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Maximizing Growth and Productivity of Rice by Using N₂-Fixing *Anabaena oryza* and *Spirulina platensis* Extract

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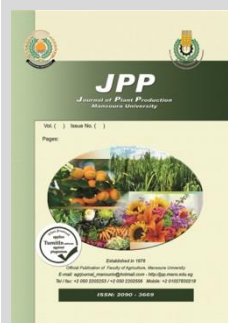
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ABSTRACT

This study highlights the role of both inoculation with N₂-Fixing cyanobacterium *Anabaena oryzae* and spraying with cyanobacterium *Spirulina platensis* extract and their combinations on growth and productivity of the two rice varieties (Sakha108 and Giza178) under different doses of nitrogen fertilizers. The experiment was laid out in a split - plot design with four replications; the two rice varieties were located in the main plots and fertilizer treatments were placed in the sub-plots. Chlorophyll content of flag leaf, plant height, number of panicle/m² and panicle length, number of filled grain/panicle, one thousand grain weight, straw and grain yields (t/ha) were measured. Bioactive compounds and amino acid production of *Spirulina* were determined by GC–MC and amino acid analyzer, respectively. The results show *Spirulina* extract contains eighteen amino acid and several bioactive molecules and application of mixture *Anabaena oryzae* and *Spirulina platensis* extract with 75% recommended dose of nitrogen or recommended dose of N fertilizer gave nearly the highest value of growth characters, grain yield and yield components compared with other treatments in both seasons. This study describes the role of *Anabaena oryzae* and spraying with *Spirulina platensis* extract through plant-microbe interactions and the effect of this reaction on the growth and productivity of rice crop.

Keywords: *Anabaena oryzae*, *Spirulina platensis* extract, growth, productivity, rice



INTRODUCTION

Rice is a main dish in Egypt and after the Egyptian population reached 100 million at the beginning of 2020 (CAPMAS, 2020) and the increase in the prices of nitrogen mineral fertilizers and the health problems resulting from excessive use, this led to the search for cheap and safe means to increase the productivity of the rice crop and overcome these problems (Chittoraa *et al.*, 2020).

Due to nitrogen fixation and release of growth substances, nitrogen-fixing cyanobacteria are one of the important microbial communities in rice fields. The nitrogen fixation of cyanobacteria strains is different from 39.0-327.7 nmol/day of ethylene (Didovich *et al.*, 2020). Nitrogen-fixing *Anabaena* reduces the use of chemical fertilizers by as much as 25% and contributes approximately 20-30 kg of nitrogen demand/Rice growth season (Singh *et al.*, 2016 and Chittapun *et al.*, 2018). The application of nitrogen-fixing cyanobacteria with low urea-N content can promote the growth of rice, increase the absorption of nitrogen, phosphorus, and potassium, increase the weight of 1,000 grains, and increase the yield of rice (Alam *et al.*, 2014), and concentration of N, P, K, Ca, Mg and Na (Jan, *et al.*, 2018). The dominant population of N₂-fixing cyanobacteria in rice fields donates a certain amount of ammonium in the rice soil (Hendrayanti *et al.*, 2019). Cyanobacteria enhance the stability of soil aggregates, microbial community, soil fertility, production of vitamins, amino acids, polypeptides, biotin, proteins, total soluble sugars, antibacterial and antifungal, and polymers that improve soil structure and exoenzyme activity (Chamizo *et al.*, 2018).

Application of *Spirulina platensis* was improved the chlorophyll a, b, leaf area, gibberellin, carotenoids, and height of the plant (Yanni *et al.*, 2020). The foliar spray of *Spirulina* extract is pollution-free, cheap, improve the mineral nutrients in plants, and uses renewable resources to protect soil fertility (Anitha *et al.*, 2016). The application of *Spirulina* extract polysaccharides on plants increases the dry weight and length of shoot, carotenoid, chlorophyll, and protein content of the plant. *Spirulina platensis* increase the content of unsaturated fatty acids (UFA) in plants and the improvement of linolenic acid. The highest phytosterol enhancement was observed in plant treated with *Spirulina platensis* (Rachidia *et al.*, 2020). The plant uses the amino acids as a source of nitrogen compared with mineral nitrogen. Therefore, external amino acids will reduce the release of ammonium salts and the synthesis of root tissues (Mohamed and Mohamed 2012) and enhance the dry weight of plant (Shafeek *et al.*, 2012).

Spirulina microalgae are rich in organic nitrogen such as amino acids. Amino acids have biological effects in plants, such as detoxification of toxins and heavy metals (Rizwan *et al.*, 2017; Bashir *et al.*, 2018 and Hussain *et al.*, 2018), chlorophyll synthesis (Amin *et al.*, 2011), increase the nutrient absorption, vitamin biosynthesis, and enhancing dry matter of plant (Khalilzadeh *et al.*, 2012), maintain cell division, help cell division and expand the channel into plant cells, thereby dividing and expanding the hormone structure, and transforming into cell division, differentiation, and growth efficient polyamines (Kakkar *et al.*, 2000), and resistant stress conditions (Souri and Hatamian 2019). Amino acids foliar increase the concentrations of gibberellic acid and indole acetic

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acid and increase the uptake NPK by plant (Talaat *et al.*, 2005 and Hua-Jing *et al.*, 2007). Methionine enters the synthesis of plant growth regulators (such as cytokinins, auxins), and increases the NPK content and the dry weight of plant shoots (Chen *et al.*, 2005 and El-Awadi *et al.*, 2011). The surface region of leaf plants is strengthened by tryptophan and phenylalanine (Dahab and El-Aziz 2006). The important role of cysteine in the cytoplasm and cytoplasm is the improvement of plant cell mitochondria and hairy roots (Romero *et al.*, 2014). Glycine or glutamine as foliar application stimulates growth of plant (Noroozlo *et al.*, 2019). Treating soil with glycine can obtain higher N, Ca, K, P, Fe, Mg, Zn, plant height, fresh weight of roots, and antioxidant activity of vitamin C (Mohammadipour and Souri 2019). The application of proline and glycine betaine maximizes the ability of the rice plant to tolerate the salinity (Hasanuzzaman *et al.*, 2019). This study shows that the effects of *Anabaena* and *Spirulina* extract in improving rice growth and productivity to reduce nitrogen fertilizer usage will help reduce production costs and pollution.

