



Influence of Rhizobacteria on Soil Ion Concentration under Paddy Cultivation

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ABSTRACT

Sustainable agriculture requires nutrient management options that can increase crop yields that are profitable for N nutrient availability and are environmentally friendly. However, N nutrient is dynamic and easy to lose, and excessive use of chemical N fertilizers has a negative impact on the environment. Biofertilizer by rhizobacteria is an effective way to maintain a reliable N-supply for rice growth. This study investigates the effect of a different combination of rhizobacterial fertilizers on nitrogen ion concentration in the soil and total rice production. The field experiment was conducted in two seasons. There were three treatments, including PGPR with 25% CF, PGPR with 50% CF, and 100% CF. The soil sample was taken from each treatment in six growth stages of rice i.e. 15, 36, 50, 72, 100, and 118 days after transplanting. Soil chemicals NH_4^+ and NO_3^- were analyzed by Ion Chromatography. The results showed that the uniformity of NH_4^+ and NO_3^- in the soil were the highest in fertilizer application with PGPR. The application of PGPR with 50% CF confirms that it can increase rice yields by 25.5% and 12.9%, respectively. The application of rhizobacteria can reduce the use of chemical fertilizers.

INTRODUCTION

Sustainable agriculture is one of the most critical efforts globally because it has a sustainable role in providing for agricultural needs. Some of the essential functions of sustainable agriculture are maintaining the environment, maintaining food security globally, and achieving a sustainable part. One of the main crops important in terms of food security globally is rice because it is one of the primary food commodities for over half of the world's population (Zhang et al., 2019). The growing global population and the increasing demand for food in the world is

one of the main challenges of agriculture (Rouphael & Colla, 2020). The increasing population has led to a tendency to use chemical fertilizers to increase crop production (Savci, 2012). Intensive use of chemical fertilizers can negatively impact the environment, especially soil degradation, microorganisms in the soil, and plants. Chemical fertilizers will also change fertile soil to acid soil (Slepetiene et al., 2020).

The status of nutrient availability is significant for plants, one of which is nitrogen ions in the soil. In general, plants can absorb nitrogen in Ammonium (NH_4^+) and Nitrate (NO_3^-). Nitrogen is one of the plant

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nutrients which is very important to increase crop yield, but the nitrogen has natural dynamics and is easily lost due to several factors. The use of intensive chemical fertilizers, especially nitrogen, makes it one of the biggest challenges in rice cultivation. One of the efforts to support the sustainability of agriculture is the use of biological fertilizers to increase soil fertility; utilizing renewable inputs can maximize ecological benefits and reduce environmental hazards. The plant rhizosphere is an ecological soil environment important for plant-microbial interactions and nutrients in the soil. It includes the colonization of various microorganisms in the soil, which can lead to associative, neutral, or parasitic symbiotic relationships in the soil and nutrient status. Rhizobacteria are bacteria that have many benefits for plant growth directly or indirectly which produce growth regulators that can be used successfully to increase plant growth (Konkolewska *et al.*, 2020; Liu *et al.*, 2021; Yagmur & Gunes, 2021; & Zhou, Song, Li, & Kuipers, 2021). PGPR is widely recognized as one of the best alternatives that can reduce chemical fertilizers, increase growth, and increase crop yields in a sustainable way (Basu *et al.*, 2021). PGPR has many roles in encouraging plant growth, including dissolving phosphorus, nitrogen fixation, phytoremediation, producing siderophore and also producing plant phytohormones such as indole acetic acid (IAA), antibiotics, and making plants resistant to disease and environmental stress (Sukul *et al.*, 2021). The application of PGRP as biological fertilizer can increase plant roots which can increase root growth thereby increasing water and nutrient uptake (Goswami & Deka, 2020; Nawaz *et al.*, 2020).

