EFFECT OF IRRIGATION, CHEMICAL FERTILIZATION, AND PROBIOTICS IN RICE FIELDS SOIL PROPERTIES

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ABSTRACT

Rice crop requires a large amount of base fertilizer through slow-release behaviour fertilizer. Implementation of the system of rice intensification with probiotics can be a potential innovation as a suitable strategy to improve soil properties and increase rice yields in an environmentally friendly manner. The aim of this study was to determine nitrogen fertilizer in the soil through impacts of two different rice cultivation methods particularly rice conventional and system of rice intensification. A field experiment was conducted in 2018 August until December. The experiment included three treatments viz. conventional method with 100% chemical fertilizer as a control, SRI method with 50% chemical fertilizer + 100% probiotics, and SRI method with 25% chemical fertilizer + 100% probiotics. Soil sample were taken from each treatments in six growth stages of rice i.e. 20, 30, 41, 62, 91, and 105 days after transplanting. Soil chemical NO_3^- was analysed by Ion Chromatography and organic content was analysed by Raman Spectroscopy. The results showed that soil texture tends to be uniform in SRI Field and NO_3^- was higher in SRI Field compared to conventional fields. Integration SRI with probiotics and chemical fertilizer can release NO_3^- that can be taken by the plants and reduce the use of chemical fertilizer by up to 75%. In conclusion, the study indicated that the SRI method with the addition of probiotics and chemical fertilizer had performed better than rice conventional. Moreover, probiotics have been contributed to support SRI method to distribute fertilizer evenly as compared with conventional method. Thus this method becomes sustainable management practice in rice field.

Keywords: System of rice intensification, Probiotics, Raman Spectroscopy, Ion Chromatography

1. INTRODUCTION

Rice (*Oryzasativa* L.) is the staple food for more than half population of the world. Commonly, rice is cultivated by conventional and System of Rice Intensification (SRI) method. Interestingly, SRI method with some innovations has been widely adopted and showed significanteffect on rice yield (Thakur *et al*., 2011; Glover, 2011; Chapagain*et al*., 2011; Ly *et al*., 2012; Ndiiri*et al*., 2013; Wu *et al*., 2015; Hidayati*et al*., 2016; Rui-Qi*et al*. 2018; Khadka and Uphoff, 2019) and water management (Thakur *et al*., 2011; Hameed *et al*., 2011; Adusimilli and Bhagya, 2011). Hence, SRI is approved to be a strategy to improve rice yield with more productive and more sustainable through reduce chemical fertilizer, water, and seed use. It has been proposed that SRI method as great potential to create a better growing environmental condition for rice cultivation in the future (Glover, 2011; Chapagain*et al*., 2011; Wu *et al*., 2015; Shiang-Min *et al*., 2018) compared with the conventional method. However,

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continuously application SRI method with chemical fertilizer will have a negativ effect on soil degradation and the environment in the future. Therefore, one approach to support the SRI method is the use of integrated microorganism by plant growth promoting rhizosphere (PGPR).

The PGPR is already known as bacteria that havea direct and indirect effect on plant growth and is suitable to replace the chemical fertilizers for the improvement of rice yield in an eco-friendly manner (Glick, 2012; Kundan*et al*., 2015; Khan *et al*., 2017). Recent of study, the PGPR are revealed significantly effect on rice yield and decrease the chemical fertilizer i.e. by enhancing Nitrogen fixation and/or Phosphate solubilization (Baker and Gehan, 2011; Lavakus*et al*., 2014; Prasnna*et al*., 2015; Khan *et al*., 2017). Nitrogen is playing a crucial role in determining the rice yield production by biochemical and physiological functions of the plant (Hassan *et al*., 2014; Leghari*et al*., 2016). Subsequently, the plant can absorb Nitrogen by two forms of inorganic ions, one of them is NO_3^- (Torres-Oliver *et al*., 2014). As a result, the existence of PGPR is expected to produce nitrogen available in the soil for plants uptake and it can reduce the use of chemical fertilizers.