MATERIALS AND METHODS

A dual season field experiment was conducted at the Experimental Farm Sakha Agriculture Research Station, Kafr El-Sheikh, Egypt, during 2018 and 2019 rice growing seasons. To study the impact of N₂-Fixing *Anabaena oryzae* and *Spirulina platensis* extract and their combination in the presence of different nitrogen doses on yield of both rice varieties; Sakha108 (V1) and Giza178 (V2). Treatments of *Anabaena oryzae*, *Spirulina platensis* extract and their integration in the presence of different nitrogen doses are shown in Table 1. The experiment was laid out in a split plot design with four replications; the two rice varieties were located in the main plots and fertilizer treatments were placed in the sub-plots. Pre-germinated seeds of the two rice varieties at the rate of 120 kg seeds/ha, were broadcasted manually in the prepared nursery on 10th of May in both 2018 and 2019 seasons. The seedbed of transplanting method was prepared and well ploughed; dry leveled, submerged by water then water leveled. Calcium mono phosphate (15.5% P₂O₅) at the rate of 36 kg P₂O₅/ha was added during dry land preparation. Nitrogen in the form of urea (46.5%) was added into the dry soil then incorporated just before flooding at the rate of 80 kg N/ha, and ZnSO₄ H₂O at the rate 57 kg/ha. The permanent field was prepared as mentioned in nursery. Calcium mono

phosphate (15.50 % P₂O₅) was added at the rate of 36.90 kg P₂O₅/ha during land preparation. Nitrogen fertilizer at the rate of 165 kg N/ha as form of urea was added according to the treatments as shown in Table 1.

Table 1. The different treatments used in this study in 2018 and 2019 seasons.

Treatment	
T1	25% recommended dose of N as urea + <i>Spirulina platensis</i> extract
T2	50% recommended + <i>Spirulina platensis</i> extract
T3	75 % recommended + <i>Spirulina platensis</i> extract
T4	25% recommended + <i>Anabaena oryzae</i>
T5	50% recommended + <i>Anabaena oryzae</i>
T6	75% recommended + <i>Anabaena oryzae</i>
T7	25% recommended + mixed (<i>Spirulina platensis</i> extract + <i>Anabaena oryzae</i>)
T8	50% recommended + mixed (<i>Spirulina platensis</i> extract + <i>Anabaena oryzae</i>)
T9	75% recommended + mixed (<i>Spirulina platensis</i> extract + <i>Anabaena oryzae</i>)
T10	Recommended dose of nitrogen

The *Spirulina platensis* extract was applied as foliar spray three times after 15 , 30 and 60 days after transplanting (DAT) at a concentration of 2.4 liters extract/480 liter water/ha, and the control spray with water. *Anabaena oryzae* inoculation was carried out 10 days after transplanting (DAT) at the rate 15 kg of soil based cyanobacteria inoculum (SBI) ha⁻¹. The chemical composition of *Spirulina platensis* extract is shown in Table 2.

The studied characters were: Chlorophyll content (SPAD) was determined at late booting using chlorophyll meter (model SPAD-502) Minolta camera Co. Ltd., Japan. At harvest the plant height/cm, number of panicles were counted, panicle length (cm), panicle weight (g), number of filled grains/panicle, 1000-Grain weight (g), grain and straw yield (t/ha) were estimated according to (IRRI STS, 1996). Some chemical analyses of the experimental soil site were determined in soil past extract before experiments according to Richards (1969) are presented in Table 3. The collected data were subjected to statistical analysis according to the procedure described by Gomez and Gomez (1984), using Genstat 5 release 3 for Windows, VSN International, Hemel Hempstead, UK. The differences among the treatment were compared by multiple comparison tests using Duncan’s Multiple Range Test (DMRT) (Duncan, 1955).

Table 2. Chemical composition of *Spirulina platensis*

composition of <i>Spirulina platensis</i>	Retention time (min)	% area of bioactive compound
Docosane	16.63	0.66
Hexadecanoic acid, methyl ester	21.49	29.26
7,10-Hexadecadienoic acid, methyl ester	21.68	8.29
Phytol	23.94	4.28
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	24.36	34.40
9,12,15-Octadecatrienoic acid, methyl ester. (Z,Z,Z)	24.75	2.89
1-Nonadecene	15.25	1.59
psi.,psi.-Carotene, 3,4-didehydro-1,2-dihydro-1-methoxy	4.79	0.58
19-Norethindrone, O-methyloxime	7.29	1.23
Glafenin	9.92	0.92
Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-,17-[O (phenylmethyl) oxime], (3à,5à)	10.30	0.82
Trimethylsilyl 3-methoxy-2-(2-oxo-2-((trimethylsilyl)oxy)ethoxy)benzoate	12.37	0.90
Silicone oil	15.14	0.55
Toosendanin	23.12	1.32
Propanoic acid	23.63	0.38
Linoleic acid ethyl ester	24.86	0.40
6,9-octadecadienoic acid, methyl ester	25.97	1.80
Promecarb 2,4-dinitrophenylether	30.74	1.23
Lycoxanthin	39.41	0.43

Table 3. Some chemical analyses of the experimental soil before planting in 2018 and 2019 seasons.

Soil chemical properties	2018 season	2019 season
pH(1:2.5)	8.12	8.35
Ec (ds.m ⁻¹)	3.09	2.90
Available ammonium (ppm)	16.00	17.50
Available Nitrate (ppm)	13.30	14.10
Available P (ppm)	5.70	6.00
Available K (ppm)	440.50	455.10
Anions (meq.L ⁻¹)		
CO ₃ ⁻	--	--
HCO ₃ ⁻	6.50	5.77
Cl ⁻	8.80	8.30
SO ₄ ⁻	15.63	14.90
Cations(meq.L ⁻¹)		
Ca ⁺⁺	6.30	5.80
Mg ⁺⁺	4.10	3.70
Na ⁺⁺	19.13	17.70
K ⁺	1.40	1.70
Available micronutrients (ppm)		
Fe	6.00	6.50
Mn	3.70	3.60
Zn	1.00	1.12

***Spirulina platensis* and *Anabaena oryzae* cultivation**

Spirulina platensis extract was grown in the Zarrouk medium (Zarrouk, 1966). 100 ml of the appropriate medium was autoclaved for 20 min. at 121°C. Every flask was inoculated with a culture of 10 ml containing 10⁷–10⁸ colony-forming units /ml, incubated for 7days and then used as an inoculum for 1L of the specific medium, incubated for 7days and transported to a 20 l white polyethylene container, incubated for 7days then, inoculated in a glass basin of 60× 60×120 cm. contain the 100 L specific medium, incubated for 25 days. The cultures were grown under controlled laboratory conditions of 30±2°C and continuous illumination of 5500–6500 Lux.

