EFFECT OF COATING OF SYNTHETIC NPK FERTILIZER WITH PLANT GROWTH PROMOTIN BACTERIA (PGPB) ON GROWTH, YIELD OF RICE UNDER ALUM STRESS

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SUMMARY

Microorganisms in the rhizosphere soil play a key role in maintaining the soil fertility, which is a key for successful crop production to meet the increasing global food demand. The aims of this study were to boost growth attributes, yield and nutrient uptake of rice by different treatment combination of plant growth promoting bacteria (PGPB) and NPK fertilizer doses. The experiment was set up as one - way factorial design (Randomized Complete Block Design), including 4 different treatments with 4 replications spreading over two seasons: winter season 2018 to 2019. The current study was done on rice cultivar (Dai Thom 8) at Long An province, a product was formulated by coating NPK with three bacterial strains of PGPB (Plant Growth Promoting Bacteria) compared the control fertilizer to improve rice productivity under a alum environment. The effects of three PGPB strains (MC/LH 2/1, SP2/LH 2/1 and ED/LH 2/1) on vegetative growth, yield of rice were tabulated. Results showed that the treatment with ASS-MC/LH2/1 produced the highest significant values of plant height, effective tiller and number of panicles per hill. The highest yield was obtained from ASS-MC/LH2/1 treatment (6.14 ton/ha), followed by ASS-SP2/LH2/1 (5.82 ton/ha). The ASS-ED/LH2/1 and control treatments recorded the lowest yield.

Keyword: Alkaline soil, NPK, Plant Growth Promoting Bacteria, rice, yield.

1. INTRODUCTION

Alum stress is one of the major abiotic stresses threatening sustainable crop production worldwide. The extent of alum affected area is expected to cover about 50% of total agricultural land by 2050. Alum stress produces various detrimental effects on plants' physiological, biochemical, and molecular features and reduces productivity. The poor plant growth under alum stress is due to reduced nutrient mobilization, hormonal imbalance, and formation of reactive oxygen species (ROS), ionic and alumic toxicity, and osmotic stress. Additionally, alum also modulates hysicochemical properties and reduces the microbial diversity of soil and thus decreases soil health. Plants harbor diverse microbes in their rhizosphere, and these have the potential to cope with the alum stress. These alum-tolerant plant growth-promoting bacteria (PGPB) assist the plants in withstanding alum conditions (Madhulika Singh and Neha Tiwari, 2021)

As the word population is still increasing and will be about 9.5 billion by 2050 (FAO, 2021), and expansion of arable land is hardly realistic in densely populated Asia, crop intensification is often the main vehicle for increasing food output. Rice is the staple food of billions of people in Asia, and rice cropping was indeed intensified in many Asian countries during the last 30 years of the 20th century. This intensification of crop production, in combination with unbalanced fertilization, has resulted in increasing the use of chemical fertilizers, pesticides and affecting environment. In sustainable farming systems, biofertilizers are alternatives to chemical fertilizers that enhance plant growth, yield and quality (Ajmal et al., 2018). Microorganisms are also essential for the promotion of plant nutrient circulation, reducing the high demand for chemical fertilizers (Çakmakçı et al., 2006;

Javaid and Mahmood, 2010).

The use of agroecological practices such as inoculation by plant growth-promoting bacteria (PGPB) can represent a sustainable alternative for increase nutrient use efficiency in tropical agriculture (Galindo et al., 2018 a, b, 2019 a,b; Martins et al., 2018). The use of these PGPB is growing, particularly in Latin America, for different crops (Souza et al., 2015; Martins et al., 2018; Galindo et al., 2019a). Several PGPB genera show association with different species of agricultural importance, such as *Azospirillum*, *Bacillus* and *Pseudomonas* (Zeffa et al., 2018). These bacteria can stimulate plant growth by a series of mechanisms, including but not restricted, the production of phytohormones, such as salicylic acid, gibberellins, cytokinins and indole-3-acetic acid (IAA) (Cassán and DiazZorita, 2016; Fukami et al., 2017), phosphate solubilization (Ludueña et al., 2018; Qi et al., 2018), nutrient availability increase (Galindo et al., 2018b), production of indolic compounds and siderophores (Ambrosini and Passaglia, 2017), increase on 1-aminocyclopropane-1-carboxylate deaminase activity (Ambrosini and Passaglia, 2017), biological nitrogen fixation (BNF) (Pankievicz et al., 2015), biological control of plants, production of natural antibiotics and protective effect against secondary soil phytopathogens (Zhou et al., 2016; Mishra and Arora, 2018; Shameer and Prasad, 2018).

