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

Howida B. I. El-Habet* and A. Y. Elsadany

1 Rice Research & Training Center, Sakha Agricultural Research Station, Field Crops Research Institute, Agric. Res. Center, Giza, Egypt.
2 Cyanobacteria Lab., Microbiology Dept., Sakha Agricultural Research Station, Soils, Water and Environment Research Institute, Agric. Res. Center, Giza, Egypt.

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 (Chittorara 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, phosphorous, 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., 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

* Corresponding author.
E-mail address: howidaelnamaky@gmail.com
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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 application 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 *N*₂-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 (15.50 % 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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>25% recommended dose of N as urea + <em>Spirulina platensis</em> extract</td>
</tr>
<tr>
<td>T2</td>
<td>50% recommended + <em>Spirulina platensis</em> extract</td>
</tr>
<tr>
<td>T3</td>
<td>75% recommended + <em>Spirulina platensis</em> extract</td>
</tr>
<tr>
<td>T4</td>
<td>25% recommended + <em>Anabaena oryzae</em></td>
</tr>
<tr>
<td>T5</td>
<td>50% recommended + <em>Anabaena oryzae</em></td>
</tr>
<tr>
<td>T6</td>
<td>75% recommended + <em>Anabaena oryzae</em></td>
</tr>
<tr>
<td>T7</td>
<td>25% recommended + mixed (<em>Spirulina platensis</em> extract + <em>Anabaena oryzae</em>)</td>
</tr>
<tr>
<td>T8</td>
<td>50% recommended + mixed (<em>Spirulina platensis</em> extract + <em>Anabaena oryzae</em>)</td>
</tr>
<tr>
<td>T9</td>
<td>75% recommended + mixed (<em>Spirulina platensis</em> extract + <em>Anabaena oryzae</em>)</td>
</tr>
<tr>
<td>T10</td>
<td>Recommended dose of nitrogen</td>
</tr>
</tbody>
</table>

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* extract

<table>
<thead>
<tr>
<th>Composition of <em>Spirulina platensis</em></th>
<th>Retention time (min)</th>
<th>% area of bioactive compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Docosane</td>
<td>16.63</td>
<td>0.66</td>
</tr>
<tr>
<td>Hexadecanoic acid, methyl ester</td>
<td>21.49</td>
<td>29.26</td>
</tr>
<tr>
<td>7,10-Hexadecadienoic acid, methyl ester</td>
<td>21.68</td>
<td>8.29</td>
</tr>
<tr>
<td>Phytol</td>
<td>23.94</td>
<td>4.28</td>
</tr>
<tr>
<td>9,12-Octadecadienoic acid (Z,Z)-, methyl ester</td>
<td>24.36</td>
<td>34.40</td>
</tr>
<tr>
<td>9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)</td>
<td>24.75</td>
<td>2.89</td>
</tr>
<tr>
<td>1-Nonadecene</td>
<td>15.75</td>
<td>1.59</td>
</tr>
<tr>
<td>psi₅, psi₆, Carotene, 3,4-didehydro-1,2-dihydro-1-methoxy</td>
<td>4.79</td>
<td>0.58</td>
</tr>
<tr>
<td>19-Norethindrone, O-methyloxime</td>
<td>7.29</td>
<td>1.23</td>
</tr>
<tr>
<td>Glaefin</td>
<td>9.92</td>
<td>0.92</td>
</tr>
<tr>
<td>Androstane-11,17-dione, 3-((trimethylsilyl)oxy)-,17-O (phenylmethyl) oxime, (3a,5a)</td>
<td>10.30</td>
<td>0.82</td>
</tr>
<tr>
<td>Trimethylsilyl 3-methoxy-2-(2-oxo-2-((trimethylsilyl)oxy)ethoxy)benzoate</td>
<td>12.37</td>
<td>0.90</td>
</tr>
<tr>
<td>Silicone oil</td>
<td>15.14</td>
<td>0.55</td>
</tr>
<tr>
<td>Toosendanin</td>
<td>23.12</td>
<td>1.32</td>
</tr>
<tr>
<td>Propanoic acid</td>
<td>23.63</td>
<td>0.38</td>
</tr>
<tr>
<td>Linoleic acid ethyl ester</td>
<td>24.86</td>
<td>0.40</td>
</tr>
<tr>
<td>6,9-Octadecadienoic acid, methyl ester</td>
<td>25.97</td>
<td>1.80</td>
</tr>
<tr>
<td>Promecarb 2,4-dinitrophenylether</td>
<td>30.74</td>
<td>1.23</td>
</tr>
<tr>
<td>Lycopenin</td>
<td>39.41</td>
<td>0.43</td>
</tr>
</tbody>
</table>
Table 3. Some chemical analyses of the experimental soil before planting in 2018 and 2019 seasons.