Anabaena oryzae was grown in modified Watanabe medium (El- Nawawy *et al*, 1958) for 10 days under controlled laboratory conditions of 30 ± 2 °C and continuous illumination of 5500–6500 Lux. Soil as the cyanobacteria carrier, 2.5 cm of soil is spread in try (0.5× 1.0 m) and covered

with 5 cm tap water and supplied with phosphate (0.2g Na₂HPO₄ /L), molybdenum (0.2 mg MoO/L) and 1.0 g carbofuran. After the soil settles down and the water in the trays becomes clear, each tray was then inoculated with 100 ml cyanobacteria culture of *Anabaena oryzae*. The trays were kept in the open air up to 15 days and collected to dry.

Preparation of *Spirulina* extract

First, use a blender to extract the *Spirulina platensis* material (1000 g), and then use a mortar and pestle to extract. It was filtered through a cotton cloth to remove debris and designated as 100% soluble water concentration (SWC) and used concentration 100 % in this study were prepared by adding distilled water and refrigerated between 0 – 4 °C until use (Pise and Sabale 2010).

GC-MS analysis

The chemical composition *Spirulina platensis* extract was performed using Trace GC-TSQ Quantum mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50°C and then increased by 5°C /min to 200 °C hold for 2 min. increased to the final temperature 290°C by 30°C /min and hold for 2 min. The injector and MS transfer line temperatures were kept at 270, 260°C respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min and diluted samples of 1 µl were injected automatically using AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–500 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

Amino acids analysis

The amino acids of *Spirulina* extract were measured by the high-performance amino acid analyzer (Biochrom 30) according to (AOAC 2012).

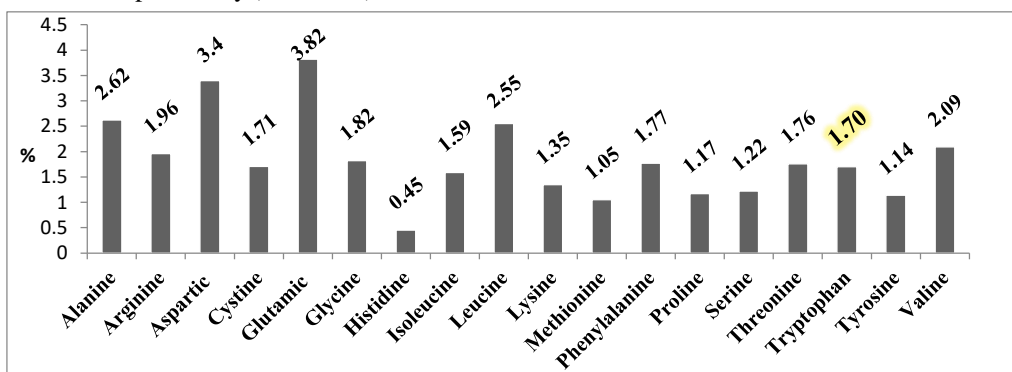


Figure 1. Amino acid production by *Spirulina*

Some chemical component of the *Spirulina* extract

The contents of P, K, Mn and Fe using ICP (optima 2000 DV– Perkin Elmer)(AOAC, 2012).

Potassium 189.7 (ppm), Phosphorus 52.3(ppm), Manganese 7.7 (ppm) and Iron 20 (ppm).

Total protein content measured using the Kjeldahl method (Randhir and Pradhan, 1981). Total protein 36.4%

Economic feasibility

An economic analysis was conducted using the mentioned formulas to measure the net return and the benefit-cost ratio for each procedure (Cimmyt, 1988).

Cost of cultivation (L.E. ha⁻¹): The cost of farming for each treatment is calculated in Egyptian pounds (L.E.). Data on the cost of inputs, which calculated as a rental cost land preparation, seeding, planting, irrigation, fertilizers, weeding, harvesting, transportation, and other expenses.

Gross return (L.E. ha⁻¹): Estimated based on the harvested straw and grain yield (t ha⁻¹) in Egyptian pounds.. One ton of straw yield =200 L.E., one ton of grain yield =4000 L.E. in both seasons, the average prices were taken from survey market prices of rice.

Net return (L.E / ha): Net return was estimated as the difference between total revenue from the sale of harvested rice grain and total costs (fixed and variable cost of rice yield).

Benefit-cost ratio: Calculated by the formula, B: C ratio = Gross return/Cost of cultivation

RESULTS AND DISCUSSION

From the data shown in Table 4, it is obvious that there are significant differences in the chlorophyll content of flag leaves (SPAD), plant height (cm), and number of panicle/m² of rice varieties. Compared with Giza178, Sakha108 has the highest chlorophyll content and plant height. It might be due to the genetic background in this character. Compared with

other treatments, the combination of *Spirulina platensis* extract and *Anabaena oryzae* with 75% recommended dose of N (T9) resulted in the highest value of chlorophyll content, plant height, panicle number /m² and panicle length.. This is because the foliar spray of *spirulina platensis* extract contains several amino acids, bioactive compounds which increase plant growth (Rizwan *et al*, 2017), improves chlorophyll a and b (Bashir *et al*, 2018) and enhances plant biomass (Yanni *et al*, 2020). Also, the continuous supply of nitrogen by *Anabaena* stimulates the secretion of growth substances, which increases the absorption of N, P and K nutrients (Zebo *et al*, 1998).

