or quadratic (second order). The predicted maximum averages ($\hat{Y}_{max}$) which could have been obtained due to the addition of the predicted maximum N level (X_{max}) were calculated according to Neter *et al*. (1990) as explained by Abdul Galil *et al*. (2003) using the following equations:

$$\hat{Y}_{max} = \hat{Y}_0 + b^2 / 4c \qquad X_{max} = X_0 + b / 2c \ (u)$$

where: $\hat{Y}_0$ = Grain yield at the lowest N level , *i.e.* zero kg N/ fad (ton/ fad).
b = Measures the linear component of the response equation.
c = Measures the quadratic component of the response equation.
u = unit of N = 25 kg N/ fad.

Data in Table 1 show some soil physical and chemical properties of the experimental field in the two seasons.

TABLE 1. Soil mechanical and chemical analyses of the experimental sites at 30 cm soil depth in the two seasons.

Properties	2014	2015
Soil analysis:		
Texture:	Clay	Clay
Sand (%)	24.64	20.61
Silt (%)	29.51	31.82
Clay (%)	45.85	47.57
pH (soil susbintion)[a]	7.95	8.02
EC (dS m^{-1})[b] 1: 5 solution	1.82	1.94
Field capacity (FC),%	26.43	25.92
Available N (mg kg^{-1})	60.5	57.32
Available P (mg kg^{-1})	9.74	8.15
Available K (mg kg^{-1})	146.9	149.3
Organic matter (%)	1.05	1.03

a: Soil susbintion b: Soil extract
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Results and Discussion

Growth and physiological attributes of rice

Plant height

Biofertilization effect : Effect of inoculation with *Anabaena* through soil or spraying with *Anabaena extract* either alone or in combination with application mineral N-fertilizer on plant height of rice plants is given in Table 2. Data showed significant differences among bio-fertilization treatments in plant height where the tallest plants were obtained for treatment including inoculation with *Anabaena* through soil along with spraying with *Anabaena* extract. In this respect, Whitton, (2000) reported that rice significantly responded to cyanobacterial inoculants, as the rice field conditions provide an ideal environment for the luxurious growth of blue–green algae. The enhancement in plant elongation of rice could be due to the excretion of growth promoting regulators, vitamins, amino acids, polysaccharides, antifungal and antibacterial compounds etc. (Roger *et al.*, 1993 and Adam, 1999).

TABLE 2. Plant height, Ch. a and Ch. b of rice as affected by biofertilization and nitrogen fertilization level and their interactions in the two seasons.

Main effects and interactions	Plant height			Ch. a			Ch. b		
	2014	2015	Combined	2014	2015	Combined	2014	2015	Combined
Biofertilization treatments (B):									
Control	96.89 c	96.60 d	96.74 d	1.409 d	1.394 d	1.401d	0.277d	0.246 d	0.271d
Anabaena oryzae	99.35 b	99.04 b	99.19 b	1.589 b	1.570 b	1.579 b	0.330 b	0.316 b	0.323 b
Anabaena oryzae extract	95.17 d	97.92 c	98.04 c	1.458 c	1.444 c	1.451 c	0.311 c	0.296 c	0.304 c
Anabaena + *Anabaena* extract	100.53 a	100.13a	100.33 a	1.625 a	1.603 a	1.614 a	0.377 a	0.365 a	0.371 a
F.test	**	**	**	**	**	**	**	**	**
Nitrogen level (N):									
Check	92.89 d	92.61 d	92.75 c	1.264c	1.244 c	1.254 c	0.194 d	0.180 d	0.187 d
25 kg N/ fad	97.81 c	97.95 b	92.08 d	1.487b	1.469 b	1.478 b	0.310 c	0.296 c	0.303 c
50 kg N/ fad	106.04 a	105.59 a	105.82 a	1.835a	1.823 a	1.829 a	0.472 a	0.457 a	0.465 a
75 kg N/ fad	98.21 b	97.53 c	97.67 b	1.494b	1.475 b	1.484 b	0.320 b	0.308 b	0.314 b
F.test	**	**	**	**	**	**	**	**	**
Interactions:									
B x M	**	**	**	**	**	**	**	**	**

* indicate significancy at 0.05 levels of differences.
** indicate significancy at 0.01 levels of differences.
N.S. indicate insignificancy of differences.

Nitrogen level effect : Addition of 50 kg N/ fad gave the highest plant height average with significant difference among nitrogen levels (Table 2). The increase in plant height in this regard may be due to the activation of cell division and cell enlargement (Patil, 1985). Therefore, plant height increase in response to nitrogen application may be due to increase in availability of nitrogen which enhanced leaf area that resulted in enhanced photosynthesis which in turn enhanced dry matter accumulation in favour of plant elongation (Behind *et al.,* 2005). These results are supported by the findings of Rupp & Hubner (1995).

Interaction effect : According to the interaction between bio-fertilization and mineral nitrogen fertilization on plant it is interesting to mention that the inoculation with bio-fertilizer *Anabaena* through soil along with spraying with *Anabaena* extract in combination with the addition of 50 kg N/ fad significantly increased plant height where the highest average was attained followed by inoculation with *Anabaena* in combination with 50 kg N/ fad. The increase of plant height of rice plant obtained as a result of cyanobacterial inoculation combined with addition of 50 kg N/ fad are in agreement with previous findings such as Roger & Kulasooriya (1980) and Cong *et al.* (2009). The results obtained using a combination of bio-fertilizers and synthetic nitrogen fertilizer indicate clear economical and environmental advantages of adopting this agronomic practice.

Photosynthetic pigments

Biofertilization effect : The effect of mineral nitrogen, biofertilizers and their interaction on pigments, *i.e.* chlorophyll a, b and total chlorophyll (a + b) as well as carotniods in rice leaves are shown in Tables 2 and 3. It is clear from the data that inoculation with biofertilizer *Anabaena* through soil in addition with spraying *Anabaena* extract increased significantly chlorophyll a, b and total chlorophyll (a + b) as well as carotniods in rice leaves compared with the check without biofertilization. In this respect Panwar (2000) reported that inoculation with *Azospirillum brasilense* and *Bacillus subtilis* enhanced cholorphyll content of wheat plant. Also, Pereira *et al.,* (2009), found that the use of biofertilizers based on local strains of cyanobacteria was effective to increase the nitrogen use efficiency in rice. It seems reasonable to suggest that, significant increase in photosynthetic pigments a result of biofertilizer treatments may be due to the role of nitrogen in the increase of photosynthetic activity of the chloroplast.

Nitrogen level effect : It is conspicuous from the data that application of mineral nitrogen significantly increased chlorophyll a, b and total chlorophyll a + b as well as carotenoids as compared to the control (without fertilization), addition of 50 kg N/ fad gave the highest averages of photosynthetic pigments followed by 75 kg N/ fad (Tables 2 and 3). These results are in accordance with those found by Khalil (1982) who found that concentrations of chlorophyll a, b and total chlorophyll (a+b) in leaves tissue of tomato were increased gradually with increasing nitrogen application to the highest level, *i.e.* 150 kg/fed. In addition, application of nitrogen directly increased the chlorophyll content and leaf surface area resulting in increased photosynthesis process leading to more sugar formation (Dikshit & Paliwal, 1989). Nitrogen nutrition influences the content of photosynthetic pigments, the synthesis of the enzymes taking part in the carbon reduction, the formation of the membrane system of chloroplasts, etc. Thus the increase in growth owing to the application of N-fertilizers may be attributed to the fact that these nutrients being important constituents of nucleotides, proteins, chlorophyll and enzymes, involve in various metabolic processes which have direct impact on vegetative and reproductive phases of plants (Mengel & Kirkby,1996). Verma *et al*. (2004) recorded that the N content in the third leaf and chlorophyll a content increased with increasing nitrogen rate.

