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Impact of three strains of *Bacillus* as bio NPK fertilizers and three levels of mineral NPK fertilizers on growth, chemical compositions and yield of Sakha 106 rice cultivar.

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Abstract: Two field experiments were conducted at the experimental farm of Rice Research and Training Center (RRTC), Sakha, Kafrelsheikh, Egypt during 2013 and 2014 rice seasons to study the effect of three strains application of *Bacillus* as Plant Growth Promoting Rhizobacteria (PGPR) in permanent field as well as their combinations with mineral NPK fertilizers on growth, chemical compositions, yield and its attributes of Sakha 106 rice cultivar. *Bacillus* as bio NPK fertilizer sources split to three strains; *Bacillus subtilis* as the nitrogen fertilizer source, *Bacillus megatherium* as the phosphorus fertilizer source and *Bacillus circulans* as the potassium fertilizer source. Also, the application of mineral NPK and the previously mentioned bacteria improved the growth characters such as dry matter accumulation (g m^{-2}), leaf area index (LAI), total chlorophyll content (SPAD), number of tillers m^{-2} and plant height (cm) at harvest; also yield and its attributes such as number of panicles m^{-2}, number of grains $panicle^{-1}$, percentage of filled grains, 1000 grain weight, grain and straw yield as well as chemical compositions as NPK content in both grain and straw (%). The highest values of these characters mostly obtained from the combination of 75% of mineral NPK + *Bacillus subtilis, Bacillus megatherium* and *Bacillus circulans* as bio NPK fertilizer or may be more than the application of recommended of mineral NPK (100% NPK) by the other using these strains of bacteria save about 25% of mineral NPK.
Key words: Rice, Biofertilizers, *Bacillus subtilis*, *Bacillus megatherium*, *Bacillus circulans*, Plant Growth Promoting Rhizobacteria (PGPR), mineral NPK fertilizers.

Introduction

Rice (*Oryza sativa* L.) is probably the most important cereal in the world and serves as food for about 50% of the world's population[1]. It is one of the major crops in the world as a staple food source and is widely cultivated in Egypt.

The growing population in Egypt and the world demands an equal growth in the rate of production of food crops to meet the ever increasing demand for food. In order to increase world food production in a sustainable manner, farmers have to use balanced fertilizer timely. And it is exciting that much of cereals especially rice is produced in countries where rapidly growing population, coupled to limited amounts of land and scares resources. One of the most important factors in the generation of high yields from rice cultivars is nitrogen fertilizer as well as phosphorus and potassium as macronutrients. That is why farmers are applying high amounts of the fertilizers which is very costly and make the environment hazardous especially when use discriminately. Crop scientists all over the world are facing this alarming situation and they are trying to overcome this condition by exploring alternative sources which is cost effective and save the environment. Biofertilizer, an alternative source of NPK fertilizer, especially plant growth promoting rhizobacteria (PGPR). The potential negative effect of chemical fertilizers on the global environment and the cost associated with production had lead to research with the objective of replacing chemical fertilizers with bacterial inoculants.

Biofertilizers play an important role for supplementing the essential plant nutrients for sustainable agriculture, economy and eco-friendly environment. Biofertilizers are becoming increasingly popular in many countries and for many crops, they are defined as products containing active or latent strains of soil microorganisms, either bacteria alone or in combination with algae or fungi that increase the plant availability and uptake of mineral nutrients[2,3,4]

Plant growth promoting rhizobacteria (PGPR) affect plant growth by producing and releasing secondary metabolites (plant growth regulators, phytohormones and biologically active substances), facilitating the availability and uptake of certain nutrients from the root environment and inhibiting plant pathogenic organisms in the rhizosphere. At the same time, plants produce root exudation containing e.g. sugars, amino acids, organic acids, vitamins, enzymes and organic or inorganic ions. PGPR are free living bacteria, isolated from the rhizosphere, which when applied to seeds or crops, enhanced the growth of the plant or reduce the damage from soil–borne plant pathogens.

The demand of PGPR biofertilizers has been increasing day by day with increase in the importance of organic agriculture with minimum inputs of chemicals. PGPRs such as *Bacillus* holds promise as more eco-friendly and sustainable tools in agriculture.

Bacillus has great potential uses in agriculture. Its members are able to produce antimicrobial metabolites to control plant pathogens. These different types of microorganisms are of economic importance in improving crop productivity and can replace costly chemical fertilizers, improving water utilization, lowering production costs, and reducing environmental pollution.