Table 4. The mean values of chlorophyll content of flag leaf, plant height, number of panicle/m² and panicle length of both rice varieties as affected by the application of *Spirulina platensis* extract and *Anabaena oryzae* with different nitrogen doses in 2018 and 2019 seasons

Variety (A)	Chlorophyll content (SPAD) of flag leaf		Plant height (cm)		Number of panicle/m ²		Panicle length (cm)	
	2018	2019	2018	2019	2018	2019	2018	2019
Sakha108 (V1)	44.26a	44.43a	96.33a	97.84a	688.20a	692.3a	23.27a	23.43a
Giza178 (V2)	41.74b	42.08b	90.80b	92.40b	669.00b	675.1b	22.94a	23.17a
F. test	*	*	*	*	*	*	ns	ns
Treatment (B)	Chlorophyll content (SPAD)		Plant height (cm)		Number of panicle/m ²		Panicle length (cm)	
T1	38.5f	38.70g	84.16e	85.15e	632.50g	636.5 g	21.75f	22.15d
T2	41.50d	41.05e	89.00d	89.65d	652.00f	657.5f	22.90de	23.20bc
T3	42.90c	42.55d	92.50c	94.40c	682.50d	688.5d	23.20cd	23.55b
T4	40.35e	40.00f	88.16d	89.90d	652.50f	657.0f	22.75e	22.95c
T5	43.10c	42.70d	92.66c	94.15c	679.00d	683.5d	23.20cd	23.55b
T6	44.60b	44.70c	98.00b	99.65b	704.00b	704.5c	23.55b	23.60ab
T7	41.23d	41.75e	92.50c	93.65c	663.00e	669.0e	22.85e	23.10de
T8	45.20b	46.65b	98.60b	100.00b	704.00b	708.0bc	23.45bc	23.1bc
T9	47.25a	48.05a	101.50a	104.15a	715.50a	720.0a	23.88a	24.10a
T10	45.40b	46.40b	98.50b	100.50b	706.00b	712.5 b	23.55b	23.35bc
F. test	**	**	**	**	**	**	**	**
Interaction :A x B	*	*	*	*	*	*	*	*

* and ** indicate significant and highly significant at $\alpha = 0.05$ level and $\alpha = 0.01$ level probability, respectively. In same column, means with the same letter (s) are not significantly different at 5% level, according to Duncan's multiple range tests

Where is:

- T1: 25% recommended dose of N + *Spirulina platensis* extract
- T2: 50% recommended + *Spirulina platensis* extract
- T3: 75% recommended + *Spirulina platensis* extract
- T4: 25% recommended + *Anabaena oryzae*
- T5: 50% recommended + *Anabaena oryzae*

- T6: 75% recommended + *Anabaena oryzae*
- T7: 25% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T8: 50% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T9: 75% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T10: Recommended dose of N

Data in Table 5 indicate that the chlorophyll content of flag leaf and plant height were significantly as affected by the interaction between rice varieties and fertilizer treatments (*Spirulina platensis* extract, *Anabaena oryzae* with different nitrogen doses) in both seasons. T9 (75% recommended dose of N combined with *Spirulina platensis* extract and *Anabaena oryzae*.) recorded the maximum values of chlorophyll content and plant height followed by T10 (recommended dose of N)

and T8 (50% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*) for two varieties. It means that the application of nitrogen fertilizer (urea) with *Anabaena* inoculation improve growth and productivity of rice plant. This is due to ability of *Anabaena oryzae* to fix nitrogen and carbon dioxide, and produce ammonia and oxygen (Phathka *et al*, 2018 and Godlewska *et al*, 2019).

Table 5. Chlorophyll content of flag leaf and plant height as affected by the interaction between rice varieties and *Spirulina platensis* extract, *Anabaena oryzae* with different nitrogen doses in 2018 and 2019 seasons

Treatment	Chlorophyll content (SPAD)				plant height (cm)			
	2018		2019		2018		2019	
	Sakha108	Giza178	Sakha108	Giza178	Sakha108	Giza178	Sakha108	Giza178
T1	40.00hi	37.00j	40.10ij	37.30i	84.33i	84.00i	85.30j	85.00j
T2	43.50cd	39.50hi	42.80fg	39.30jk	91.00f	87.00gh	92.30g	87.00ij
T3	44.30c	41.50fg	43.50f	41.60h	95.00e	90.00f	97.30de	91.50gh
T4	41.50fg	39.20i	41.00hi	39.00k	90.33f	86.00h	91.50gh	88.30i
T5	43.91cd	42.30ef	43.40f	42.00gh	94.33e	91.00f	95.00ef	93.30fg
T6	46.20b	43.00de	45.90cd	43.50f	101.00c	95.00e	103.00b	96.30de
T7	41.96ef	40.50gh	42.00gh	41.50h	97.00d	88.00g	98.00cd	89.30hi
T8	46.30b	44.10cd	48.00b	45.30de	102.33bc	95.00e	104.00b	96.00de
T9	48.50a	46.00b	49.50a	46.60c	105.00a	98.00d	108.00a	100.30c
T10	46.50 b	44.30c	48.10b	44.70e	103.00b	94.00e	104.30b	97.30de

Where is:

- T1: 25% recommended dose of N + *Spirulina platensis* extract
- T2: 50% recommended + *Spirulina platensis* extract
- T3: 75% recommended + *Spirulina platensis* extract
- T4: 25% recommended + *Anabaena oryzae*
- T5: 50% recommended + *Anabaena oryzae*

- T6: 75% recommended + *Anabaena oryzae*
- T7: 25% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T8: 50% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T9: 75% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T10: Recommended dose of N

These results agreed with Noroozlo *et al.*, (2019) who found that foliar application of *Spirulina* extract increase the chlorophyll content in the leaves of plant, especially when integrated with appropriate doses of N (50 and 75%) resulted in a higher content of a green pigment (13 and 16.5% more, respectively) in comparison with 100% of N (Yassen *et al.*, 2018). In addition to, *Spirulina* extract contains amino acids (as shown in Fig.1) that enhance growth of plant Soil treatment with glycine (amino acid) gave high values of N, Ca, K, P, Fe, Mg, Zn, plant height, shoot and root fresh weights, vitamin C and antioxidant activity of rice plant (Mohammadipour and Souri 2019 a, b).

Table 6 indicate that there is a significant difference in number of panicle and panicle length as affected by the interaction between rice varieties and fertilizer treatments (*Spirulina platensis* extract, *Anabaena oryzae* with different nitrogen doses) in 2018 and 2019 seasons. Sakha108 recorded

the highest number of panicle with T9 (75% recommended + *Spirulina platensis* extract + *Anabaena oryzae*) without any significant differences with T8 (50% recommended N + *Spirulina platensis* extract + *Anabaena oryzae*) and T10 (Recommended dose of N) in both seasons. Whereas, the highest number panicle length were recorded with T9 followed by T8 and T10. Giza 178 rice cultivar gave maximum number of panicle and panicle length with T9 in both seasons. Inoculated rice plant with growth-promoting rhizobacteria (PGPR) increase the grain yield about 23.63 % compared with the un-inoculated treatment because continuous supply the rice plant by Auxins (indole acetic acid) or hormones (Gibberellins) which cause the increasing the volume of the root exposed to plant absorption and thus increasing the plant's ability to absorb a large amount of nutrients and increasing the added fertilizers (Chi *et al.*, 2005 and Godlewska *et al.*, 2019).