TABLE 3. Ch. a+b and carotniods of rice as affected by biofertilization and nitrogen fertilization level and their interactions in the two seasons .

Main effects and interactions	Ch. a+b			Carotniods		
	2014	2015	Combined	2014	2015	Combined
<u>Biofertilization treatments (B):</u>						
Control	1.658 d	1.656 d	1.671 d	1.294 c	1.279 c	1.286 d
Anabaena oryzae	1.918 b	1.885 b	1.901 b	1.386 b	1.371 b	1.379 b
Anabaena oryzae extract	1.768 c	1.739 c	1.753 c	1.356 b	1.340 b	1.348 c
Anabaena + *Anabaena* extract	2.000 a	1.965 a	1.982 a	1.529 a	1.549 a	1.539 a
F.test	**	**	**	**	**	**
<u>Nitrogen level (N):</u>						
Check	1.457 d	1.424 d	1.441 d	1.165 d	1.152 d	1.159 d
25 kg N/ fad	1.795 c	1.763 c	1.779 c	1.319 c	1.300 c	1.309 c
50 kg N/ fad	2.305 a	2.278 a	2.291 a	1.720 a	1.700 a	1.710 a
75 kg N/ fad	1.812 b	1.781 b	1.797 b	1.361 b	1.386 b	1.374 b
F.test	**	**	**	**	**	**
<u>Interactions:</u>						
B x M	**	**	**	**	**	**

* indicate significancy at 0.05 levels of differences
** indicate significancy at 0.01 levels of differences
N.S. indicate insignificancy of differences

Interaction effect : Regarding the interaction between bio-fertilizer and mineral nitrogen fertilizer significantly increased photosynthetic pigments, these result agree with Agamy (2004) who found that application of NPK in combined with biofertilizer increased chlorophyll content of sweet fennel. The changes in chlorophyll concentration might be ascribed to the influence of mineral and biofertilizers on the development processes leading to synthesis of chloroplasts and chlorophyll, and/or to effects on activities of chloroplast enzymes. Furthermore, the increase of chlorophyll concentration could be attributed to the increase of N and Mg concentration which known to be main components of chlorophyll molecule (Mohsen & Aly , 2004).

Carbohydrate fractions

Biofertilization effect : Data presented in Table 4 show the effect of inoculation with of *Anabaena* through soil and spraying with *Anabaena* extract either alone or in combinations together with application mineral N-fertilizer on total carbohydrate and total sugar in shoots of rice plants. It could be generally noticed that application of bio-fertilizer significantly increased total carbohydrate and total sugar of rice shoots as compared to the control plants inoculated with *Anabaena* through soil alone or in combined with *Anabaena* extract gave the best results of total carbohydrate and total sugar of rice shoots. These result agree with that found by Kavitha *et al.* (2013) who reported that total carbohydrate of the *Amaranthus tristis* was highly increased by application of *Azospirillum*. Also, Safinaz & Ragaa (2013) found that inoculation by *Laurencia obtusa, Jania rubens, Corallina elongata* either singly, and their combinations had significant potential as biofertilizers on sugar content of maize.

TABLE 4. Total carbohydrate and total sugar of rice as affected by biofertilization and nitrogen fertilization level and their interactions in the two seasons .

Main effects and interactions	Total carbohydrate			Total sugar		
	2014	2015	Combined	2014	2015	Combined
Biofertilization treatments (B):						
Control	27.40 c	27.22 c	27.31 c	20.06 c	19.95 b	20.01 c
Anabaena oryzae	28.01 b	27.70 b	27.85 b	20.27 b	20.24 a	20.26 b
Anabaena oryzae extract	27.99 b	27.90 b	27.94 b	19.96 d	19.92 b	19.94 d
Anabaena + *Anabaena* extract	28.56 a	28.46 a	28.51 a	20.45 a	20.32 a	20.54 a
F.test	**	**	**	**	**	**
Nitrogen level (N):						
Check	26.09 c	25.75 c	25.92 d	19.11 d	19.02 d	19.06d
25 kg N/ fad	27.46 b	27.37 b	27.41 c	20.11 b	20.06 b	20.09 b
50 kg N/ fad	30.73 a	30.51 a	30.62 a	21.61 a	21.57 a	21.58 a
75 kg N/ fad	27.67 b	27.46 b	27.66 b	19.52 c	19.49 c	19.50 c
F.test	**	**	**	**	**	**
Interactions:						
B x M	**	**	**	**	**	**

*indicate significancy at 0.05 levels of differences.
**indicate significancy at 0.01 levels of differences.
N.S. indicate insignificancy of differences.

Nitrogen level effect : Application mineral nitrogen significantly increased total carbohydrate and total sugar in shoots of rice plants as compared to control plants (zero nitrogen). In this respect addition of 50 kg N/ fad gave the highest averages of total carbohydrate while the highest averages of total sugar found in 50 kg N/ fad followed by addition of 25 kg N/ fad (Table 4). These results are in agreement with the findings of Gwal *et al.* (1999) who studied the effect of mineral fertilization on total carbohydrate of wheat plants. They found that total carbohydrate was increased with increasing mineral fertilizers rates.

The same trends were reported by Yakardi *et al.* (1992) on faba bean. The increases in carbohydrate by the increase of mineral N fertilizer may be attributed to the nitrogen which increased the capacity of plants to absorb nutrients by the increase of roots surface unit of the soil volume as well as the highest capacity of the plants supplied with nitrogen in building metabolites which in turn much contributed to the increase of nutrient uptake (Mandour *et al.,* 1986).

Interaction effect : Concerning the interaction effect of bio-fertilizer and mineral nitrogen fertilizer, generally the use of bio-fertilizer in combined with mineral nitrogen fertilizer significantly increased total carbohydrate and total sugar in shoots of rice plants and the best treatment was inoculation with *Anabaena* + spraying *Anabaena* extract + 50 kg N/fad. The increase in total carbohydrate as a result to mineral and biofertilizers was previously mentioned by Dessouky (2002) who studied the effect of mineral nitrogen, inoculation with *Azotobacter chroococum* on *Borago afficinalis* L. plant and found increase in total carbohydrate. The same results were found by Ebrahim & Aly (2004) on wheat.