Maintenance of soil properties is essential in sustainable agriculture. Increasing crop production while maintaining soil quality and not damaging the environment will support sustainable agricultural systems worldwide (Kumar & Ashraf, 2017). The use of biological fertilizers can dissolve and mobilize nutrients (Sattar *et al.*, 2019) to reduce costs and the use of ordinary fertilizers (Singh & Gupta, 2018). It is crucial to determine the constant availability of nutrients in the soil. In rice cultivation, the availability of nutrients that are not uniform in the land will result in not uniform growth of rice plants, so it is essential to have uniformity of nutrients in the soil. This limitation can be overcome by using modern approaches and techniques for soil analysis with Ion Chromatography (IC). This method can provide fertilization reference by simpler and easier cultivation guidelines, which

can be used to understand the availability of nutrients in the soil accurately. This study examined the effect of differences in doses of rhizobacteria and chemical fertilizers on the availability of ionic concentrations (NH_4^+ and NO_3^-) in the soil. Moreover, the study aimed to observe the relationship between rhizobacteria, soil ion concentration, and total rice production. The results may provide new insight into rhizobacteria in increasing the effect of N and reducing the use of chemical fertilizers.

MATERIALS AND METHODS

Experimental Site and Design

This experiment was conducted from March to November 2019 in the Paddy Field Guanshan Township of Taitung County, Taiwan (GPS location 23°06'24.0" 121° 12'10'36.8"E). Analysis of soil nutrients was done at the Laboratory Biotechnology Department, National Pingtung University of Science and Technology (NPUST). The research experiment was carried out in two seasons; the first rice cultivated was sown at the end of March and harvested in June. The second rice produced was planted in the second week of August and harvested in November. There were three treatments: PGPR with 25 % CF (Chemical Fertilizer), PGPR with 50% CF, and 100% CF as control. The type of rice used in this experiment was Kaohsiung 147; Chemical fertilizer including Urea, Potassium, and Phosphate for probiotics, was enriched with plant growth rhizobacteria (PGPR). Chemical fertilizer was applied as basal during panicle initiation, flowering stage, and followed by probiotics. During the growing season, hand weeding was done to control weeds.

Soil Samples Collection

Soil samples in the three fields were collected at every growth stage of the rice crop at 15, 36, 50, 72, 100, and 118 days after transplanting. In each field, soil samples were taken from 0–20 cm depth at six points. Soil samples were collected after separating from plant debris, then the soil samples were put in plastic and brought to the laboratory for soil analysis using Ion Chromatography.

Ion Chromatography

Soil analysis was determined using Ion Chromatography (IC) by placing 20 g of soil samples, adding 80 ml Milli-Q in Erlenmeyer flash, and put in an ultrasonic oscillator for 90 minutes. The supernatant was decanted and filtered through Whatman filter paper by 1 μm and put sample into the

new Falcon tube, and centrifuged supernatant with 12.000 rpm for 10 minutes. Filter the supernatant using a membrane filter 0.22 μm and placed the supernatant in a conical tube and store it in the freezer at 20 $^{\circ}\text{C}$. In this research, the analysis of soil ion concentration through Ion Chromatography was carried out using standard ion solutions (0.1, 0.5, 1.0, 5.0, and 10.0 ppm).

Statistical Analysis

The data from various treatments were statistically analyzed with SPSS (version 20: IBM, Armonk, New York, USA). The treatment means were compared with ANOVA using the Least Significant Difference Test (LSD) at the 5% significance level.

RESULTS AND DISCUSSION

Effect of Rhizobacteria on NH_4^+ and NO_3^- Concentration

One of the main benefits of rhizobacteria is to provide plant nutrition. Some rhizobacteria species can release nitrogen and other nutrients from organic matter. PGPR acts as a direct growth promoter in plants because it can increase the availability of plant nutrients by tying up the supply for plant growth (Kumar, 2016). Several types of nitrogen-fixing bacteria that interact with plant roots can convert nitrogen from the ammonium form to the nitrate form and back again under certain soil conditions. The use of PGPR as a biofertilizer can increase plant growth, crop yields, increase soil fertility and also as a biocontrol (Soumare *et al.*, 2020). Bacteria are essential in water dynamics, nutrient cycling, and pathogen suppression. One of the causes of decreased crop yields is the unavailability of N in the soil. Haghshenas & Malidarreh (2021) reported that one way to increase grain yield in rice cultivation can be done by increasing nitrogen fertilization. Most of the nitrogen in the soil comes from free nitrogen from the air, and a small part comes from organic matter. The free nitrogen from the air can enter the soil in various ways, including anchoring by microorganisms, both symbiotic and non-symbiotic, through rainwater and fertilizers applied to the soil. Plants can absorb nitrogen in the form of NH_4^+ and NO_3^- (Tegeger & Masclaux-Daubresse, 2018). The ions come from the transformation process of organic form and fertilizer. This transformation goes through stages, namely mineralization, nitrification, denitrification. Organic nitrogen mineralization occurs through the process of degradation of proteins, amino sugars,