Table 6. Number of panicles and panicle length (cm) as affected by the interaction between rice varieties and *Spirulina platensis* extract, *Anabaena oryzae* with different nitrogen doses in 2018 and 2019 seasons

Treatment	Number of panicles/m ²				Panicle length (cm)			
	2018		2019		2018		2019	
	Sakha108	Giza178	Sakha108	Giza178	Sakha108	Giza178	Sakha108	Giza178
T1	645.0j	620.0k	650.0j	623.0k	22.00i	21.50j	22.30ef	22.00f
T2	665.0g-i	639.0j	670.0hi	645.0j	23.00e-h	22.80gh	23.30d-d	23.10b-d
T3	695.0cd	670.0fg	699.0cd	678.0f-h	23.40b-e	23.00e-h	23.80ab	23.30b-d
T4	660.0hi	645.0j	665.0i	649.0j	22.90f-h	22.60h	23.10b-d	22.80de
T5	683.0e	675.0f	687.0de	680.0fg	23.20c-g	23.20c-g	23.56b-d	23.50b-d
T6	702.0bc	696.0cd	707.0bc	702.0b-d	23.50b-d	23.60bc	23.50b-d	23.70bc
T7	668.0fh	658.0i	672.0g-i	666.0i	22.90f-h	22.80gh	23.20b-d	23.00c-e
T8	718.0a	690.0d	721.00a	695.0de	23.80b	23.10d-g	23.50b-d	23.40b-d
T9	726.0a	705.0b	730.00a	710.0b	24.26a	23.50b-d	24.50a	23.70bc
T10	720.0a	692.0d	722.00a	703.0b-d	23.80b	23.30c-f	23.50b-d	23.20b-d

Where is:

- T1: 25% recommended dose of N + *Spirulina platensis* extract
- T2: 50% recommended + *Spirulina platensis* extract
- T3: 75% recommended + *Spirulina platensis* extract
- T4: 25% recommended + *Anabaena oryzae*
- T5: 50% recommended + *Anabaena oryzae*
- T6: 75% recommended + *Anabaena oryzae*

- T7: 25% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T8: 50% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T9: 75% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T10: Recommended dose of N

Data in Table 7 shows the mean values of number of filled grain/panicle, 1000 grain weight (g), straw and grain yields (t/ha) of both rice varieties. There was a significant difference between two tested varieties in number of filled grain/panicle, 1000 grain weight (g), straw and grain yield (t/ha). Sakha108 gave the greatest 1000 grain weight, straw and grain yield while, Giza178 gave the highest number of filled grain per panicle in both seasons. It could be attributed to the variation in genetic back ground between two rice varieties. Data also, indicate that the application of *Spirulina platensis* extract and *Anabaena oryzae* integrated with 75% recommended dose of N (T9) gave highest number of filled grain/panicle, 1000 grain weight, straw and grain yield followed by T10 (recommended dose of N) compared with other treatments in two studied seasons. It clears from the results that *Spirulina platensis* extract and *Anabaena oryzae* integrated with 75% recommended of N (T9) was most outstanding being significantly superior to the rest of the treatments. This is the due ability of *Anabaena oryzae* to N₂-fixation, secrete indole acetic acid (IAA) and regulate the cell division, leaf length, and light response. Use plants with IAA-producing bacteria to increase growth and yield. A similar result has been cited by (Fernandes *et al.*, 2018 and Sutariati *et al.*, 2019). In addition to, *Spirulina* extract amino acids

(methionine, glycine, tryptophan and phenylalanine) increase the growth substances and enhance number of leaves and leaf area/plant (Yassen *et al.*, 2018 and Noroozlo *et al.*, 2019). Amino acids are the source of nitrogen, which the plant can utilize more quickly than mineral nitrogen. However, external amino acids reduce the flow of ammonium and the transcription of the root tissue (Mohamed and Mohamed, 2012) and enhance the dry weight of plant (Shafeek *et al.*, 2012). It is clear from results that combined *spirulina platensis* extract and *Anabaena oryzae* with nitrogen is necessary to emerge more tillers of rice varieties and cause promotion for rice roots by increasing the root depth and volume consequently increase the sufficient uptake of both water and nutrients to make continuous supply to up ground parts of rice plants. A similar result has been obtained by (Mishra and Pabbi, 2004; Hegazi *et al.*, 2010 and du Jardin, 2015).

Number of filled grain/panicle and 1000 grain weight (g) as affected by the interaction between rice varieties and fertilizer treatments (*Spirulina platensis* extract, *Anabaena oryzae* with different nitrogen doses) are presented in Table 8. The greatest number of filled grain/panicle was found with Giza 178 and Sakha108 rice varieties when received *Spirulina platensis* extract and *Anabaena oryzae* integrated with 75% recommended of N (T9) followed by 50%

recommended of N + *Spirulina platensis* extract + *Anabaena oryzae* (T8) and recommended dose of N (T10) in two studied seasons. It could be attributed to the increase in activity of *spirulina platensis* extract and *Anabaena oryzae* when combined with 75 or 50% recommended of N because of the continuous supply of N to plants by adequate amount by the

promoting substance at different stage of rice resulted increase in the physiological processes in rice plants beside the improve in the root and shoot morphology resulted an increase in the water and nutrients uptake and photosynthesis. These results are in harmony with those obtained by Yanni *et al*, (1997) and Biswas *et al*, (2000).