N, P and K content

Biofertilization effect : Effect of inoculation with *Anabaena* through soil and spraying with *Anabaena* extract either alone or in combination together with application of mineral N-fertilizer on N, P, and K content of shoots of rice plants is given in Table 5. Data showed that significant differences among bio-fertilizer treatments in N, P, and K content while the best result of bio-fertilizer was obtained for treatment inoculated with *Anabaena* and spraying with *Anabaena* extract treatment followed by that inoculated with *Anabaena* treatment. In this respect, Safinaz & Ragaa (2013) found that inoculation by *Laurencia obtusa, Jania rubens, Corallina elongata* either singly, and their combinations have significant potential as biofertilizers on NPK content of maize plant. Also, Hanafy *et al.* (1997) suggested that addition of biofertilizers increased the ability to convert N_2 to NH_4 and thus make it available to plant. Also many investigators showed that inoculation of biofertilizers increased N concentration in potato, (Abd El-Ati *et al.,* 1996), and in wheat (El-Hawary *et al.,* 2007).

TABLE 5. N, P and K content in shoot of rice as affected by biofertilization and nitrogen fertilization level and their interactions in the two seasons.

Main effects and interactions	N %			P %			K %		
	2014	2015	Combined	2014	2015	Combined	2014	2015	Combined
Biofertilization treatments (B):									
Control	2.776 d	2.760 d	2.768 d	0.661 d	0.651 d	0.656 d	2.096 d	2.075 d	2.086 d
Anabaena oryzae	2.958 b	2.941 b	2.949 b	0.727 b	0.719 b	0.723 b	2.235 b	2.226 b	2.231 b
Anabaena oryzae extract	2.805 c	2.789 c	2.797 c	0.690 c	0.679 c	0.684 c	2.147 c	2.134 c	2.141 c
Anabaena + *Anabaena* extract	3.179 a	3.159 a	3.169 a	0.764 a	0.757 a	0.761 a	2.346 a	3.322 a	2.334 a
F.test	**	**	**	**	**	**	**	**	**
Nitrogen level (N):									
Check	2.041 d	2.029 d	2.035 d	0.527 c	0.515 d	0.521 d	1.615 c	1.591 c	1.603 c
25 kg N/ fad	2.896 c	2.866 c	2.881 c	0.696 b	0.686 c	0.691 c	2.168 b	2.150 b	2.159 b
50 kg N/ fad	3.871 a	3.859 a	3.865 a	0.916 a	0.911 a	0.914 a	2.890 a	2.886 a	2.888 a
75 kg N/ fad	2.909 b	2.895 b	2.902 b	0.702 b	0.694 b	0.698 b	2.152 b	2.130 b	2.141 b
F.test	**	**	**	**	**	**	**	**	**
Interactions:									
B x M	**	**	**	**	**	**	**	**	**

* indicate significancy at 0.05 levels of differences.

** indicate significancy at 0.01 levels of differences.

N.S. indicate insignificancy of differences.

Nitrogen level effect : Application of 50 kg N/ fad gave the highest N, P, and K content averages with significant differences among nitrogen levels treatments (Table 5). These results are in agreement with the finding of Soares *et al.* (1985) who reported that increasing N fertilizer increased leaf N content of tomato plant. Similar findings were also reported by Fetouh & Abdel-Fattah (1996).

Interaction effect : Regarding the interaction between bio-fertilizer and mineral nitrogen fertilizer significantly increased N, P, and K content of shoots of rice plant.

Yield and yield attributes of rice

The yield and yield attributes of rice as affected by biofertilization treatments and N levels are presented in Tables 6, 7 and 8.

Flag leaf area, panicle length and number of filled and unfilled grains per panicle

Biofertilization effect : Biofertilization treatments did not reflect any significant effect on flag leaf area, panicle length and number of unfilled grains per panicle in both seasons and number of filled grains per panicle in the second season. However, the combined analysis detected significant increase in flag leaf area and number of filled and unfilled grains per panicle due to the biofertilization treatments (Table 6). The use of the *Anabaena* through soil recorded the highest flag leaf area average but decreased the number of filled and unfilled grains per panicle as compared to the check treatment. Also, the use of the anabaena + extract treatment or the check treatment recorded at par higher averages for the of filled and unfilled grains per panicle. These results refer to failure of biofertilization as expressed in the significant decrease of the number of filled grains per panicle due to the use of *Anabaena* through soil and the equality of the check treatment average with the biofertilization one. The decrease in the number of unfilled grains per panicle due to the foliar application of *Anabaena* extract might refer to some beneficial effect of the use of *Anabaena* through rice foliage rather than soil biofertilization.

Nitrogen level effect : Significant differences could be detected in flag leaf area, panicle length and number of filled and unfilled grains per panicle in both seasons and their combined. The flag leaf area was significantly increased due to the increase of N level to 25 kg N/ fad. Similar effect was observed in panicle length and number of filled and unfilled grains per panicle where the further increase in N level did not add a further significant increase in these averages (Table 6). The increase of the N level beyond 25 kg N/ fad might have had caused an increase in the number of fertile florets (sink size) per panicle which was over the capacity of the rice plant (source) where a large number had been failed to fill all grains per panicle. Similar results were reported by Hossain *et al*. (2001), Abd El-Rahman *et al*. (2002), Sharief *et al*.

(2006), Metwally *et al*. (2011), Paudel *et al*. (2012), Uddin *et al*. (2013) but, these results are not in accordance with those reported by Abou-Khalifa (2012) and Zayed *et al*. (2013) as they reported more response to added N than that observed herein.

Interaction effect : According to the combined analysis, flag leaf area (Table 6-a), panicle length (Table 6-b), number of filled and unfilled grains per panicle (Tables 6-c and 6-d) were significantly affected by the biofertilization treatments x N level interaction. It is quite clear from Tables 6-a to 6-d that, the flag leaf area, panicle length and number of filled and unfilled grains per panicle tended to increase significantly with the increase of N level but, with different magnitudes which varied according to the biofertilization treatment. A look in Table 6-a regarding this interaction effect on the flag leaf area showed linearity in the increase of flag leaf area due to the increase of N level in the check and the mixture treatments, however, quadratic increase in the soil biofertilization treatment and the foliar application treatment where this area could be maximized due to a predicted maximum additions of 41.41 and 34.08 kg N/ fad where the flag leaf area could be maximized to 28.65 and 27.27 cm^2, respectively. It is evident from Table 6-b that, the response equations showed positive diminishing increase with the increase of N level. For the foliar application treatment which recorded the highest average, panicle length could be maximized to 23.26 cm due to the addition of a predicted maximum N level of 40.98 kg N/ fad compared with 46.69 kg N/ fad needed to maximize the panicle length to 23.16 cm for the check. For the other two biofertilization treatments, higher N levels were predicted to maximize the panicle length. Moreover, It is evident from Table 6-c that, the interaction effect on the number of filled grains per panicle showed linearity in the increase of this number due to the increase of N level in the foliar application treatment and the mixture treatment, however, quadratic increase in the check and the soil biofertilization treatments where this number could be maximized due to predicted maximum additions of 25.16 and 41.5 kg N/ fad where these numbers could be maximized to 172.4 and 156.2, respectively. For the soil application treatment which recorded the lowest unfilled grains number/ panicle (Table 6-d) this number could be maximized to 14.64 due to the addition of a predicted maximum N level of 29.86 kg N/ fad compared with 39.05 kg N/ fad needed to maximize the number of unfilled/ panicle to 26.75 for the check. These interactions and the response equations clearly indicate the need of N but to a certain lower levels than the maximum tried herein (75 kg N/ fad). These lower N levels were more effective and efficient when combined with biofertilization particularly when tried fully through rice foliage.