nucleic acids into NH_4^+ in the form of nitrogen minerals. Ammonium is immobilized or accumulated in the soil depending on the nitrogen needs of microorganisms to grow. The formation of NH_4^+ is caused by several possible pathways to break down ions, including the ions taken up by plants, used by microorganisms when there is an excess of carbon sources, bound in exchange complexes, or can be exchanged with cations in soil solution, react with humus, experience volatility, undergoing a nitrification process. Another form of nitrogen that can be used by plants is NO_3^- . This ion comes from the nitrification process by microorganisms by utilizing NH_4^+ as a substrate. Nitrate is easily lost in the soil through washing and reduction. The role of microorganisms in plant nutrition is enormous because microorganisms are always present and live in soil and plant interactions in the soil.

NH_4^+ and NO_3^- concentration during the first season

Ion concentration of NH_4^+ on days 72, 100, and 118 (Fig. 1) confirmed that there was a significant difference ($p < 0.05$) between each treatment. The Duncan test shows that the highest concentration value on day 72 was found on PGPR with 50% CF and followed by control, and the lowest was on PGPR with 25% CF. On days 100 and 118, the highest significant data was found in the control treatment, followed by PGPR with 50% CF, and the lowest was in the PGPR treatment with 25% CF. On days 15 to 36, the concentration of NH_4^+ decreased because the plants could cause it had absorbed NH_4^+ . The ion concentration in the soil is unstable because N in the soil is mobile and dynamic. It can easily change from one form to another, from NH_4^+ to NO_3^- (Cao *et al.*, 2016). Fig. 2 shows the concentration of NO_3^- in all treatments and days after plantation is not significantly different.

However, the results of the analysis show a relationship between NO_3^- and NH_4^+ , the NO_3^- concentration. The graph (Fig. 2) shows the difference with the NH_4^+ concentration. On the 15 to 36 days after plantation, the NH_4^+ concentration decreased (Fig. 1), but the NO_3^- concentration showed an increasing value. It is presumably because of the nitrifying bacteria transform of NH_4^+ into NO_3^- , which rice plants can use. The nitrification process occurs in the soil, where ammonium is transformed into nitrate as the main form of nitrogen that is easily absorbed by plants (Xu, Fan, & Miller, 2012). Decreasing the concentration

of NH_4^+ will increase the NO_3^- concentration. The process of transforming nitrogen forms occurs through a biochemical cycle. According to Norton & Stark (2011), environmental factors such as oxygen/carbon dioxide concentration, temperature, humidity, organic matter, and clay content strongly influence nitrification. Increasing the availability of NO_3^- on the 15 to 36 days is very important because

on the 15 days is the tillering stage and the 30 days is the panicle initiation. After that, it will be continued at the booting stage. The availability of nitrogen in this period is essential. In this period of rice growth, plants need sufficient nutrients to support vegetative and productive change. The availability of nitrogen for plants will support growth and result in high production (Haghshenas & Malidarreh, 2021).