Table 7. Number of filled grains/panicle, 1000 grain weight, straw and grain yield (t/ha) of both rice varieties as affected by the application of *Spirulina platensis* extract, *Anabaena oryzae* with different nitrogen doses in 2018 and 2019 seasons

Variety (A)	Number of filled grain/panicle		thousand grain weight (g)		Straw yield t/ha		Grain yield (t/ha)	
	2018	2019	2018	2019	2018	2019	2018	2019
Sakha108	115.6b	116.90b	27.90a	28.30a	13.08a	13.24a	10.58a	10.60a
Giza178	128.30a	129.60a	26.10b	24.45b	11.11b	11.43b	9.27b	9.45b
F. test	*	*	*	*	*	*	*	*
Treatment (B)								
T1	103.70g	107.00h	23.95f	23.58f	10.75e	11.02g	8.35f	8.40g
T2	115.50e	117.00f	26.00c	25.88de	11.45f	11.60f	9.25e	9.39e
T3	126.50c	128.50d	28.00b	26.46c	11.98cd	12.22de	10.05c	10.20c
T4	110.00f	111.50g	24.90d	24.53ef	11.60ef	11.76f	8.57f	8.77f
T5	121.00d	123.00e	27.90b	27.62abc	12.15c	12.25de	9.55d	9.75d
T6	130.00b	132.50b	28.80a	28.38ab	12.50b	12.60cd	10.30c	10.47c
T7	114.00e	116.00f	25.60c	25.74de	11.80de	11.97ef	9.05e	9.13e
T8	131.5b	131.00bc	27.90b	25.40de	12.55b	12.95bc	11.10b	11.10b
T9	136.50a	137.00a	29.10a	28.79a	13.40a	13.62a	11.70a	11.67a
T10	131.00b	129.00cd	27.60b	27.20bcd	12.80b	13.35ab	11.30b	11.27b
F. test	**	**	**	**	**	**	**	**
Interaction :A x B	*	*	*	*	*	*	*	*

* and ** indicate significant and highly significant at $\alpha = 0.05$ level and $\alpha = 0.01$ level probability, respectively. In same column, means with the same letter (s) are not significantly different at 5% level, according to Duncan's multiple range tests

Where is:

- T1: 25% recommended dose of N + *Spirulina platensis* extract
- T2: 50% recommended + *Spirulina platensis* extract
- T3: 75% recommended + *Spirulina platensis* extract
- T4: 25% recommended + *Anabaena oryzae*
- T5: 50% recommended + *Anabaena oryzae*

- T6: 75% recommended + *Anabaena oryzae*
- T7: 25% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T8: 50% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T9: 75% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T10: Recommended dose of N

Table 8. Number of filled grain/panicle and 1000 grain weight (g) as affected by the interaction between rice varieties and *Spirulina platensis* extract, *Anabaena oryzae* with different nitrogen doses in 2018 and 2019 seasons

Treatment	Number of filled grain/panicle				1000 grain weight (g)			
	2018		2019		2018		2019	
	Sakha108	Giza178	Sakha108	Giza178	Sakha108	Giza178	Sakha108	Giza178
T1	95.00l	112.33hi	98.00l	116.00i	25.50h	22.40k	26.00fg	21.16k
T2	106.00jk	125.00de	107.00k	127.00ef	27.50ef	24.50i	28.30b-e	23.46ij
T3	122.00ef	131.00c	125.00fg	132.00cd	28.50cd	27.50ef	29.00b-d	23.92hi
T4	104.00k	116.00gh	106.00k	117.00i	26.20g	23.60j	26.80e-g	22.26jk
T5	114.00h	128.00cd	117.00i	129.00de	28.50cd	27.50ef	29.20b-d	26.04fg
T6	125.00de	135.00b	128.00ef	137.00b	29.10b	28.50cd	29.80ab	26.96de
T7	109.00ij	119.00fg	111.00j	121.00h	27.20f	24.00ij	28.20b-e	23.28ij
T8	125.00de	138.00b	125.00fg	137.00b	28.80bc	27.00f	28.50b-e	2.30gh
T9	130.00c	143.00a	129.00de	145.00a	29.70a	28.50a	31.00a	26.59efg
T10	126.00d	136.00b	123.00gh	135.00bc	28.00de	27.20f	29.00b-d	25.39fgh

Where is:

- T1: 25% recommended dose of N + *Spirulina platensis* extract
- T2: 50% recommended + *Spirulina platensis* extract
- T3: 75% recommended + *Spirulina platensis* extract
- T4: 25% recommended + *Anabaena oryzae*
- T5: 50% recommended + *Anabaena oryzae*

- T6: 75% recommended + *Anabaena oryzae*
- T7: 25% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T8: 50% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T9: 75% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T10: Recommended dose of N

Straw and grain yields as affected by the interaction between rice varieties and fertilizer treatments (*Spirulina platensis* extract, *Anabaena oryzae* with different nitrogen doses) are presented in Table 9. The results indicate that *Spirulina platensis* extract and *Anabaena oryzae* integrated with 75% recommended of N (T9) gave the highest values of straw and grain yields followed by 50% recommended + *Spirulina platensis* extract + *Anabaena oryzae* T8 and recommended dose of nitrogen (T10) compared with other the treatments in both seasons. This is due to the N₂-fixing *Anabaena oryzae* fix of nitrogen, improve the soil microbial

community, improving physicochemical properties of soil, controlling soil-borne diseases, added organic matter, release growth-promoting substances, solubilize the insoluble phosphates, and soil fertility. This is reflected in plant growth and thus on its productivity (Chittoraa *et al*, 2020 and Didovich, *et al*, 2020), enhance the growth, plant height, weight of 1000 grain, and grain yield of rice (Singh *et al*, 2016; Chittapun *et al*, 2018 and Jan *et al*, 2018). *Spirulina platensis* can also be used as a source of macro- and micronutrients for plants such as vitamins, amino acids, polypeptides, phytohormones (gibberellins, auxins, and

cytokinins), antioxidants and compounds with antibacterial and antifungal properties as shown in Table 2. These results are harmony with those obtained by (Bhowmik *et al*, 2010; Osman *et al*, 2016 and Nawrocka *et al*, 2017). The effects of *spirulina platensis* and *Anabaena oryzae* treatments on grain

yield, depending on the inorganic fertilizer treatment (urea). Gopalakrishnan *et al*, (2012) who stated that use of biofertilizers in combined with chemical fertilizer significantly improve rice grain yield and its attributes.