TABLE 6. Flag leaf area, panicle length, filled and unfilled grains number per panicle of rice as affected by biofertilization and nitrogen fertilization level and their interactions in the two seasons.

Main effects and interactions	Flag leaf area (cm^2)			Panicle length (cm)			Filled grains no./ panicle			Unfilled grains no./ panicle		
	2014	2015	Combined	2014	2015	Combined	2014	2015	Combined	2014	2015	Combined
<u>Biofertilization treatments (B):</u>												
Control	21.16	20.76	20.96 ab	23.14	22.97	23.05	164.0 a	167.1	165.6 a	22.63	22.25	22.44 a
Anabaena oryzae	23.71	24.68	24.19 a	22.53	22.20	22.36	136.0 b	136.6	136.3 b	12.25	8.13	10.19 b
Anabaena oryzae extract	21.04	24.23	22.64 ab	22.05	22.89	22.47	154.4 ab	155.4	154.9 a	11.88	16.13	14.0 b
Anabaena + *Anabaena* extract	20.56	21.21	20.89 b	22.81	22.29	22.55	172.8 a	154.1	163.4 a	21.00	17.25	19.13 a
F.test	N.S.	N.S.	*	N.S.	N.S.	N.S.	*	N.S.	**	N.S.	N.S.	*
<u>Nitrogen level (N):</u>												
Check	20.50	19.86 b	20.18 b	22.23	21.81 b	22.02 b	157.5	142.8 b	150.1	13.50 d	11.75 d	12.63 c
25 kg N/ fad	22.36	26.89 a	24.62 a	22.50	23.23 a	22.86 a	148.4	163.5 a	156.0	21.50 a	21.88 a	21.69 a
50 kg N/ fad	21.96	23.21 b	22.59 a	22.91	22.91 a	22.91 a	157.8	160.0 a	158.9	18.00 b	17.38 b	17.69 b
75 kg N/ fad	21.65	20.93 b	21.29 ab	22.88	22.40 ab	22.64 a	163.5	147.0 a	155.3	14.75 c	12.75 c	13.75 c
F.test	N.S.	**	**	N.S.	**	**	N.S.	**	N.S.	**	**	**
<u>Interactions:</u>												
B x M	N.S.	**	**(6-a)	N.S.	N.S.	**(6-b)	N.S.	N.S.	**(6-c)	**	**	**(2-d)

*indicate significancy at 0.05 levels of differences.
**indicate significancy at 0.01 levels of differences.
N.S. indicate insignificancy of differences.

TABLE 6-a. Flag leaf area of rice as affected by the biofertilization treatments and nitrogen level interaction and the response equation, predicted maximums average and predicted maximum N levels (combined data).

Biofertilization treatments	N level				$\hat{Y} = a + bx - c\,x^2$	$\hat{Y}_{max}$	X_{max}
	Check	25 kg N/ fad	50 kg N/ fad	75 kg N/ fad			
Control	A 20.57 a	A 19.79 b	A 19.63 b	A 23.86 a	20.76 + 2.79 x	-	-
Anabaena oryzae	B 18.31 a	A 29.39 a	A 25.99 a	AB 23.09 a	19.06 + 11.58 x – 3.5 x^2	28.65	41.41
Anabaena oryzae extract	B 19.34 a	A 30.22 a	B 22.35 ab	B 18.64 a	20.49 + 9.95 x – 3.65 x^2	27.27	34.08
Anabaena + *Anabaena* extract	A 22.50 a	A 19.09 b	A 22.38 ab	A 19.58 a	21.86 + 1.0 x	-	-

$\hat{Y}_{max}$: predicted maximums average . X_{max}: predicted maximum N level.

TABLE 6-b. Panicle length of rice as affected by the biofertilization treatments and nitrogen level interaction and the response equation, predicted maximums average and predicted maximum N levels (combined data).

Biofertilization treatments	N level				$\hat{Y} = a + bx - c\,x^2$	$\hat{Y}_{max}$	X_{max}
	Check	25 kg N/ fad	50 kg N/ fad	75 kg N/ fad			
Control	A 22.88 a	A 23.13 ab	A 23.13 a	A 23.07 a	22.89 + 0.29 x – 0.08 x^2	23.16	46.69
Anabaena oryzae	B 21.44 b	A 22.63 ab	A 22.63 a	A 22.75 a	21.51 + 1.20 x – 0.27 x^2	22.84	55.86
Anabaena oryzae extract	B 21.44 b	A 23.44 a	A 22.75 a	AB 22.25 a	21.58 + 2.05 x – 0.63 x^2	23.26	40.98
Anabaena + *Anabaena* extract	A 22.32 ab	A 22.25 b	A 23.13 a	A 22.50 a	22.20 + 0.56 x – 0.14 x^2	22.76	50.18

$\hat{Y}_{max}$: predicted maximums average . X_{max}: predicted maximu N level.

TABLE 6-c. Filled grains number per panicle of rice as affected by the biofertilization treatments and nitrogen level interaction and the response equation, predicted maximums average and predicted maximum N levels (combined data).

Biofertilization treatments	N level				$\underline{\hat{Y} = a + bx - c\,x^2}$	$\underline{\hat{Y}_{max}}$	$\underline{X_{max}}$
	Check	25 kg N/ fad	50 kg N/ fad	75 kg N/ fad			
Control	A 169.0 a	A 172.8 a	A 168.8 a	A 159.8 a	$169.14 + 6.44\,x - 3.20\,x^2$	172.4	25.16
Anabaena oryzae	B 113.8 b	A 148.0 b	A 155.8 a	AB 127.8 b	$113.3 + 51.63\,x - 15.55\,x^2$	156.2	41.5
Anabaena oryzae extract	A 156.8 a	A 149.0 ab	A 157.0 a	A 156.8 a	$155.6 + 4.90\,x$	-	-
Anabaena + *Anabaena* extract	A 161.0 a	A 154.0 ab	A 162.0 a	A 176.8 a	$160.6 + 10.81\,x$	-	-

$\hat{Y}_{max}$: predicted maximums average. X_{max}: predicted maximum N level .

TABLE 6-d. Unfilled grains number per panicle of rice as affected by the biofertilization treatments and nitrogen level interaction and the response equation, predicted maximums average and predicted maximum N levels (combined data).

Biofertilization treatments	N level				$\underline{\hat{Y} = a + bx - c\,x^2}$	$\underline{\hat{Y}_{max}}$	$\underline{X_{max}}$
	Check	25 kg N/ fad	50 kg N/ fad	75 kg N/ fad			
Control	C 15.0 a	A 35.75 a	C 16.0 b	B 23.0 a	$18.36 + 10.74\,x - 3.44\,x^2$	26.75	39.05
Anabaena oryzae	B 8.75 b	A 18.0 b	B 9.0 c	B 5.0 c	$9.91 + 7.91\,x - 3.31x^2$	14.64	29.86
Anabaena oryzae extract	B 8.0 b	A 18.0 b	A 20.0 b	B 10.0 c	$7.8 + 15.8\,x - 5.0\,x^2$	20.28	39.50
Anabaena + *Anabaena* extract	B 18.75 a	B 15.0 b	A 25.75 a	B 17.0 b	$17.05 + 4.3\,x - 1.25\,x^2$	20.75	43.0

$\hat{Y}_{max}$: predicted maximums average. X_{max}: predicted maximum N level .