<table>
<thead>
<tr>
<th>Soil chemical properties</th>
<th>2018 season</th>
<th>2019 season</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (1:2.5)</td>
<td>8.12</td>
<td>8.35</td>
</tr>
<tr>
<td>EC (dSm-1)</td>
<td>3.09</td>
<td>2.90</td>
</tr>
<tr>
<td>Available ammonium (ppm)</td>
<td>16.00</td>
<td>17.50</td>
</tr>
<tr>
<td>Available Nitrate (ppm)</td>
<td>13.30</td>
<td>14.10</td>
</tr>
<tr>
<td>Available P (ppm)</td>
<td>5.70</td>
<td>6.00</td>
</tr>
<tr>
<td>Available K (ppm)</td>
<td>440.50</td>
<td>455.10</td>
</tr>
<tr>
<td>Available micronutrients (ppm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anions (meq.L⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO₃⁻</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>6.50</td>
<td>5.77</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>8.80</td>
<td>8.30</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>15.63</td>
<td>14.90</td>
</tr>
<tr>
<td>Cations (meq.L⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>6.30</td>
<td>5.80</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>4.10</td>
<td>3.70</td>
</tr>
<tr>
<td>Na⁺</td>
<td>19.13</td>
<td>17.70</td>
</tr>
<tr>
<td>K⁺</td>
<td>1.40</td>
<td>1.70</td>
</tr>
<tr>
<td>Available micronutrients (ppm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>6.00</td>
<td>6.50</td>
</tr>
<tr>
<td>Mn</td>
<td>3.70</td>
<td>3.60</td>
</tr>
<tr>
<td>Zn</td>
<td>1.00</td>
<td>1.12</td>
</tr>
</tbody>
</table>

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 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).

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

<table>
<thead>
<tr>
<th>Variety (A)</th>
<th>Chlorophyll content (SPAD) of flag leaf</th>
<th>Plant height (cm)</th>
<th>Number of panicle/m²</th>
<th>Panicle length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sakha108 (V1)</td>
<td>44.26a</td>
<td>44.43a</td>
<td>96.33a</td>
<td>97.84a</td>
</tr>
<tr>
<td>Giza178 (V2)</td>
<td>41.74b</td>
<td>42.08b</td>
<td>90.80b</td>
<td>92.40b</td>
</tr>
</tbody>
</table>

F-test: * indicate significant and highly significant at α = 0.05 level and α = 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.

Interaction: A x B

Data in Table 5 indicate that the chlorophyll content of flag leaf and plant height were significantly 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 Godlewski 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chlorophyll content (SPAD)</th>
<th>Plant height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sakha108</td>
<td>40.00a</td>
<td>37.00a</td>
</tr>
<tr>
<td>Giza178</td>
<td>43.00c</td>
<td>39.50c</td>
</tr>
<tr>
<td>T1</td>
<td>44.30c</td>
<td>41.50c</td>
</tr>
<tr>
<td>T2</td>
<td>45.00c</td>
<td>42.00c</td>
</tr>
<tr>
<td>T3</td>
<td>46.20c</td>
<td>43.00d</td>
</tr>
<tr>
<td>T4</td>
<td>47.90c</td>
<td>44.50c</td>
</tr>
<tr>
<td>T5</td>
<td>48.50c</td>
<td>45.00c</td>
</tr>
<tr>
<td>T6</td>
<td>46.50c</td>
<td>44.30c</td>
</tr>
<tr>
<td>T7</td>
<td>47.50c</td>
<td>46.00c</td>
</tr>
<tr>
<td>T8</td>
<td>46.50c</td>
<td>44.30c</td>
</tr>
</tbody>
</table>