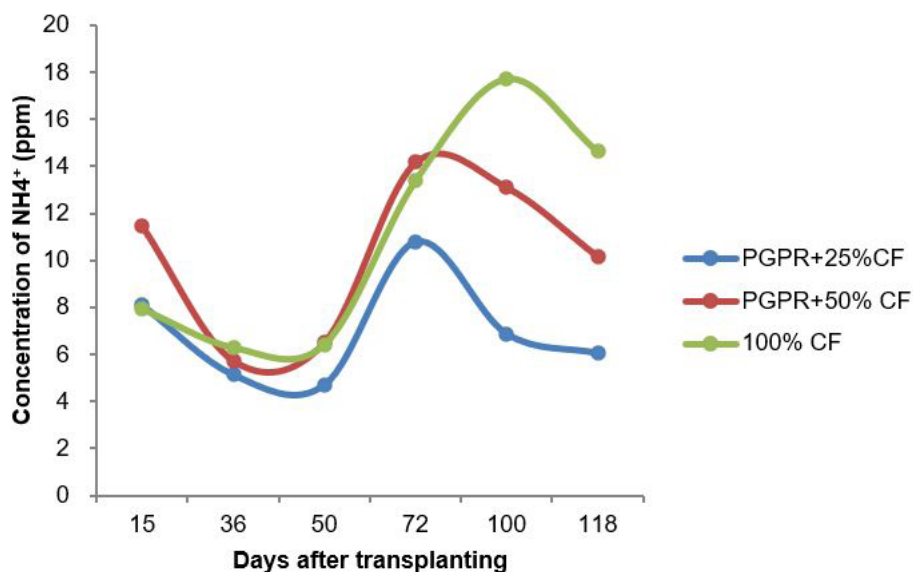


Fig. 1. The NH_4^+ concentration for the three treatments determined using Ion Chromatography beginning from day 15 to 118 during the first growing season.

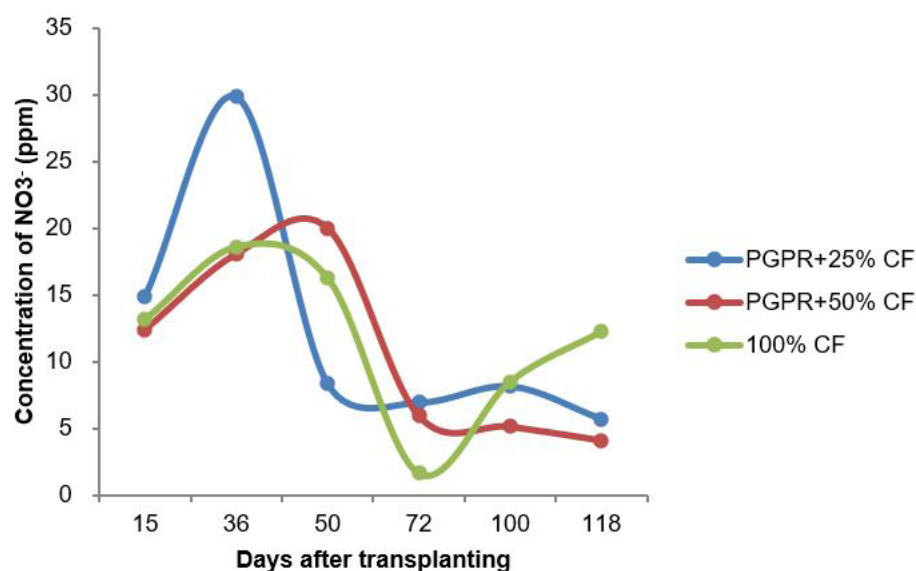


Fig. 2. The NO_3^- concentration for the three treatments determined using Ion Chromatography beginning from day 15 to 118 during the first growing season.

NH₄⁺ and NO₃⁻ Concentration during the Second Season

NH₄⁺ concentration in the second season showed the days 36, 50, and 100 significant differences between each treatment. On the 36 days after transplanting, the concentration of NH₄⁺ was the highest in the control treatment, followed by PGPR with 25% CF and PGPR with 50% CF. Likewise, on the 50 days, the Duncan test showed a significant difference, the highest concentration was found in PGPR with 25% CF followed by PGPR 50%, and the lowest was in the control treatment. On day 100 the NH₄⁺ was the highest in PGPR treatment with 50% CF and followed by PGPR with 25% and the lowest in control. Of the three essential data, on the 36 day, the control has the highest availability among others. It is suspected that the application of chemical fertilizers has a fast reaction in plants, but it is likely that they will quickly disappear due to environmental conditions, while the nitrogen content in the PGPR application is thought to have not been dissolved. However, on days 50 and 100, the application with the addition of PGPR had the highest NH₄⁺ availability compared to control. It is assumed that the nitrogen content has dissolved, and the presence of PGPR in the soil has helped increase nitrogen availability due to interactions in the soil. The availability of nitrogen on days 50 to 100 is very important in the 50 day period, which

is the booting stage towards flowering. If nitrogen nutrients are available to plants, it will help increase flowering in rice plants and increase the grain yield. PGPR is thought to help provide nitrogen in the soil that can be absorbed by plants.