Materials and Methods

Two field experiments were conducted at the experimental farm of Rice Research and Training Center (RRTC), Sakha, Kafrelsheikh, Egypt during 2013 and 2014 rice seasons to study the impact of three strains of *Bacillus* as bio NPK fertilizer sources as well as their combinations with mineral NPK fertilizers on growth, chemical compositions, yield and its attributes of Sakha 106 rice cultivar in permanent field. The preceding crop was barley in both seasons.

Land of nursery site was well prepared (plowed and dry leveled). Nitrogen, phosphorus and potassium fertilizer were applied as recommended. A seed of Sakha 106 rice cultivar does soaked 24 hr. and incubates 48 hr. pre-germinated seeds was broadcasted in nursery soil after slight wet leveling and zinc was applied before seed broadcasting as recommended while weeds were controlled as hand weeding.

The permanent field of experiment was well prepared and both phosphorus and potassium were applied during land preparation, while the application of both nitrogen and biofertilizer were applied as experimented treatments.

Experimental design: Experiment was laid out in Randomized Complete Block Design (RCBD) with four replications in the two seasons, as follows:

T_1: 100% NPK; T_2: 75% NPK; T_3: 50% NPK; T_4: 50% NPK + *Bacillus subtilis* (B_1); T_5: 50% NPK + *Bacillus megatherium* (B_2); T_6: 50% NPK + *Bacillus circulans* (B_3); T_7: 50% NPK + B_1 + B_2 + B_3; T_8: 75% NPK + *Bacillus subtilis* (B_1); T_9: 75% NPK + *Bacillus megatherium* (B_2); T_{10}: 75% NPK + *Bacillus circulans* (B_3) and T_{11}: 75% NPK + B_1 + B_2 + B_3.

I. Growth characters: Dry matter accumulation (g m^{-2}), leaf area index (LAI) and total chlorophyll content (SPAD) at 65 days after sowing (DAS) as well as number of tillers m^{-2} and plant height (cm) at harvest were recorded.

II. Chemical compositions: Nitrogen content; nitrogen in grain and straw was determined at harvest using orange–G dye method[5] and measured by spectrophotometer, Phosphorus content; phosphorus in grain and straw was extracted as described[6] and measured by spectrophotometer using ascorbic acid method[7] and potassium content; potassium in grain and straw was determined using the Flame photometer method[6].

III. Yield attributes: Number of panicles m^{-2} at harvest, number of total grains panicle^{-1}, 1000– grain weight (g) were determined and percentage of filled grains was calculated as follows:

$$\text{Percentage of filled grains} = \frac{\text{No. of filled grains panicle}^{-1}}{\text{Total No. of grains panicle}^{-1}} \times 100$$

IV. Yield: Straw yield (t fed^{-1}) and Grain yield (t fed^{-1}); At harvest, the central area of 10 m^2 from each plot were manually harvested then left for air drying for three days then mechanically threshed and the grain weight was recorded and adjusted to have 14% moisture content.

Statistical analysis: All collected data were statistically analyzed according to the technique of analysis of variance as a Randomized Complete Block Design (RCBD) analysis for the two seasons and the Duncan's Multiple Range Test (DMRT)[8] was used to test the difference between the treatment means[9]. All statistical analysis was performed using analysis of variance technique by means of "MSTATC" computer software package.

Results and Discussion

I. Growth characters:

Dry matter accumulation, leaf area index and total chlorophyll content, number of tillers m^{-2} and plant height as affected by the application of mineral NPK and the three strains of *Bacillus* as bio NPK fertilizer sources; presented in Table 1. It can be also observed that the application of 75% of mineral NPK plus *Bacillus subtilis, Bacillus megatherium* and *Bacillus circulans* as bio NPK fertilizer or the application of recommended of mineral NPK (100% NPK) produced the greatest values of all the studied characters of Sakha 106 rice cultivar than 75% NPK plus *Bacillus subtilis* during 2013 and 2014 seasons. It means that using *Bacillus* PGPR bacteria save about 25% of mineral NPK. The increase in the growth studied characters might be due to improve in fertility and nutrients uptake due to the secretion of oxins or hormones plus N fixation. The tillering, plant height, biomass and yield were significantly increased by inoculation[10]. As discussed above same result has been reported[11] that plant growth-promoting rhizobacteria (PGPR) enhanced the rice growth. The use of PGPR as inoculants biofertilizers is an efficient approach to replace chemical fertilizers.