Panicles number per m^2 , 1000 grain weight and panicle grain weights

Biofertilization effect : Biofertilization was without significant effect on the number of panicles per m^2 in both seasons, however, the combined analysis detected a significant decrease in this number due to the combined biofertilization with *Anabaena* through soil and foliar of its extract through foliage compared with the rest of treatments and the check without biofertilization. Furthermore, biofertilization treatments had a significant effect on the 1000-grain weight in both seasons and their combined where this weight was significantly increased due to soil application or foliar spraying with *Anabaena* compared with the check without biofertilization (Table 7). This possibly explains the higher efficiency of the soil inoculation with *Anabaena* or its foliar sprays in increasing grain filling. Similar results were reported by Hossain *et al*. (2001), Wijebandara *et al*. (2008), Paudel *et al*. (2012), Farah *et al*. (2014) and Neni *et al*. (2014). However, in both seasons and their combined, the biofertilization treatments were without significant effect on panicle grain weight. These results are not in accordance with those reported by Abd El-All *et al*. (2013) who showed that, using biofertilizers alone significantly increased the weight of grains per panicle. The increase in 1000 grain weight observed herein compensated the decrease of their number where the grain weight per panicle was not significantly affected.

Nitrogen level effect : In both seasons and their combined, a significant increase could be detected in the number of panicles per m^2 and panicle grain weight due to the increase in N level (Table 7). However, N level did not significantly affect 1000-grain weight in both seasons but, the combined analysis detected a significant increase due to the increase N level up to 25 kg N/ fad. This N level recorded significantly higher panicle grain weight than the check though the highest number of panicles per m^2 was recorded due to addition of 75 kg N/ fad. These results are in harmony with those obtained by Sharief *et al.* (2006), Wijebandara *et al.* (2008), Metwally *et al.* (2011), Abou-Khalifa (2012), Zayed *et al.* (2013) and Farah *et al.* (2014).

Interaction effect : It is evident from Tables 7-a, 7-b and 7-c that the rice yield components were significantly increased with the increase of the N level but, with different magnitudes in the different biofertilization treatments. A look in Table 7-a regarding this interaction effect on the number of panicles/ m^2 showed linearity in the increase of this number due to the increase of N level in the soil biofertilization and the mixture treatments, but however, quadratic increase in the check and foliar application treatment where this number could be maximized due to predicted maximum additions of 53.65 and 44.11 kg N/ fad where the numbers could be maximized to 917.1 and 906.3 panicle/ m^2, respectively. In the full foliar biofertilization application treatment, the highest 1000-grain weight could have been maximized to 22.98 g due to the addition of a predicted maximum N level of 29.17 kg N/ fad compared with 28.04 kg N/ fad needed to maximize this weight to 21.93 g for the mixture treatment. Moreover, Table 7-b showed linearity in the increase of the 1000-grain weight due to the increase of N level in the check and the soil biofertilization treatments.

TABLE 7. Number of panicle per m^2, 1000-grains weight and panicle grain weight of rice as affected by biofertilization and nitrogen fertilization level and their interactions in the two seasons.

Main effects and interactions	Panicles no./ m^2			1000-grains weight (g)			Panicle grain weight (g)		
	2014	2015	Combined	2014	2015	Combined	2014	2015	Combined
Biofertilization treatments (B):									
Control	865.8	884.5	875.1 a	21.61 b	22.21 ab	21.91 b	3.32	3.41	3.37
Anabaena oryzae	880.0	901.6	890.7 a	22.64 a	23.0 a	22.82 a	3.14	3.06	3.10
Anabaena oryzae extract	893.9	868.6	881.4 a	22.79 a	22.55 a	22.67 a	3.32	3.42	3.37
Anabaena + *Anabaena* extract	820.5	836.1	828.3 b	21.25 b	21.41 b	21.33 c	3.86	3.31	3.58
F.test	N.S.	N.S.	**	**	*	**	N.S.	N.S.	N.S.
Nitrogen level (N):									
Check	842.3 b	801.6 b	821.9 c	21.78	21.83	21.80 b	3.30 ab	3.02 b	3.16 b
25 kg N/ fad	859.6 b	890.9 a	875.3 b	21.98	22.47	22.23 ab	3.22 b	3.45 a	3.34 a
50 kg N/ fad	842.4 b	889.3 a	865.8 b	21.94	22.24	22.09 ab	3.49 a	3.47 a	3.48 a
75 kg N/ fad	915.6 a	909.4 a	912.5 a	22.60	22.64	22.62 a	3.63 a	3.26 a	3.44 a
F.test	*	**	**	N.S.	N.S.	*	**	**	**
Interactions:									
B x M	**	N.S.	** (7-a)	**	N.S.	**(7-b)	**	N.S.	**(7-c)

* indicate significancy at 0.05 levels of differences.
** indicate significancy at 0.01 levels of differences.
N.S. indicate insignificancy of differences.

TABLE 7-a. Panicles number per m^2 of rice as affected by the biofertilization treatments and nitrogen level interaction and the response equation, predicted maximums average and predicted maximum N levels (combined data).

Biofertilization treatments	N level				$\hat{Y} = a + bx - c x^2$	$\hat{Y}_{max}$	X_{max}
	Check	25 kg N/ fad	50 kg N/ fad	75 kg N/ fad			
Control	B 800.0 ab	AB 887.8 a	A 912.8 a	A 900.0 a	$801.3 + 107.9\ x - 25.15\ x^2$	917.1	53.65
Anabaena oryzae	AB 900.0 a	AB 887.8 a	B 825.0 b	A 950.0 a	$911.9 + 94.18\ x$	-	-
Anabaena oryzae extract	A 850.0 a	A 887.8 a	A 912.8 a	A 875.0 a	$847.5 + 66.7\ x - 18.9\ x^2$	906.3	44.11
Anabaena + *Anabaena* extract	B 737.8 b	A 837.8 a	AB 812.8 b	A 925.0 a	$750.9 + 44.51\ x$	-	-

$\hat{Y}_{max}$: predicted maximums average . X_{max}: predicted maximum N level .

TABLE 7-b. 1000- grain weight of rice as affected by the biofertilization treatments and nitrogen level interaction and the response equation, predicted maximums average and predicted maximum N levels (combined data).

Biofertilization treatments	N level				$\hat{Y} = a + bx - c x^2$	$\hat{Y}_{max}$	X_{max}
	Check	25 kg N/ fad	50 kg N/ fad	75 kg N/ fad			
Control	AB 22.06 a	B 20.93 b	AB 22.23 a	A 22.42 b	$21.88 + 0.75\ x$	-	-
Anabaena oryzae	B 22.45 a	B 22.36 b	B 22.44 a	A 24.04 a	$22.52 + 0.78\ x$	-	-
Anabaena oryzae extract	AB 22.29 a	A 24.13 a	B 21.67 a	AB 22.61 b	$22.68 + 0.53\ x - 0.23\ x^2$	22.98	29.17
Anabaena + *Anabaena* extract	B 20.41 b	AB 21.48 b	A 22.02 a	AB 21.41 b	$20.38 + 1.61\ x - 0.42\ x^2$	21.93	28.04

$\hat{Y}_{max}$: predicted maximums average . X_{max}: predicted maximum N level .