The NO₃⁻ concentration in the second season was significantly different between each treatment on day 50. The highest NO₃⁻ concentration was found in the PGPR application with 25% CF, followed by control and PGPR with 50% CF. The PGPR application with 25% CF has higher availability among others. It is suspected that the existence of PGPR increases the availability of NO₃⁻ because only this application has the highest amount of PGPR and the least chemical fertilizers, among others. In this study, PGPR was thought to work effectively on day 50.

Meanwhile, on other days there was no significant difference between each treatment. The graphs in Fig. 3 and Fig. 4 show that at 15 days, the control treatment had the highest concentration compared to the PGPR treatment. This is also presumed due to the fast chemical fertilizer reaction while PGPR has not reacted. On the other hand, on day 50, the PGPR treatment worked effectively. Treatment using chemical fertilizers can rapidly lose N fertilizer due to several environmental factors that affect it. The loss of N fertilizer is mainly in the form of NH₄⁺, not NO₃⁻. It was similar to the studies reported by Cao & Yin (2015) and Cao *et al.* (2017).

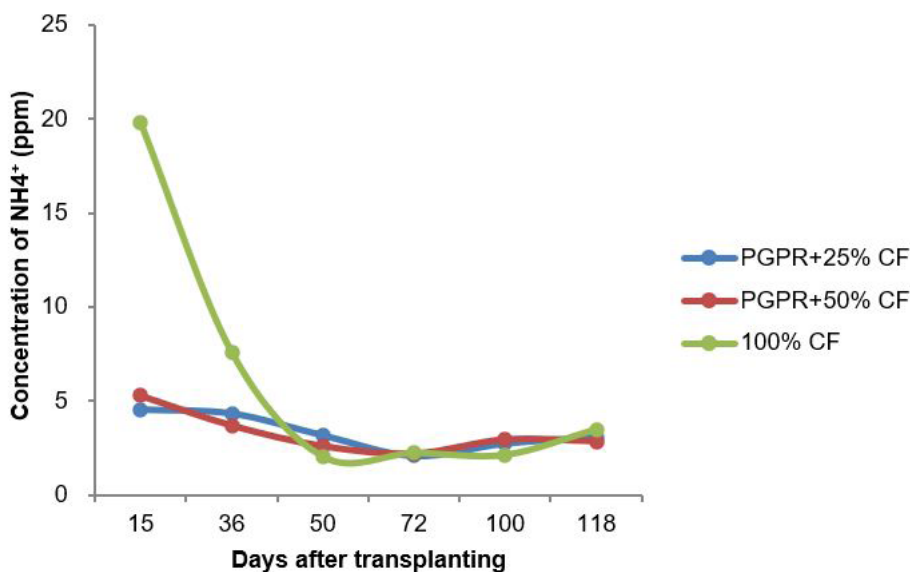


Fig. 3. The NH₄⁺ concentration for the three treatments determined using Ion Chromatography beginning from day 15 to 118 during the second growing season.