Where ic:

T1: 25% recommended dose of N + Spirulina platensis extract
T2: 50% recommended + Spirulina platensis extract
T3: 75% recommended + Spirulina platensis extract
T4: 100% 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 agree 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 Godlewski 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2018</th>
<th>2019</th>
<th>Panicle length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2018</td>
<td>2019</td>
<td></td>
</tr>
<tr>
<td>Sakha108</td>
<td>645.0</td>
<td>620.0</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>665.0</td>
<td>639.0</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>695.0</td>
<td>670.0</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>660.0</td>
<td>645.0</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>683.0</td>
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<td>T5</td>
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<td>696.0</td>
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<td>668.0</td>
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<td>T7</td>
<td>718.0</td>
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<td>T8</td>
<td>726.0</td>
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<td>T9</td>
<td>720.0</td>
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<td>T10</td>
<td>620.0</td>
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<td>T11</td>
<td>645.0</td>
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<td>T12</td>
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<td>T13</td>
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<td>T16</td>
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<td>T17</td>
<td>668.0</td>
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<tr>
<td>T18</td>
<td>718.0</td>
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<tr>
<td>T19</td>
<td>726.0</td>
<td>705.0</td>
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<tr>
<td>T20</td>
<td>720.0</td>
<td>692.0</td>
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</tr>
</tbody>
</table>

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, Giza78 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%
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

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Sakha108</td>
<td>115.06</td>
<td>116.90b</td>
<td>27.90a</td>
<td>28.30a</td>
<td>13.08a</td>
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<tr>
<td>Giza178</td>
<td>128.30a</td>
<td>129.60a</td>
<td>26.10b</td>
<td>24.45b</td>
<td>11.11b</td>
<td>11.43b</td>
<td>9.27b</td>
<td>9.45b</td>
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<tr>
<td>F. test</td>
<td>*</td>
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</thead>
<tbody>
<tr>
<td>T1: 25%</td>
<td>103.70g</td>
<td>107.00h</td>
<td>23.95f</td>
<td>23.58f</td>
<td>10.75c</td>
<td>11.02g</td>
<td>8.35f</td>
<td>8.40g</td>
</tr>
<tr>
<td>T2: 50%</td>
<td>115.50e</td>
<td>117.00f</td>
<td>26.00c</td>
<td>25.88de</td>
<td>11.45f</td>
<td>11.60f</td>
<td>9.25c</td>
<td>9.39e</td>
</tr>
<tr>
<td>T3: 75%</td>
<td>126.50c</td>
<td>128.50d</td>
<td>28.00b</td>
<td>26.46c</td>
<td>11.98c</td>
<td>12.22d</td>
<td>10.05c</td>
<td>10.20c</td>
</tr>
<tr>
<td>T4: 100%</td>
<td>110.00f</td>
<td>111.50g</td>
<td>24.90d</td>
<td>24.53f</td>
<td>11.60f</td>
<td>11.76f</td>
<td>8.57f</td>
<td>8.77f</td>
</tr>
<tr>
<td>T5: 125%</td>
<td>121.00d</td>
<td>123.00e</td>
<td>27.90b</td>
<td>27.62ab</td>
<td>12.15c</td>
<td>12.25d</td>
<td>9.55d</td>
<td>9.75d</td>
</tr>
<tr>
<td>T6: 150%</td>
<td>130.00c</td>
<td>132.50b</td>
<td>28.80a</td>
<td>28.38ab</td>
<td>12.50b</td>
<td>12.60c</td>
<td>10.30c</td>
<td>10.47c</td>
</tr>
<tr>
<td>T7: 175%</td>
<td>114.00b</td>
<td>116.00f</td>
<td>25.60c</td>
<td>25.74de</td>
<td>11.80de</td>
<td>11.97ef</td>
<td>9.05e</td>
<td>9.13e</td>
</tr>
<tr>
<td>T8: 200%</td>
<td>131.5b</td>
<td>110.00e</td>
<td>29.70a</td>
<td>25.40de</td>
<td>12.55b</td>
<td>12.95bc</td>
<td>11.10b</td>
<td>11.10b</td>
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<tr>
<td>T9: 250%</td>
<td>136.50a</td>
<td>137.00a</td>
<td>29.10a</td>
<td>28.79a</td>
<td>13.40a</td>
<td>13.62a</td>
<td>11.70a</td>
<td>11.67a</td>
</tr>
<tr>
<td>T10: 300%</td>
<td>131.00b</td>
<td>129.00cd</td>
<td>27.60b</td>
<td>27.20bcd</td>
<td>12.80b</td>
<td>13.35ab</td>
<td>11.30b</td>
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<tr>
<td>F. test</td>
<td>**</td>
<td>**</td>
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<tbody>
<tr>
<td>* and ** indicate significant and highly significant at α = 0.05 level and α = 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</td>
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</table>