II. Chemical compositions

Nitrogen, phosphorus and potassium content as affected by the application of mineral NPK and the three strains of *Bacillus* as bio NPK fertilizer sources. Data in Table 2 indicated that the application of 75% of mineral NPK plus *Bacillus subtilis, Bacillus megatherium* and *Bacillus circulans* produced the greatest values of NPK analyses in grain and straw of Sakha 106 rice cultivar and surpassed recommended dose of mineral NPK in 2013 and 2014 seasons. NPK content in grains and straw significantly increase with treated inoculation[12]**.** The direct mechanisms of plant growth promotion may involve the synthesis of substances by the bacterium or facilitation of the uptake of nutrients from the environment[4], increasing NPK content in grain and straw due to the ability of tested bacteria to fix nitrogen, solubalization of phosphorus and mineralization of other nutrients, sequestering of iron cytokinins, gibberellins, production of hormones e.g. auxin i.e. indole acetic acid (IAA), abscisic acid (ABA), gibberellic acid and cytokinins, production of ACC-deaminase[13]**.** Use of these microorganisms as environment friendly biofertilizer helps to reduce the much expensive phosphatic fertilizers. Phosphorus biofertilizer could help increase availability of accumulated phosphate by solubilization efficiency of biological nitrogen fixation and increase availability of K, Fe, Zn etc., through production of plant growth promoting substances[14, 15]

III. Yield attributes

Number of panicles m^{-2}, number of total grains $panicle^{-1}$, 1000– grain weight (g) and percentage of filled grains as affected by the application of mineral NPK and the three strains of *Bacillus* as bio NPK fertilizer sources; presented in Table 3. It can be also observed that the application of 75% of mineral NPK plus *Bacillus subtilis, Bacillus megatherium* and *Bacillus circulans* produced the greatest values of all the studied characters except 1000 grain weight reached to the maximum value when Sakha 106 rice cultivar treated with the tested bacteria with 50% of mineral NPK or 50% of mineral NPK treatment only. One thousand grain weight which increased under the application of the lowest levels of fertilizer, these might be due to the less number of spikelets consequently increased in filling due to the adequate amount of photosynthetic products[4,16,17]. It means that using *Bacillus* PGPR bacteria save about 25% of mineral NPK. The increase in the Studied characters except 1000 grain weight stimulate the source parameters resulted in increase the number of panicles plus increase photosynthesis consequently increase the filling % and number of filled grains. Also, these increases might be due to improve in fertility and nutrients uptake due to the secretion of oxins or hormones plus N fixation. In overall, the inoculation of *Bacillus* as PGPR in rice plant may increase number of panicles m^{-2}, number of grains $panicle^{-1}$, percentage of filled grains because of enhancement of plant biomass, root elongation, uptake of NPK and ultimately increase all characters without deteriorating soil health. Subsequently, they act as potential PGPR biofertilizers and antagonistic agents to save synthetic chemical fertilizers and pesticides, respectively and become an ecofriendly alternative in upcoming days[18, 4]

IV. Yield

Yield is the final indicator of crop performance under different crop management practices. Grain and straw yield (ten ha^{-1}) were significantly improved with *Bacillus* as PGPR. Data in Table 3 clarified that grain and straw yield as affected by the application of mineral NPK and the three strains of *Bacillus* as bio NPK fertilizer sources. It is clear that the application of 75% of mineral NPK plus *Bacillus subtilis, Bacillus megatherium* and *Bacillus circulans* produced the greatest grain and straw yield without any significant difference with recommended dose of mineral NPK treatment or 75% NPK + *Bacillus subtilis*. The same trend was observed in the two studied seasons. Using this strain of bacteria save about 25% of mineral NPK as well as improve the soil fertility[4]**.** The inoculation of *Bacillus* as PGPR in rice plant may increase grain and straw yield. Subsequently, they act as potential *Bacillus* biofertilizers and antagonistic agents to save synthetic mineral fertilizers[18]

Table 1: Dry matter accumulation (DMA), Leaf area index (LAI), Total chlorophyll content, number of tillers m^{-2} and plant height of Sakha 106 rice cultivar asaffected by the combination with bio and mineral NPK fertilizers in 2013 and 2014 seasons.