The *Azospirillum* spp. is considered one of the most studied plant growth promoter genera (Galindo et al., 2016, 2017). An analysis of field trials conducted worldwide for over 20 years, where various non-legume crops were inoculated with *Azospirillum* spp. under different weather and soil conditions, concluded that crop yield can increase up to 30% with inoculation (Fukami et al., 2016). Also, positive results in corn development and yield has been reported with *Azospirillum brasilense* inoculation (strains Ab-V5 and Ab-V6) under tropical conditions (Martins et al., 2018; Oliveira et al., 2018; Galindo et al., 2019b). However, greater responses with other PGPB can be achieved (Pankievicz et al., 2019). New research investigating *Bacillus* spp. and *Pseudomonas* spp. as beneficial PGPB are being conducted, especially for annual crops (Oliveira et al., 2019; Pankievicz et al., 2019; Tavanti et al., 2020). For example, under tropical conditions, *Bacillus subtilis* inoculation (strains Pant001 and QST713) associated with *Bradyrhizobium japonicum* has been reported to increase soybean [*Glycine max* (L.) Merr.] yield compared to single inoculation with *B. japonicum*, besides improving seed quality due to the increase in total storage proteins concentration, seedling emergence percentage and seed vigor (Tavanti et al., 2020). Traoré et al., (2016) reported improved corn seed germination, plant growth, plant production (increase yield by 42%) grain and shoot P biomass content of 34 and 64%, respectively with *B. subtilis* inoculation (strain DSM10). Lima et al., (2019) verified that *B. subtilis* (strains AP-3 and PRBS-1) promoted common bean (*Phaseolus vulgaris* L.) and corn growth, increasing the water use efficiency, leaf water content and the regulation of stomata, without damaging photosynthetic rates. Zarei et al., (2019) concluded that *Pseudomonas fluorescens* (P1, P3, P8, and P14 – prepared from the collection of Vali-eAsr University of Rafsanjan) can improve plant water deficit stress tolerance, P solubilization and siderophore production, leading to an increased sweet corn (*Zea mays* L. var saccharata) growth and yield. Differently, Oliveira et al. (2019), the researchs of PGPB inoculation in soybean (*A. brasilense*, *B. amyloliquefacens*, *B. licheniformis*, *B. pumilus*, *B. subtilis* e *P. fluorescens*) associated with *B. japonicum* has not verifiedplant development and increased grain yield compared to single inoculation with *B. japonicum.*

To Understand the success or failure of inoculation requires to understand the complex interactions between the roots of inoculated plants, the specificity between hosts (plant) and PGPB, and the major microbial communities in the rhizosphere (Bashan et al*.,* 2004; Florio et al*.,* 2017, 2019). Therefore, in this study, the experiment with coating NPK with three bacterial strains of PGPB in tropical conditions should be performed, since new reports can be largely applicable to other important producing countries.

2. RESEARCH METHODOLOGY

Experimental setup and treaments

The study was conducted at Long An province ($10^0$41'29.93''N, $106^0$12'17.4''E), Mekong Delta, Vietnam during December 2018 to March 2019. The experimental site was primarily used to produce annual crops. It receives a mean annual rainfall of 120 mm with mean annual temperatures of 27°C.