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

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Sakha108</td>
<td>95.00l</td>
<td>112.33hi</td>
<td>98.00l</td>
<td>116.00k</td>
<td>25.50h</td>
<td>22.40k</td>
<td>26.00g</td>
<td>21.16k</td>
</tr>
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<td>Giza178</td>
<td>106.00j</td>
<td>125.00de</td>
<td>107.00k</td>
<td>127.00ef</td>
<td>27.50ef</td>
<td>24.50i</td>
<td>28.30e</td>
<td>23.46j</td>
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<tr>
<td>Sakha108</td>
<td>112.00ef</td>
<td>131.00c</td>
<td>125.00g</td>
<td>132.00cd</td>
<td>28.50cd</td>
<td>27.50ef</td>
<td>29.00e-d</td>
<td>23.29hi</td>
</tr>
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<td>Giza178</td>
<td>104.00k</td>
<td>116.00gh</td>
<td>106.00k</td>
<td>117.00o</td>
<td>26.20g</td>
<td>23.60j</td>
<td>26.80e-g</td>
<td>22.26jk</td>
</tr>
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<td>Sakha108</td>
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<td>128.00cd</td>
<td>117.00n</td>
<td>129.00de</td>
<td>28.50cd</td>
<td>27.50ef</td>
<td>29.20e-d</td>
<td>26.04fg</td>
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<td>135.00b</td>
<td>128.00ef</td>
<td>137.00o</td>
<td>29.10b</td>
<td>28.50cd</td>
<td>29.80ab</td>
<td>26.96de</td>
</tr>
<tr>
<td>T1: 25% recommended dose of N + Spirulina platensis extract</td>
<td>T6: 75% recommended + Anabaena oryzae</td>
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</tr>
<tr>
<td>T2: 50% recommended + Spirulina platensis extract</td>
<td>T7: 25% recommended + (Spirulina platensis extract + Anabaena oryzae)</td>
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</tr>
<tr>
<td>T3: 75% recommended + Spirulina platensis extract</td>
<td>T8: 50% recommended + (Spirulina platensis extract + Anabaena oryzae)</td>
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<td></td>
</tr>
<tr>
<td>T4: 25% recommended + Anabaena oryzae</td>
<td>T9: 75% recommended + (Spirulina platensis extract + Anabaena oryzae)</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>T5: 50% recommended + Anabaena oryzae</td>
<td>T10: Recommended dose of N</td>
<td></td>
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</tr>
</tbody>
</table>