Treatments	DMA ($g\ m^{-2}$)		LAI		Chlorophyll content (SPAD)		No. of tillers m^{-2}		Plant height (cm)	
	Season									
	2013	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**
100% NPK	1238.0 ab	1223.0 a	3.493 ab	3.330 b	39.74 a	38.35 ab	602.8 a	597.2 a	102.0 ab	102.0 a
75% NPK	945.8 e	916.7 ef	2.370 fg	2.370 ef	36.40 bc	35.11 de	475.0 de	469.4 e	93.00 ef	92.33 e
50% NPK	712.3 g	677.1 h	2.093 g	2.077 f	33.56 d	34.08 e	375.0 g	361.1 h	90.33 g	90.00 f
50% NPK + *Bacillus subtilis* (B_1)	946.5 e	925.6 ef	2.707 ef	2.590 de	36.73 bc	36.92 bc	469.4 e	463.9 e	94.00 de	93.33 de
50% NPK + *Bacillus megatherium* (B_2)	916.7 ef	887.5 fg	2.247 g	2.273 ef	35.20 cd	35.09 de	427.8 f	419.4 f	91.67 fg	90.33 f
50% NPK + *Bacillus circulans* (B_3)	854.2 f	825.0 g	2.230 g	2.113 f	34.67 d	35.86 cd	394.4 g	402.8 g	92.33 ef	90.33 f
50% NPK + $B_1 + B_2 + B_3$	1044.0 d	1029.0 cd	2.997 de	2.957 cd	37.36 b	37.15 bc	519.4 c	508.3 c	97.00 c	95.67 c
75% NPK + *Bacillus subtilis* (B_1)	1157.0 bc	1123.0 b	3.433 bc	3.363 b	39.62 a	39.02 a	561.1 b	569.4 b	100.7 b	98.33 b
75% NPK + *Bacillus megatherium* (B_2)	1087.0 cd	1063.0 bc	3.097 cd	3.193 bc	38.13 ab	38.39 ab	527.8 c	508.3 c	101.3 ab	96.67 bc
75% NPK + *Bacillus circulans* (B_3)	1004.0 de	975.6 de	2.823 de	2.807 d	37.12 b	36.92 bc	502.8 cd	488.9 d	95.00 d	95.00 cd
75% NPK + $B_1 + B_2 + B_3$	1303.0 a	1244.0 a	3.830 a	3.727 a	39.88 a	39.91 a	597.2 a	586.1 a	102.7 a	102.0 a
F. Test	**	**	**	**	**	**	**	**	**	**

*, ** and NS indicate $P < 0.05$, $P < 0.01$ and not significant, respectively. Means of each factor designated by the same latter are not significantly different at 5% level using Duncan's Multiple Range Test (DMRT).

Table 2: Nitrogen (N) content (%), phosphorus (P) content (%) and potassium (K) content (%) in grain and straw of Sakha 106 rice cultivar as affected by the combination with bio and mineral NPK fertilizers in 2013 and 2014 seasons.

Treatments	N % in grain		N % in straw		P % in grain		P % in straw		K % in grain		K % in straw	
	Season											
	2013	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**
100% NPK	1.053 b	1.067 b	0.427 e	0.590 a	0.209 b	0.207 b	0.099 a	0.097 ab	0.291 ab	0.290 a	1.788 b	1.754 ab
75% NPK	0.797 gh	0.797 e	0.473 c-e	0.433 d	0.174 ef	0.170 ef	0.069 cd	0.068 ef	0.235 e	0.229 de	1.372 f	1.320 fg
50% NPK	0.780 h	0.803 e	0.417 e	0.483 cd	0.139 h	0.129 h	0.051 e	0.049 h	0.189 g	0.186 f	1.162 h	1.147 h
50% NPK + *Bacillus subtilis* (B_1)	0.903 de	0.847 de	0.520 c	0.527 bc	0.185 de	0.179 de	0.071 cd	0.069 ef	0.240 e	0.238 d	1.434 ef	1.411 ef
50% NPK + *Bacillus megatherium* (B_2)	0.913 de	0.860 de	0.513 c	0.487 cd	0.168 fg	0.162 f	0.065 d	0.062 fg	0.219 f	0.218 de	1.244 g	1.212 gh
50% NPK + *Bacillus circulans* (B_3)	0.837 fg	0.890 d	0.450 de	0.526 bc	0.157 g	0.150 g	0.059 de	0.054 gh	0.214 f	0.210 e	1.201 gh	1.884 a
50% NPK + $B_1 + B_2 + B_3$	0.923 cd	0.973 c	0.530 c	0.570 ab	0.195 cd	0.197 bc	0.085 b	0.083 cd	0.268 d	0.265 bc	1.571 d	1.553 cd
75% NPK + *Bacillus subtilis* (B_1)	0.977 c	1.000 bc	0.603 b	0.610 a	0.204 bc	0.203 b	0.098 a	0.095 ab	0.285 bc	0.281 ab	1.680 c	1.623 bc
75% NPK + *Bacillus megatherium* (B_2)	0.913 de	0.983 c	0.497 cd	0.600 a	0.201 bc	0.200 bc	0.090 ab	0.089 bc	0.279 c	0.277 a-c	1.610 cd	1.608 c
75% NPK + *Bacillus circulans* (B_3)	0.863 ef	0.830 de	0.491 cd	0.467 d	0.192 cd	0.189 cd	0.079 bc	0.078 de	0.261 d	0.259 c	1.483 e	1.459 de
75% NPK + $B_1 + B_2 + B_3$	1.140 a	1.143 a	0.687 a	0.556 ab	0.221 a	0.219 a	0.101 a	0.099 a	0.299 a	0.296 a	1.926 a	1.878 a
F. Test	**	**	**	**	**	**	**	**	**	**	**	**