The experimental design was a completely randomized block design with four treatments and four replicates. Four treatments consisted of coating of synthetic NPK fertilizer with Plant Growth Promoting Bacteria (PGPB) (ASS, 100 kg N/ha + 60 kg P_2O_5/ha + 60 kg K_2O/ha), ASS + MC/LH 2/1, ASS + SP2/LH 2/1, ASS + ED/LH 2/1. Each experimental plot was 50 m^2. ASS was selected based on the National Technical Regulaton on Testing for Value of Cultivation and Use of Rice Varieties of Vietnam. The coated NPK fertilizer with PGPB as Biofertilizer was obtained from the PetroVietnam Fertilizer and Chemicals Corporration (PVFCCo).

Seed of Dai Thom 8 variety was used. The seeds of rice were spread on the media surface and covered with a thin soil layer. Watering the media was ensured to keep the field capacity. AAS fertilizer is divided into 3 times of top dressing at the following stages: 07 days after sowing (7 DAS); 22 days after sowing (22 DAS) and 40 days after sowing (40 DAS). Technical measures such as planting density, fertilizing, watering, pest control, and others were applied according to common procedures at the test site and uniform for the experimental formulations.

Soil analysis

Soil samples were collected, mixed and a random subsample was used to determine soil chemical attributes before the beginning of field trial in the 0.3 – 0.6 m depth. This samples were collected with soil core sample type cup auger (0.4 m x 0.10 m–cup length and diameter, respectively), randomized in the entire experimental site, regardless of experimental blocks and plots. After samples being collected and mixed, the sub-sample was dried in the shade and soil chemical attributes were determined according to the Raij et al*.,* (2001) methodology. The following results were verified in *Table 1*.

Table 1. Chemical properties of the experimental soil

Parameter	Properties/content	Frame Of Reference
pH KCl	4.89	TCVN 5979-2007
pH H_2O	4.29	TCVN 5979-2007
Fe_2O_3	3.32%	TCVN 6649-2000
$Al_2O_3^*$	17.50%	Ref. TCVN 7370-1:2004

Growth, yield and yield components

Parameters measured for physiological characteristics of rice were: (i) plant height (cm); (ii) tillers number; and (iii) percentage of productive tillers. Harvesting were carried out when 90% of the grains had turned hard, clear and free from greenish tint (Panda, 2010). The following yield components analysis were determined: (i) panicle number per hill; (ii) determination of grains per panicle was done by threshing the grains from the samples and unfilled grains were separated manually from filled grains; (iii) percentage of filled grains was calculated using a formula (filled grains per panicle/total grains per panicle) x 100 (Yoshida et al., 1976); and (iv) 1,000 grain weight (g). Grain yield (tons ha^{-1}) was calculated using this equation proposed by Yoshida (1981):

$$Y = N * W * F * 10^{-5}$$

Where Y = grain yield (tons ha^{-1})

N = panicle number m^{-2}

W = 1,000 grain weight (g) and

F = filled grain per panicle (%)

Fertilizer use efficiency

Fertilizer use efficiency = Yield bumper/kg of fertilizer used.

Yield bumper (ton/ha) = Yield of trial fertilizer treatment – Yield of control treatment.

Statistical Analyses

The experiment was arranged in a completely randomized design (CRD). The data were analyzed by one-way analysis of variance (ANOVA). Significant differences between means were compared using Duncan multiple range test at $P \leq 0.05$. Statistical analysis was performed by using SPSS software version 16.0.