Elmi Junita Tarigan et al.: *Rhizobacteria and Sustainable Agriculture*

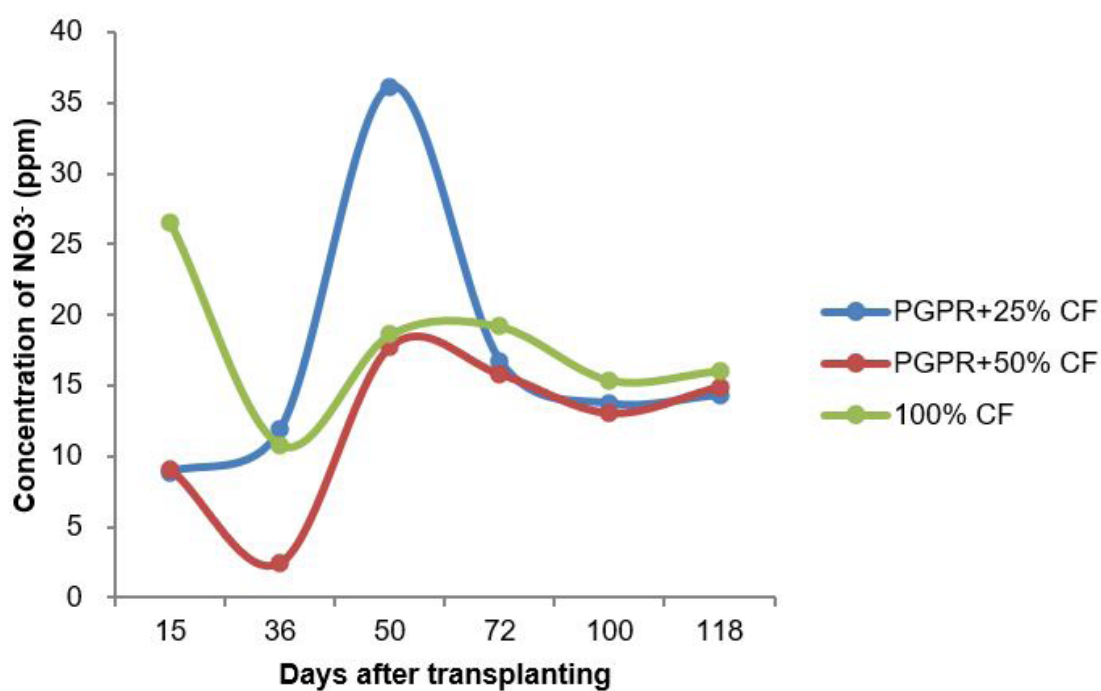


Fig. 4. The concentration NO_3^- for the three treatments determined using Ion Chromatography beginning from day 15 to 118 during the second season.

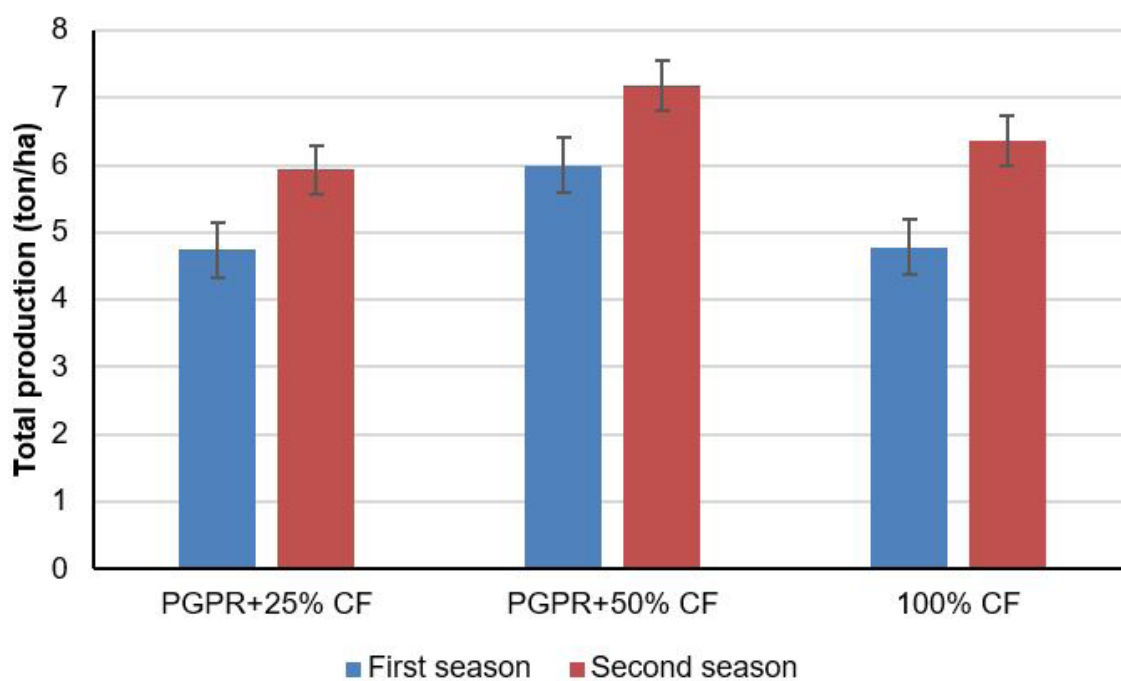


Fig. 5. The total production of rice for the three treatment during two seasons

Effect of Different Fertilizer Dosages on Rice Grain Yield

The research results in Fig. 5 show the total yield of rice production in the first season and the second season. The production yields in the first and second seasons had the same highest yields in the PGPR with 50% CF treatment. In the first season, rice production PGPR with 50% CF treatment produced was the highest of yield (5.99 t/ha), followed by 100% CF and PGPR with 25% CF respectively (4.78 t/ha) and (4.74 t/ha). PGPR with 50% confirms that it can increase rice yields by 25.5%. In the second season, the highest production of yield was (7.19 t/ha) on the application PGPR with 50% CF, followed by 100% CF (6.37 t/ha) and application PGPR with 25% CF (5.93 t/ha). The application of PGPR with 50% CF can increase the percentage of production by (12.9%). However, in the second season, rice yields were higher than the first season for each treatment. The yields on the PGPR application with 25% CF and PGPR with 50% CF increased in the second season 80% and 83%, respectively. It assumed that the application of PGPR in the first season had affected the second season.