TABLE 7-c. Panicle grain weight of rice as affected by the biofertilization treatments and nitrogen level interaction and the response equation, predicted maximums average and predicted maximum N levels (combined data).

Biofertilization treatments	N level				$\hat{Y} = a + bx - c x^2$	$\hat{Y}_{max}$	X_{max}
	Check	25 kg N/ fad	50 kg N/ fad	75 kg N/ fad			
Control	A 3.35 a	A 3.47 ab	A 3.49 a	A 3.17 b	$3.34 + 0.28\ x - 0.11\ x^2$	3.51	31.59
Anabaena oryzae	B 2.83 b	A 3.04 b	A 3.45 a	A 3.08 b	$2.80 + 0.55\ x - 0.15\ x^2$	3.30	47.5
Anabaena oryzae extract	B 3.03 ab	A 3.57 a	A 3.52 a	AB 3.37 b	$3.05 + 0.62\ x - 0.17\ x^2$	3.60	44.53
Anabaena + *Anabaena* extract	B 3.45 a	B 3.28 ab	B 3.45 a	A 4.16 a	$3.46 + 0.43\ x$	-	-

$\hat{Y}_{max}$: predicted maximums average . X_{max}: predicted maximum N level .

It is evident from Table 7-c that when biofertilization was tried, no significant increase in the panicle grain weight could be obtained beyond addition of 25 kg N except the mixture treatment which responded to addition of 75 kg N/ fad. This interaction effect showed linearity in the increase the panicle grain weight due to the increase of N level in the mixture treatment, however, quadratic increase in the soil biofertilization treatment and the foliar application treatments where this weight could be maximized due to predicted maximum additions of 47.5 and 44.53 kg N/ fad where the weight could have been maximized to 3.30 and 3.60 g, respectively. To summarize the effect of this interaction on the number of panicles per m^2, 1000 grain weight and grain weight per panicle, higher N levels were needed to maximize the averages of these yield attributes in the check biofertilized treatment than in the rest of the biofertilization treatments. Though different magnitudes of response to added N were detected where a complementary effect could be observed between mineral and biofertilization.

Grain yield/ fad

Biofertilization effect : Significant variations could be detected in the second season and were reflected in the combined analysis in the grain yield/fad. The use of the extract of *Anabaena* recorded the highest grain yield average whereas the rest of the biofertilization treatments and the control recorded at par lower averages in the second season. However, the combined analysis detected significant differences among the four biofertilization treatments where again the use of the extract recorded the highest average whereas the use *Anabaena* as soil biofertilization treatment recorded the lowest average. Also, the control and the *Anabaena* + extract treatments recorded at par intermediate averages (Table 8).

TABLE 8. Grain, straw and total yields per fad and harvest index of rice as affected by biofertilization and nitrogen fertilization level and their interactions in the two seasons.

Main effects and interactions	Grain yield (ton/ fad)			Straw yield (ton/ fad)			Total yield (ton/ fad)			Harvest index (%)		
	2014	2015	Combined	2014	2015	Combined	2014	2015	Combined	2014	2015	Combined
Biofertilization treatments (B):												
Control	4.15	3.58 b	3.87 b	5.43	4.59	5.01 b	9.58	8.17	8.88 b	43.49	44.01 b	43.75 b
Anabaena oryzae	3.55	3.38 b	3.46 c	4.48	4.44	4.46 b	8.03	7.82	7.92 b	44.66	44.02 b	44.34 b
Anabaena oryzae extract	4.31	4.37 a	4.34 a	5.56	6.0	5.78 a	9.87	10.4	10.1 a	43.85	42.61 b	43.23 b
Anabaena + *Anabaena* extract	4.08	3.86 b	3.97 b	4.86	4.43	4.65 b	8.94	8.29	8.61 b	45.60	46.38 a	45.99 a
F.test	N.S.	*	**	N.S.	N.S.	**	N.S.	N.S.	*	N.S.	*	**
Nitrogen level (N):												
Check	4.07 a	4.03 a	4.05 a	4.54 b	4.14 b	4.34 c	8.61 b	8.16 b	8.39 b	47.31 a	49.20 a	48.25 a
25 kg N/ fad	3.84 b	3.73 ab	3.78 b	5.19 a	5.66 a	5.42 a	9.03 ab	9.39 a	9.21 a	43.06 b	40.81 c	41.93 b
50 kg N/ fad	4.24 a	3.81 ab	4.02 a	5.54 a	5.0 b	5.27 a	9.77 a	8.80 ab	9.28 a	43.30 b	43.34 b	43.32 b
75 kg N/ fad	3.95 ab	3.62 b	3.78 b	5.07 ab	4.67 b	4.87 b	9.02 ab	8.29 ab	8.66 b	43.93 b	43.68 b	43.81 b
F.test	**	**	**	*	**	**	*	**	**	**	**	**
Interactions:												
B x M	**	**	**(8-a)	N.S.	*	**(8-b)	*	**	**(8-c)	*	*	**(8-d)

* indicate significancy at 0.05 levels of differences.
** indicate significancy at 0.01 levels of differences.
N.S. indicate insignificancy of differences.

These results are quite interesting as they clearly indicate the efficiency of certain biofertilization treatments in maximizing the grain yield of rice through foliar spray of the anabaena extract at 40 and 55 days after sowing. Soil application of *Anabaena*, however, decreased the grain yield and recorded lower grain yield/ fad than the control without biofertilization. These data refer to some benefits gained from foliar rather than soil biofertilization with anabaena either alone or in combination with anabaena extract through rice plant foliage.

Under certain conditions, soil use of algae might cause an eutrofication effect where the over growth of algae might make P unavailable and as well might reduce the content of water from dissolved oxygen in addition to causing clogging the water ways with weeds (Ferguson *et al* ., 1996). High N:P ratios with low soil silicon content was reported to cause toxic effects due to the use of algae Si to enhance their growth (Kaarstad *et al*., 1999). The soil of the present study, had a moderate level from available P (9.7 and 8.2 ppm, in the two seasons, respectively) and a high level from available N (60.5 and 57.3 ppm, in the two seasons, respectively) as shown in Table 1. This high soil N: P ratio along with a possible low level from Si (not determined) might have had enhanced the growth of algae and hence might have had decreased the availability of phosphorus. In addition the possible decrease of dissolved oxygen in water of submerged rice might have had played a negative role in the decrease of rice grain yield/ fad observed herein due to the use of blue green algae as soil treatment. The superiority of the foliar application of algae extract in maximizing the grain yield/ fad ascertain this view. It is worth to note down here, that the improvements of rice growth as expressed in the chlorophyll and carotniods contents of rice leaves at heading (Tables 2 to 5), were not reflected in almost all grain yield attributes of rice (Tables 6 to 7), probably due to a possible adverse effect imposed on rice plants during their grain filling. These improvements could hardly be observed only due to the foliar fertilization rather than soil biofertilization. Similar significant effects were reported by Castro *et al*. (2002), Ibrahim *et al*. (2004), Sharief *et al*. (2006), Subashini *et al*. (2007), Razie & Anas (2008), Sathish & Bhaskara (2012), Abd El-All *et al*. (2013) and Farah *et al*. (2014) due to biofertilization.