3. RESULTS AND DISCUSSION

Growth parameters

Most of the growth parameters, yield components and yield were significantly affected by different combined doses of standard fertilizer practice and different strains bacteria (Table 2). An increase in plant height was observed with the presence of plant growth promoting bacteria (PGPB), however, the different was not statistically significant. The effect of PGPB was highly significant for number of tillers per hill (P<0.01). About 28% increase in number of tillers per hill was observed for plant fertilized with ASS + MC/LH 2/1 compared with the control plant (only ASS). Different combined dose of standard fertilizer practice and different PGPB did not significantly increase effective tiller compared with the control (Table 2)

Table 2. Effect of coating of synthetic NPK fertilizer with PGPB on growth of rice Dai Thom 8 variety

	Plant height (cm)	No.of tillers per hill	Effective tiller (%)	No.of panicles per hill
ASS (Control)	106.5	6.1d	59.2	3.3
ASS+MC/LH/2/1	111.6	6.8b	72.8	3.7
ASS+SP2/LH/2/1	110.9	7.1a	67.8	3.5
ASS+ED/LH/2/1	110.9	6.4c	62.8	3.4
P	> 0.05	< 0.01	> 0.05	> 0.05
$LSD_{0.05}$	-	0.7	-	-

In the same average group, the letters with the same accompanying characters do not have statistical significance P < 0.01.

Yield components and yield

Most of the yield components such as number of grains per panicle, number of filled grains per panicle and panicle weight were significantly affected by PGPB application (Table 3). Number of grains per panicle were increased from 96.00 to 123.67 under ASS + PGPB treatments when compared with control (75.67 grains).

The data in Table 3 shown that there is a difference in the number of panicles per m^2 and the percentage of unfilled grains between the four treatments. However, this difference was not statistical significance compared to the control (P < 0.05). All three treatments of synthetic NPK fertilizer with PGPB gave a high total number of grains per panicle and the difference was statistically significant compared with the control at P < 0.05. The ASS + MC/LH/ 2/1 treatment had the largest total number of grains per panicle and was significantly different from the other two treatments. The ASS + SP2/LH 2/1 treatment had a larger total number of grains per panicle than the ASS + ED/LH 2/1 treatment, but this difference was not significant. The treatments of synthetic NPK fertilizer with PGPB gave a high number of filled grains per panicle and the difference was statistically significant compared with the control (P <0.05). The ASS + MC/LH 2/1 treatment had the largest total number of filled grains per panicle, so the panicle weight in this treatment was also higher than that of the other treatments (Table 3).

Table 3. Effect of coating of synthetic NPK fertilizer with PGPB on yield of Dai Thom 8 variety

Treaments	No. of panicles per m^2	No. of grains per panicle	Unfilled grains rate (%)	No. of filled grains per panicle	Panicle weight (g)	Theoretic alyield (ton ha^{-1})	Grain yield (ton ha^{-1})	Added grain yield (%)	Fertilizer use efficiency
ASS (Control)	450.67	75.67c	4.35	72.44c	2.23b	8.87	**5.30**	-	0.013
ASS+MC/LH/2/1	469.00	**123.67a**	4.14	**118.56a**	**3.40a**	**13.80**	**6.14**	**15.87**	0.016
ASS+SP2/LH/2/1	452.00	101.22b	4.67	96.33b	2.65b	11.06	**5.82**	**9.91**	0.015
ASS+ED/LH/2/1	502.00	96.00b	4.62	91.56b	2.68b	11.55	**5.67**	**7.06**	0.014
CV (%)	13.66	8.89	24.80	8.94	9.58	17.55	6.77		
LSD 0.05	-	17.60	-	16.92	0.52	-	-		

In the same average group, the values with the same accompanying characters do not have statistical significance P <0.05.

The treatments of synthetic NPK fertilizer coated with PGPB had a rice yield of 5.67 – 6.14 tons/ha. This yield was higher than the control from 7.06 to 15.87%. In which, the ASS + MC/LH 2/1 treatment gave the highest yield, reaching 6,142 tons/ha, an increase of 15.87% compared to the control. Next is the application of ASS + SP2/LH 2/1 with 5.82 tons/ha, an increase of 9.91% compared to the control. The fertilizer use efficiency of the treatment using synthetic NPK fertilizer coated with PGPB reached from 0.014 to 0.016 (kg of grain/kg of fertilizer).