PGPR in the soil rhizosphere will associate with plant roots to support the availability of nutrients in the soil, increasing plant growth and development. PGPR treatment with 25% CF confirmed a decrease in crop yields. The decline in yield in this treatment might be caused by the concentration of fertilizer applied that did not meet the nutritional needs of the plant. One of the nutrients that is thought to be insufficient is the nutrient N. Following previous researchers, the unavailable N would cause no beneficial effect produced by PGPR (Paungfoo-Lonhienne *et al.*, 2016). The unavailability of N was due to the unsuitable fertilizer concentration for supporting N in the soil. So it is necessary to have a combination with chemical fertilizers. The increase in rice production on treatment PGPR with 50% bacteria is suspected because rhizobacteria in the soil can increase soil fertility. The increase in production in this study confirms that PGPR with 50% CF is the appropriate fertilizer concentration in this study.

In addition, the results of the study were also confirmed by Kumar & Verma (2019), reporting that the interaction between PGPR and plant roots increases soil fertility from the synthesis of

various biomolecules produced. The existence of Rhizobacteria plays a vital role in supporting plant yields. This is because PGPR can play a direct or indirect role. The natural part produced by Rhizobacteria includes changes, namely to assist the absorption and increase of nutrients through the production of regulating substances or phytohormones (Gouda *et al.*, 2018). While the indirect role is that PGPR can induce plants to become resistant to pathogens so that plants can reduce the impact of disease and can support plants to grow under environmental stress (Goswami, Thakker, & Dhandhukia, 2016).

The appropriate application of PGPR will produce optimal results in providing the nutrients needed by plants and maximizing their activity in the rhizosphere. A mixture of chemical fertilizers with PGPR has benefits for plant growth. Remarkably, treatments containing PGPR resulted in higher yields than controls without rhizobacteria. These results indicate that PGPR plays a role in the efficient absorption of plant nutrients to reduce the risk of nutrient loss. Therefore, this study plays a vital role in increasing the efficiency of the use of nutrients. Previous research that reported the use of PGPR as biological fertilizer can be a biological control that can reduce the use of chemical fertilizers in crop production (Anli *et al.*, 2020; Atieno *et al.*, 2020). At this time, the use of intensive chemical fertilizers is one of the challenges in agriculture, reducing the application of nitrogen fertilizers without losing their performance (Bordoloi *et al.*, 2019). Therefore, the use of microorganisms combined with chemical fertilizers will be a suitable strategy for plants (Sahu *et al.*, 2018), especially for rice plants with a high need for nutrient amounts. This study confirms that the presence of rhizobacteria in the soil can reduce the use of intensive chemical fertilizers, provide soil ion concentrations of NH_4^+ and NO_3^- needed by plants, and maintain environmental health. Thus, this strategy will support sustainable agriculture to increase crop yields with negative impacts on the environment, increase crop yields and provide stable food needs at present and in the future (Santoyo, Hernández-Pacheco, Hernández-Salmerón, & Hernández-León, 2017).

CONCLUSION AND SUGGESTION

The results showed that the availability of NH_4^+ and NO_3^- in the soil was the highest when

Elmi Junita Tarigan *et al.*: *Rhizobacteria and Sustainable Agriculture*

fertilizing with PGPR. The application of PGPR with CF of 50% confirms that it can increase yields of 25.5%, 12.9%, respectively, in two seasons. The application of PGPR can increase the efficiency of N fertilizer and reduce the use of excessive chemical fertilizers to protect the environment and become a sustainable agricultural system.

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Elmi Junita Tarigan *et al.*: *Rhizobacteria and Sustainable Agriculture*

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Elmi Junita Tarigan *et al.*: *Rhizobacteria and Sustainable Agriculture*

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