Nitrogen level effect : Significant differences could be detected in the grain yield/ fad in both season and their combined due to mineral N fertilization level. The control or the use of 50 kg N/ fad recorded significantly higher grain yield/ fad averages than the 25 or 75 N/ fad levels (Table 8). These results clearly indicate that the over use of N beyond the 50 kg N/ fad level was not in favor of rice plant growth and hence a certain undesirable balance between the source and the sink might have had taken place due to an excessive growth of plants causing lodging a possible mutual shading effect to lower plant leaves during grain filling. This shading effect was reported to increase photosynthates losses due an enhanced respiration by lower rice plant leaves on the expense of photosynthates portioning towards rice grain and hence grain yield. Shading to lower plant leaves in submerged rice was ,also, reported to introduce soil reduction conditions which decrease the availability of N and increase the toxicity of sulfur and a number of micronutrients among them mainly manganese (Tisdal & Nelson, 1975). Similar findings were reported by Sharief *et al*. (2006), Banayo *et al*. (2012), Uddin *et al*. (2013) and Zayed *et al*. (2013). But, these results are not in accordance with those reported by Manzoor *et al*. (2006), Kandil *et al*. (2010) , Metwally *et al*. (2011) and Abou-Khalifa (2012).

Interaction effect : It is quite clear from Table 8-a that the grain yield/ fad tended to decrease significantly with the increase of N level but with different magnitudes which varied according to the biofertilization treatment except the check where it was increased. The response equations showed negative diminishing decrease with the increase of N level. For the foliar application treatment which recorded the highest grain yield/ fad, the diminishing in the yield decrease was the highest where the grain yield/ fad could have been maximized to 4.26 ton/ fad due to the addition of a predicted maximum N level of 54.08 kg N/ fad compared with 63.12 kg N/ fad needed to maximize the grain yield to 4.24 ton/ fad for the check. For the other two biofertilization treatments higher N levels were predicted to maximize the grain yield/ fad.

TABLE 8-a. Grain yield of rice as affected by the biofertilization treatments and nitrogen level interaction and the response equation, predicted maximums average and predicted maximum N levels (combined data).

Biofertilization treatments	N level				$\hat{Y} = a + bx - c x^2$	$\hat{Y}_{max}$	X_{max}
	Check	25 kg N/ fad	50 kg N/ fad	75 kg N/ fad			
Control	B 3.22 b	A 3.82 a	A 4.24 a	A 4.19 a	$3.21 + 0.82\ x - 0.16\ x^2$	4.24	63.12
Anabaena oryzae	A 3.65 b	A 3.73 a	B 3.14 b	AB 3.33 b	$3.72 - 0.24\ x + 0.03\ x^2$	3.21	108.0
Anabaena oryzae extract	A 4.62 a	B 3.92 a	A 4.67 a	B 4.16 a	$4.48 - 0.21\ x + 0.05\ x^2$	4.26	54.08
Anabaena + *Anabaena* extract	A 4.71 a	B 3.67 a	B 4.05 a	B 3.45 b	$4.59 - 0.67\ x + 0.11\ x^2$	3.57	76.14

$\hat{Y}_{max}$: predicted maximums average . X_{max}: predicted maximum N level .

These results clearly indicate that added mineral N in the biofertilized plots did reflect different magnitudes of decrease in the grain yield/ fad, probably due to different disorders to the soil nutrient availability caused by different degrees of toxicity which also varied from one biofertilization treatment to another. The best of all these treatments was the full foliar biofertilization rather than the other two soil biofertilization treatments. These results refer to a complementary effect between bio and mineral fertilization where lower N level was needed to maximize grain yield when biofertilization was tried through rice foliage than when was tried through soil.

Straw and total yields per fad and harvest index

Biofertilization effect : Biofertilization treatments did not reflect significant effect on straw and total yields per fad in both seasons and the harvest index in the first season, however, the combined analysis detected significant increase in the former yield attributes due to biofertilization (Table 8). The foliar use of the extract of *Anabaena* recorded the highest straw and total yield averages whereas the application of the *Anabaena* + foliar sprays of the anabaena extract recorded the highest harvest index average. The improved yield attributes achieved due to this foliar biofertilization treatment (Tables 6 and 7) could be due to N fixation, P solubilization and supply of growth promoting substances (Wijebandara *et al*., 2008). Similar significant results were reported by Hossain *et al*. (2001), Paudel *et al*. (2012) and Abd El-All *et al*. (2013).

Nitrogen level effect : In both seasons and their combined a significant increase could be detected in straw and total yields and harvest index where the addition of 25 kg N/ fad, produced a significant increase in straw and total yields per fad., but the further increase of N level to 50 or 75 kg N/ fad., failed to add a further significant increase in this respect (Table 8). Contrary to this, the harvest index was significantly decreased due to the increase of N level. The decrease of harvest index due to the increase of N level, could be attributed to the significant decrease observed in grain yield per fad. Moreover, this may have been due to an excessive supply of N that produced excessive vegetative growth where the increase of N level produced a significant increase in straw and total yields. Similar findings were reported by Abd El-Hamed (2002), Sharief *et al*. (2006), Wijebandara *et al*. (2008), Kandil *et al*. (2010), Metwally *et al*. (2011), Paudel *et al*. (2012) and Uddin *et al*. (2013). But, these results are not in accordance with those reported by Zayed *et al*. (2013) who mentioned that, application of mineral nitrogen fertilizer at the level of 68.75 kg N/ fad gave the highest growth parameter, as well as yield and yield components averages.

Interaction effect : According to the combined analysis, straw yield (Table 8-b), total yield (Table 8-c) and the harvest index (Table 8-d) were significantly affected by the biofertilization treatments x N level. It is evident from Table 8-b and Table 8-c that, the straw and total yields were significantly increased with increase in the N level but with different magnitudes at the different biofertilization treatments. The response equations showed diminishing increase in the straw and total yields with the increase of N level. For the foliar application treatment which recorded the highest straw and total yields, these averages could have been maximize to 6.40 and 10.67 ton/ fad due to the addition of predicted maximum N levels of 35.56 and 33.55 kg N/ fad compared with 63.45 and 63.27 kg N/ fad needed to maximize the straw and total yields to 5.69 and 9.92 ton/ fad for the check without biofertilization. For the other two biofertilization treatments, higher N levels were predicted to maximize the straw and total yields. On the other hand, harvest index tended to decrease significantly with the increase of N level but with different magnitudes which varied according to the biofertilization treatment (8-d). The response equations showed negative diminishing decrease with the increase of N level. These interactions ascertain the view that a complementary effect could have been taken place between mineral and biofertilization where lower N mineral levels were needed to maximize yield averages in the biofertilized than in the un-biofertilized plots. The best of this combination was observed in the fully foliar biofertilized treatment with the extract as the lowest N level was needed.

TABLE 8-b. Straw yield of rice as affected by the biofertilization treatments and nitrogen level interaction and the response equation, predicted maximums average and predicted maximum N levels (combined data).