Table 4. **Economic efficiency of the use synthetic NPK fertilizer coated with PGPB on Dai Thom 8 variety**

Treaments	Grain yield (ton/ha)	Gross (1,000 vnd)	Cost (1,000 vnd)	Benefit (1,000 vnd)	Benefit compared to control (1.000 vnd)
ASS (Control)	5.300	29,152	13,533	15,619	-
ASS+MC/LH/2/1	6.142	33,780	13,633	20,147	**4,527**
ASS+SP2/LH/2/1	5.825	32,040	13,633	18,407	**2,788**
ASS+ED/LH/2/1	5.675	31,210	13,633	17,577	**1,957**

Note: the price of 5.5 millon vnd/ton.

The synthetic NPK fertilizer treatments coated with PGPB gave higher economic efficiency than the control. Specifically, the ASS + MC/LH 2/1 treatment gave the highest profit, reaching 4,527 millionVND/ha. The ASS + ED/LH 2/1 treatment gave the lowest increase in profit, reaching 1,957 million VND/ha. In whileThe ASS + SP2/LH 2/1 treatment increased profit by 2,788 million VND/ha compared to the control (Table 4). After testing, all 3 treatments of synthetic NPK fertilizer with PGPB gave higher yields and profits than the control, the ASS + MC/LH 2/1 was the best, followed by the ASS + SP2/LH 2/1 and finally the ASS + ED/LH 2/1 treatment.

4. CONCLUSION

The test results of Phu My NPK fertilizers coated with microorganism for rice plants grown on alum soil in Tan Thanh district, Long An province gave better results than the control (uncoated fertilizer), however, this is not statistically significant at the $p<0.05$ level. In which, the MC/LH 2/1 coating formula is the best. It is recommended to continue testing in a wide variety of growing areas, different soils and crops to introduce the application of this microbial inoculant to recognize technical progress and serve better production.

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ẢNH HƯỞNG CỦA PHÂN BÓN NPK TỔNG HỢP BỌC VI SINH ĐẾN SINH TRƯỞNG VÀ NĂNG SUẤT LÚA TRỒNG TRONG ĐIỀU KIỆN ĐẤT PHÈN

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[2]Viện Khoa học Kỹ thuật Nông nghiệp miền Nam

TÓM TẮT

Vi sinh vật trong đất canh tác đóng vai trò hết sức quan trọng trong việc duy trì độ phì nhiêu của đất, là chìa khóa cho việc sản xuất cây trồng thành công, đáp ứng nhu cầu ngày càng tăng về lương thực thực phẩm trên phạm vi toàn cầu. Mục tiêu của nghiên cứu này là tăng cường các thuộc tính sinh trưởng, năng suất và khả năng hấp thu các chất dinh dưỡng cho cây lúa khi được bón phân NPK tổng hợp phủ các chủng vi khuẩn ở liều lượng khác nhau. Thí nghiệm được bố trí theo kiểu khối ngẫu nhiên đầy đủ, 4 lần lặp lại với 4 nghiệm thức phân bón [ASS (đối chứng), ASS-MC/LH2/1, ASS-SP2/LH2/1, ASS-ED/LH2/1] trong vụ đông từ năm 2018 - 2019 trên giống lúa Đài Thơm 8 trồng trên đất nhiễm phèn thuộc tỉnh Long An. Kết quả nghiên cứu cho thấy ảnh hưởng của ba chủng vi khuẩn đến sinh trưởng, năng suất lúa đã được ghi nhận. Nghiệm thức (ASS-MC/LH2/1) cho chiều cao cây, số nhánh hữu hiệu, số lượng cây/khóm và năng suất đạt cao nhất (6,14 tấn/ha), theo sau là ASS-SP2/LH2/1 (5,82 tấn/ha), ASS-ED/LH2/1 và cuối cùng là nghiệm thức đối chứng (ASS).

Từ khóa: Cây lúa, đất phèn, năng suất, phân bón NPK, vi khuẩn thúc đẩy tăng trưởng thực vật.

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