*, ** and NS indicate $P < 0.05$, $P < 0.01$ and not significant, respectively. Means of each factor designated by the same latter are not significantly different at 5% level using Duncan's Multiple Range Test (DMRT).

Table 3: Number of panicles m^{-2}, number of grains $panicle^{-1}$, filled grain percentage, 1000-grain weight, grain and straw yield of Sakha 106 rice cultivar as affected by the combination with bio and mineral NPK fertilizers in 2013 and 2014 seasons.

Treatments	Yield attributes								Yield			
	No. of panicles m^{-2}		No. of grains $panicle^{-1}$		Filled grain (%)		1000 grain weight (g)		Grain yield ($t\ ha^{-1}$)		Straw yield ($t\ ha^{-1}$)	
	Season											
	2013	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**
100% NPK	600.0 a	594.4 a	134.3 b	134.2 ab	96.01 a	95.70 ab	24.02 e	24.60 f	9.203 ab	9.049 ab	11.42 ab	11.41 a
75% NPK	472.2 d	469.4 bc	121.5 f	122.4 f	92.24 cd	92.46 ef	26.62 bc	26.20 c-e	8.121 de	8.154 de	9.816 e	10.04 de
50% NPK	375.0 f	358.3 e	116.1 g	114.4 h	90.54 d	90.84 g	27.41 a	27.47 a	6.229 g	6.403 g	7.683 f	7.661 f
50% NPK + *Bacillus subtilis* (B_1)	469.4 d	463.9 c	124.7 e	124.5 ef	93.40 bc	93.40 de	26.76 ab	26.52 bc	8.170 de	8.290 de	10.81 cd	10.40 cd
50% NPK + *Bacillus megatherium* (B_2)	427.8 e	419.4 d	121.1 f	122.6 f	91.79 cd	91.61 fg	26.95 ab	27.05 ab	7.922 e	7.897 e	10.11 e	10.15 de
50% NPK + *Bacillus circulans* (B_3)	391.7 ef	400.0 d	118.5 fg	117.4 g	91.43 d	91.37 g	27.41 a	27.04 ab	7.300 f	7.347 f	10.04 e	9.942 e
50% NPK + $B_1 + B_2 + B_3$	519.4 c	508.3 b	128.2 cd	127.5 de	94.96 ab	94.09 cd	26.31 bc	26.52 bc	8.728 c	8.725 bc	11.10 bc	10.72 c
75% NPK + *Bacillus subtilis* (B_1)	561.1 ab	569.4 a	133.5 b	131.9 bc	94.97 ab	94.97 bc	25.24 d	25.74 e	8.833 bc	8.923 ab	11.11 bc	11.16 ab
75% NPK + *Bacillus megatherium* (B_2)	527.8 bc	508.3 b	129.5 c	129.8 cd	94.89 ab	94.62 c	26.03 c	25.78 de	8.824 bc	8.802 bc	11.00 c	10.80 bc
75% NPK + *Bacillus circulans* (B_3)	502.8 cd	486.1 bc	125.3 de	126.1 e	94.66 ab	93.41 de	26.59 bc	26.34 cd	8.515 cd	8.411 cd	10.48 d	10.71 c
75% NPK + $B_1 + B_2 + B_3$	597.2 a	586.1 a	137.5 a	135.1 a	96.20 a	96.24 a	24.26 e	24.69 f	9.346 a	9.314 a	11.50 a	11.44 a
F. Test	**	**	**	**	**	**	**	**	**	**	**	**

*, ** and NS indicate $P < 0.05$, $P < 0.01$ and not significant, respectively. Means of each factor designated by the same latter are not significantly different at 5% level using Duncan's Multiple Range Test (DMRT).

Conclusions

The integrated use of bio NPK through three strains of *Bacillus* such as; *Bacillus subtilis, Bacillus megatherium* and *Bacillus circulans* biofertilizer as Plant Growth-Promoting Rhizobacteria (PGPR) with mineral NPK has been found promising not only in maintaining and in sustaining high productivity but in providing stability to crop of rice production and minimize the cost of inputs and environmental pollution.

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