Biofertilization treatments	**N level**				$\hat{Y} = a + bx - c x^2$	$\hat{Y}_{max}$	X_{max}
	Check	**25 kg N/ fad**	**50 kg N/ fad**	**75 kg N/ fad**			
Control	B 3.86 ab	AB 4.87 b	A 5.73 a	A 5.58 a	$3.82 + 1.47\ x - 0.29\ x^2$	5.69	63.45
Anabaena oryzae	B 3.77 b	A 5.43 b	AB 4.59 b	AB 4.05 b	$3.91 + 1.65\ x - 0.55\ x^2$	5.15	37.5
Anabaena oryzae extract	B 5.27 a	A 6.73 a	AB 5.80 a	AB 5.33 a	$5.41 + 1.37\ x - 0.48\ x^2$	6.40	35.56
Anabaena + Anabaena extract	A 4.45 ab	A 4.66 b	A 4.95 ab	A 4.53 ab	$4.41 + 0.53\ x - 0.16\ x^2$	4.85	41.71

$\hat{Y}_{max}$: predicted maximums average . X_{max}: predicted maximum N level .

TABLE 8-c. Total yield of rice as affected by the biofertilization treatments and nitrogen level interaction and the response equation, predicted maximums average and predicted maximum N levels (combined data).

Biofertilization treatments	N level				$\hat{Y} = a + bx - c x^2$	$\hat{Y}_{max}$	X_{max}
	Check	25 kg N/ fad	50 kg N/ fad	75 kg N/ fad			
Control	B 7.08 b	AB 8.70 ab	A 9.96 a	A 9.77 a	$7.03 + 2.29\ x - 0.45\ x^2$	9.92	63.27
Anabaena oryzae	AB 7.42 b	A 9.16 ab	AB 7.73 b	B 7.38 b	$7.63 + 1.41\ x - 0.52\ x^2$	8.59	33.79
Anabaena oryzae extract	A 9.89 a	A 10.65 a	A 10.46 a	A 9.50 a	$9.90 + 1.15\ x - 0.43\ x^2$	10.67	33.55
Anabaena + *Anabaena* extract	A 9.16 a	A 8.32 b	A 8.99 ab	A 7.98 b	$9.0 + 0.16\ x - 0.04\ x^2$	9.15	46.9

$\hat{Y}_{max}$: predicted maximums average . X_{max}: predicted maximum N level .

TABLE 8-d. Harvest index of rice as affected by the biofertilization treatments and nitrogen level interaction and the response equation, predicted maximums average and predicted maximum N levels (combined data).

Biofertilization treatments	N level				$\hat{Y} = a + bx - c x^2$	$\hat{Y}_{max}$	X_{max}
	Check	25 kg N/ fad	50 kg N/ fad	75 kg N/ fad			
Control	A 45.53 b	A 43.91 a	A 42.37 a	A 43.19 a	$45.64 - 2.69\ x + 0.61\ x^2$	42.69	55.04
Anabaena oryzae	A 49.17 a	B 41.91 a	B 41.33 a	B 44.95 a	$49.05 - 9.48\ x + 2.72\ x^2$	40.78	43.58
Anabaena oryzae extract	A 46.80 ab	B 37.58 b	A 44.72 a	A 43.83 a	$45.58 - 6.43\ x + 2.08\ x^2$	40.63	38.56
Anabaena + *Anabaena* extract	A 51.52 a	B 44.34 a	B 44.85 a	B 43.26 a	$51.03 - 6.62\ x + 1.40\ x^2$	43.19	59.21

$\hat{Y}_{max}$: predicted maximums average . X_{max}: predicted maximum N level.

Conclusions

The study was carried out to find out the response of rice to different biofertilization treatments and interaction effects with different nitrogen fertilization levels on growth and yield attributes of rice. According to these results, a complementary effect could be traced between the biofertilization through foliar application of *Anabaena* and the 50 kg N/ fad level to maximize the grain, straw and total yields/ fad.

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(*Received* 11/1/2016;
accepted 13/3/2016)

تأثير التفاعل بين التسميد الحيوي ومستوى التسميد النيتروجينى على نمو ومؤشرات محصول الأرز

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أجريت هذه الدراسة في مركز بحوث التجارب التابع لكلية الزراعة جامعة الزقازيق بمنطقة غزالة محافظة الشرقية وذلك خلال الموسمين الزراعيين ٢٠١٤ – ٢٠١٥ لدراسة تأثير كل من التسميد الحيوى باستخدام فطر *الانوبينا* وهو تابع لـ سيانوبكتريا حيث تم استخدام ثلاث معاملات إضافة وهي الإضافة الأرضية *للأنوبينا* بعد ١٠ أيام من الشتل ، الإضافة الورقية لمستخلص *الانوبينا* بعد ١٠ و ٢٥ يوم من الشتل والإضافة الأرضية مع الورقية لمستخلص *الانوبينا* مقارنة بعدم التسميد الحيوي و كذلك دراسة تأثير مستوى التسميد النيتروجينى (صفر ، ٢٥ ، ٥٠ و ٧٥ كجم ن/ فدان) وذلك على نمو ومحصول الأرز ومؤشرات المحصول وبعض صفات النمو والصفات الفسيولوجية.

ويمكن تلخيص النتائج المتحصل عليها على النحو التالي:

١. كان للتسميد الحيوي تأثيراً معنوياً وايجابياً على محصول الأرز/ فدان وكذلك بعض مؤشرات النمو والصفات الفسيولوجية ، حيث سجلت معاملة التسميد الورقى أعلى المتوسطات.
٢. لم يكن للتسميد النيتروجينى تأثيراً ايجابياً على محصول الأرز/ فدان وبعض مؤشرات المحصول عند الحصاد باستثناء عدد الداليات/ م^2 والذي استجاب لإضافة ٧٥ كجم ن/ فدان ، فى حين استجابت بعض مؤشرات النمو والصفات الفسيولوجية لإضافة ٥٠ كجم ن/ فدان.
٣. لوحظ تداخل فعل معنوى بين التسميد الحيوى ومستويات التسميد النيتروجينى فى جميع الصفات تحت الدراسة والتى أوضحت إمكانية خفض مستوى التسميد النيتروجيني عند التسميد الحيوى رشاً على المجموع الخضرى لنبات الأرز فى الحقل المستديم عند ١٠ و ٢٥ يوم من الشتل حيث أمكن معظمة محصول الحبوب/ فدان وكذلك محصول القش والمحصول الكلي/ فدان بإضافات نيتروجين معدنى أقل مقارنة بمعاملة عدم التسميد الحيوي وهو ما أوضحته معادلات الاستجابة. تؤكد هذه الدراسة على ضرورة ضبط مستوى التسميد النيتروجينى عند التسميد الحيوي ، حيث أدى الإسراف في إضافته إلى انخفاض متوسطات المحصول ومعظم مؤشراته عند الحصاد رغم تحسن بعض صفات النمو والصفات الفسيولوجية عند الطرد. يشير ذلك إلى عدم الاتزان بين المنبع والمصب خلال فترة امتلاء الحبوب وسمية أمونيوم محتمله فى الوسط المحيط بنباتات الأرز تحت ظروف الغمر بسبب الزيادة غير المرغوبة لنمو الطحلب عند الإضافات الأرضية مع فعالية استخدامه على صورة مستخلص عن طريق الأوراق والذي ساهم في زيادة كفاءة النيتروجين المعدني باستخدام مستويات منخفضة لمعظمة محصول الحبوب/ فدان.