Table 9. Straw and grain yield of two rice varieties (t/ha) as affected by the interaction among rice varieties, *spirulina platensis* extract, *Anabaena oryzae* and their combination in the presence of different nitrogen doses in 2018 and 2019 seasons

Treatment	Straw yield (t/ha)				Grain yield (t/ha)			
	2018		2019		2018		2019	
	Sakha108	Giza178	Sakha108	Giza178	Sakha108	Giza178	Sakha108	Giza178
T1	12.00g	9.50k	12.25f	9.80i	8.70ij	8.00k	8.60lm	8.20n
T2	12.60ef	10.30j	12.70d-f	10.50h	9.80e-g	8.70ij	9.93gh	8.85kl
T3	13.30c	10.66ij	13.45bc	11.00gh	10.60cd	9.50gh	10.75e	9.65hi
T4	12.80de	10.40j	12.93c-e	10.60h	8.90e-g	8.25jk	9.15jk	8.40mn
T5	13.40bc	10.90hi	13.50bc	11.00gh	10.10d-f	9.00i	10.30fg	9.20jk
T6	13.80ab	11.20g	13.85ab	11.35g	11.00c	9.60f-h	11.20d	9.75h
T7	12.50ef	11.10hi	12.70d-f	11.25g	9.25hi	8.85jk	9.33ij	8.93kl
T8	13.10cd	12.00g	13.30b-d	12.60ef	12.30b	10.00e-g	11.80c	10.35f
T9	14.00a	12.80de	14.25a	13.00c-e	12.80a	10.60cd	12.60a	10.75e
T10	13.30c	12.30fg	13.50bc	13.20c-e	12.40ab	10.20de	12.10b	10.45ef

Where is:

T1: 25% recommended dose of N + *Spirulina platensis* extract

T2: 50% recommended + *Spirulina platensis* extract

T3: 75% recommended + *Spirulina platensis* extract

T4: 25% recommended + *Anabaena oryzae*

T5: 50% recommended + *Anabaena oryzae*

T6: 75% recommended + *Anabaena oryzae*

T7: 25% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)

T8: 50% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)

T9: 75% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)

T10: Recommended dose of N

Results in the same table (9) also, indicate that Sakha108 gave highest grain yield compared with Giza 178 under the same treatments.

Economic feasibility

Figures 2, 3, and 4 illustrate the total revenue and benefits/costs of different treatments. The main results of this study show that the total and net benefits per hectare of Sakha108 and the benefit-cost ratio are higher than those of the Giza178 rice variety. The highest net return in Egyptian pounds can be obtained by using a mixture of 75% nitrogen (*Spirulina* extract and *Anabaena oryzae*) at the recommended

amount. The application of microorganisms or their extracts can reduce nitrogen fertilizer by about 25%. In this concern, Yaso *et al*, (2007) displayed similar findings.

Increases in gross return, net return and B: C ratio amounted to 1.68, 3.39 and 1.77 % respectively, due to foliar spray of *Spirulina platensis* extract + *Anabaena oryzae* with 75% recommended dose of N (T9) than recommended dose of nitrogen (T10). These results are harmony with those obtained by Oladele and Awodun (2014), Geris and Elsadany (2020)

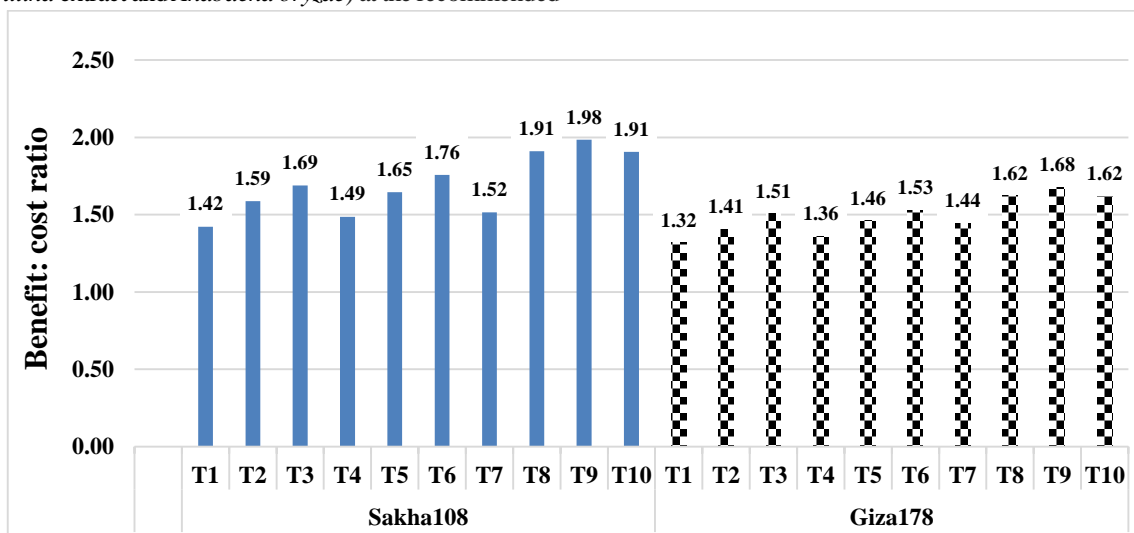


Figure 2. Benefit: cost ratio of straw and grain yield (t. ha⁻¹) as influenced by the interaction between rice varieties and the application of *Spirulina platensis* extract, *Anabaena oryzae* with different nitrogen doses as overall mean values through the two growing seasons.

T1: 25% recommended dose of N + *Spirulina platensis* extract

T2: 50% recommended + *Spirulina platensis* extract

T3: 75% recommended + *Spirulina platensis* extract

T4: 25% recommended + *Anabaena oryzae*

T5: 50% recommended + *Anabaena oryzae*

T6: 75% recommended + *Anabaena oryzae*

T7: 25% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)

T8: 50% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)

T9: 75% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)

T10: Recommended dose of N

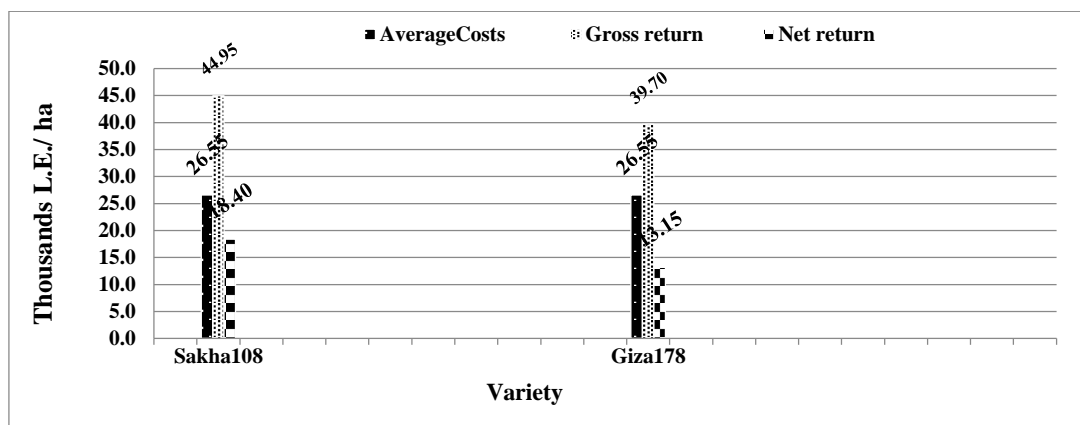


Figure 3. Cost of cultivation, gross returns and net return (L.E. ha⁻¹) of both rice varieties as overall mean values through the two growing seasons.