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). T9 and recommended dose of nitrogen (T10) compared with other 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...
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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2018 Straw yield (t/ha)</th>
<th>2019 Straw yield (t/ha)</th>
<th>2018 Grain yield (t/ha)</th>
<th>2019 Grain yield (t/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>12.00g</td>
<td>9.50k</td>
<td>12.25f</td>
<td>9.80ii</td>
</tr>
<tr>
<td>T2</td>
<td>12.60ef</td>
<td>10.30j</td>
<td>12.70d-f</td>
<td>10.50h</td>
</tr>
<tr>
<td>T3</td>
<td>13.30c</td>
<td>10.60j</td>
<td>13.45bc</td>
<td>11.00gh</td>
</tr>
<tr>
<td>T4</td>
<td>12.80de</td>
<td>10.40j</td>
<td>12.93c-e</td>
<td>10.60h</td>
</tr>
<tr>
<td>T5</td>
<td>13.40bc</td>
<td>10.90hi</td>
<td>13.50bc</td>
<td>11.00gh</td>
</tr>
<tr>
<td>T6</td>
<td>13.80ab</td>
<td>11.20g</td>
<td>13.85ab</td>
<td>11.35g</td>
</tr>
<tr>
<td>T7</td>
<td>12.50ef</td>
<td>11.10hi</td>
<td>12.70d-f</td>
<td>11.25g</td>
</tr>
<tr>
<td>T8</td>
<td>13.10cd</td>
<td>12.00g</td>
<td>13.30b-d</td>
<td>12.60ef</td>
</tr>
<tr>
<td>T9</td>
<td>14.00a</td>
<td>12.80de</td>
<td>14.25a</td>
<td>13.00c-e</td>
</tr>
<tr>
<td>T10</td>
<td>13.30c</td>
<td>12.30fg</td>
<td>13.50bc</td>
<td>13.20c-e</td>
</tr>
</tbody>
</table>

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), Geries and Elsadany (2020)
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.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Average Costs</th>
<th>Gross Return</th>
<th>Net Return</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sakha108</td>
<td>50.00</td>
<td>50.00</td>
<td>50.00</td>
</tr>
<tr>
<td>Giza178</td>
<td>50.15</td>
<td>50.15</td>
<td>50.15</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Average Costs</th>
<th>Gross Return</th>
<th>Net Return</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>25% recommended dose of N + Spirulina platensis extract</td>
<td>26.00</td>
<td>26.00</td>
</tr>
<tr>
<td>T2</td>
<td>50% recommended + Spirulina platensis extract</td>
<td>31.00</td>
<td>31.00</td>
</tr>
<tr>
<td>T3</td>
<td>50% recommended + Spirulina platensis extract</td>
<td>35.00</td>
<td>35.00</td>
</tr>
<tr>
<td>T4</td>
<td>25% recommended + Anabaena oryzae</td>
<td>38.00</td>
<td>38.00</td>
</tr>
<tr>
<td>T5</td>
<td>50% recommended + Anabaena oryzae</td>
<td>42.00</td>
<td>42.00</td>
</tr>
<tr>
<td>T6</td>
<td>75% recommended + Anabaena oryzae</td>
<td>46.00</td>
<td>46.00</td>
</tr>
<tr>
<td>T7</td>
<td>75% recommended + (Spirulina platensis extract + Anabaena oryzae)</td>
<td>50.00</td>
<td>50.00</td>
</tr>
<tr>
<td>T8</td>
<td>50% recommended + (Spirulina platensis extract + Anabaena oryzae)</td>
<td>54.00</td>
<td>54.00</td>
</tr>
<tr>
<td>T9</td>
<td>75% recommended + (Spirulina platensis extract + Anabaena oryzae)</td>
<td>58.00</td>
<td>58.00</td>
</tr>
<tr>
<td>T10</td>
<td>Recommended dose of N</td>
<td>62.00</td>
<td>62.00</td>
</tr>
</tbody>
</table>

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 وتضاعف الأرز باستخدام البيلة الليازم ومستخلصات أسبيرولينا بالينيس

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2. نمط الميكروبوزوج النباتي: معبوث البيلة النباتية وخازن الجودة الزراعية - يابسة - مصر


پاپرکاکی: سلول بیوکاترور که درجه نمایندگی و محصول چربیان نباتات را در حالت‌های مختلفی نشان می‌دهد. 

پاپرکاکی: سلول بیوکاترور که درجه نمایندگی و محصول چربیان نباتات را در حالت‌های مختلفی نشان می‌دهد. 

پاپرکاکی: سلول بیوکاترور که درجه نمایندگی و محصول چربیان نباتات را در حالت‌های مختلفی نشان می‌دهد. 

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