- T1: 25% recommended dose of N + *Spirulina platensis* extract
- T2: 50% recommended + *Spirulina platensis* extract
- T3: 75% recommended + *Spirulina platensis* extract
- T4: 25% recommended + *Anabaena oryzae*
- T5: 50% recommended + *Anabaena oryzae*
- T6: 75% recommended + *Anabaena oryzae*
- T7: 25% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T8: 50% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T9: 75% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T10: Recommended dose of N

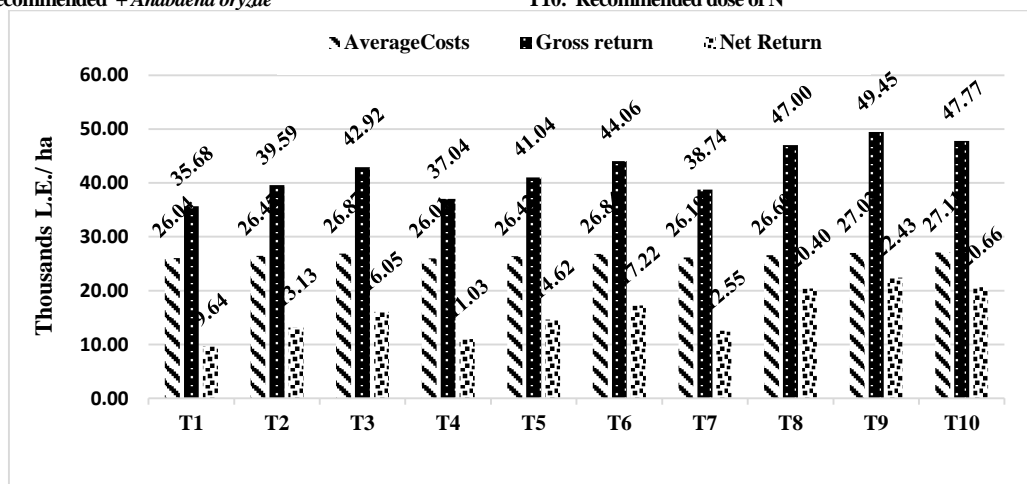


Figure 4. Cost of cultivation, gross returns and net return (L.E. ha⁻¹) as influenced by the interaction between rice varieties and the application of *Spirulina platensis* extract, *Anabaena oryzae* with different nitrogen doses as overall mean values through the two growing seasons.

- T1: 25% recommended dose of N + *Spirulina platensis* extract
- T2: 50% recommended + *Spirulina platensis* extract
- T3: 75% recommended + *Spirulina platensis* extract
- T4: 25% recommended + *Anabaena oryzae*
- T5: 50% recommended + *Anabaena oryzae*
- T6: 75% recommended + *Anabaena oryzae*
- T7: 25% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T8: 50% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T9: 75% recommended + (*Spirulina platensis* extract + *Anabaena oryzae*)
- T10: Recommended dose of N

CONCLUSION

The results show that the treatment *Spirulina platensis* extract + *Anabaena oryzae* integrated with 75% of recommended dose of nitrogen (T9) gave the highest value of grain yield, reduce nitrogen fertilizer usage and increase economic feasibility. This study concluded that the superiority of integrated use of bio and chemical fertilizers compared to chemical fertilizers alone.

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زيادة نمو و إنتاجية الأرز باستخدام انابينا اوريذا المثبتة للازوت و مستخلص اسبيرولينا بلا تينسس

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أقيمت تجربتان بمزرعة محطة البحوث الزراعية بسخا كفر الشيخ وذلك خلال موسمي 2018, 2019 للتعرف على تأثير إضافة مثبتات الازوت انابينا اوريذا و مستخلص اسبيرولينا سواء تم إضافة كلا منهما منفردا او مختلطة في وجود جرعات مختلفة من السماد المعدني (يوربا) وكانت التوليفات كالاتي: (T1) إضافة 25% من الجرعة الموصي (65كجم N/هكتار) + مستخلص سبيرولينا، (T2) إضافة 50% الجرعة الموصي بها + مستخلص سبيرولينا، (T3) إضافة 75% الجرعة الموصي بها + مستخلص سبيرولينا، (T4) إضافة 25% من الجرعة الموصي + انابينا اوريذا، (T5) إضافة 50% الجرعة الموصي بها + انابينا اوريذا، (T6) إضافة 75% الجرعة الموصي من اليوريا + انابينا اوريذا، (T7) إضافة 25% من الجرعة الموصي بها + مستخلص سبيرولينا+ انابينا اوريذا، (T8) إضافة 50% الجرعة الموصي بها + مستخلص سبيرولينا + انابينا اوريذا، (T9) إضافة 75% الجرعة الموصي بها + مستخلص سبيرولينا + انابينا اوريذا، (T10) إضافة الجرعة الموصي بها من السماد المعدني في صورة يوربا. التجربة باستخدام تصميم القطاعات العشوائية الكاملة المنشقة مرة واحدة في أربع مكررات. وتم وضع الأصناف في القطع الرئيسية (سخا108 و جيزة 178) وضعت معاملات السماد في القطع الفرعية. تم أخذ بعض القياسات وهي طول النبات (سم) عند الحصاد، محتوى كلورفيل بورقة العنق و عدد السنابل /م² عند الحصاد، طول السنبل (سم)، وزن السنبل (جم)، عدد الحبوب الممتلئة /سنبل، وزن الألف حبة، ومحصول الحبوب والقش طن/ هكتار. وأشارت النتائج إلى إضافة 75% الجرعة الموصي بها من سماد اليوريا + مستخلص سبيرولينا + مثبتات الازوت انابينا (T9) أو إضافة الجرعة الموصي بها من سماد اليوريا (T10) قد أعطت أعلى النتائج بالنسبة لمعظم الصفات تحت الدراسة والمحصول ومكونة لكلا الصنفين، بجانب التقليل في التكاليف الاقتصادية.