The objectives of the present study were to investigate the rice growth and yield in response to nitrogen fertilizer in the soil through conventional method and SRI method with probiotic and different concentration of chemical fertilizers then analysed using Raman spectroscopy and Ion Chromatography. Raman spectroscopy can do rapid screening and detects the chemical properties on soil and iron chromatography can identify nutrient content in the soil.

2. METHODS

2.1 Field Trials and Experimental Site

This research was conducted from August to December 2018 in Guanshan, Taitung, Taiwan (23.101789, 121.204465). The treatment of this experiment was included, (1) conventional with 100% of chemical fertilizer as a control, (2) SRI with 50% of chemical fertilizer and 100% of probiotics, and (3) SRI with 25% of chemical fertilizer and 100% of probiotics. The materials used were rice seed (Kaohsiung 147); chemical fertilizer including 45% Urea, 60% Potassium, and 36% Phosphate; for probiotic was enriched with plant growth promoting rhizosphere (PGPR). Chemical fertilizer was applied as basal, during panicle initiation, at the flowering stage, and followed by probiotics. Irrigation and weeding were done when it was necessary.

2.2. Soil Samples Collection

The soil was collected six times during the stage of rice development. Each sample was collected at 20, 30, 41, 62, 91 and 105 days after transplanting. Six sub-samples per plot were collected at 15-20 cm depth. The soil samples were placed in plastic and stored at 25℃ for nitrogen analysis by Raman spectroscopy and Ion Chromatography.

2.3 RamanSpectroscopy

Raman spectroscopy is used to determine ion by using dry soil. 10 g of soil was placed into the watch glass and placed in an oven at a temperature of 60℃ for 4 days. Afterwards, grind the soil become a fine texture, and placed the soil in Kraft paper bag to homogenized, and storage at 25℃ in incubator prior to analysis.

Raman spectroscopy analysis were performed by using a ProTT-EZRaman-A6® at a wavelength of 785 nm. The data were acquired by using the SLSR ReaderV8.5.6 software. The peak of Raman intensity was collected sequentially every 60 seconds. This process was repeated 20 times

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2.4 Ion Chromatography

Ion Chromatography is used to compare the accuracy and reproducibility of the Raman Spectroscopy to measure NO_3^- in soil. 20 g of soil sample was placed in Erlenmeyer flash and add 80 ml milliQ. After that, put the soil in to an ultrasonic oscillator for one-half hour. The supernatant was decanted and filtered through Whatman filter paper by 11 µm of paper size to separate the soil from the supernatant. The supernatant is centrifuged with 12000 rpm for 10 minutes to separate the soil and the water. The supernatant is filtered by 0.45 µm and 0.22 µm of membrane filter size and placed in a conical tube. The supernatant was storage at the freezer with -20℃ to NO_3^- analysis. In this experiment, the standard solution for iron chromatography was prepare made mixing standard solution of ions under test by 0.1, 0.5, 1.0 5.0 and 10.0 ppm.

3. RESULTS AND DISCUSSION

3.1 Effect of Rice Cultivation on Growth and Yield of Rice

Table1 presents a comparison of vegetative and generative growth for SRI and conventional paddy cultivation. SRI method with 25% of chemical fertilizer was able to increase vegetative growth on panicle number per hill, ration of productive tiller and average panicle length, also in generative growth on rice ears weight, grain weight and grain number compared to that of SRI with 50% of chemical fertilizer and conventional method. However, 1000-grain weight is found in SRI method with 50% of chemical analysis.

Table 1. Effect of Rice Cultivation Methods on Rice Growth and Yield

Parameter (105 days after transplant)	Conventional farming	SRI+25% fertilizer	SRI+50% fertilizer
Panicle number per hill (no.)	18.83 ± 1.27	20.67 ± 0.82	17 ± 0.94
Ratio of productive tiller (%)	87.31 ± 5.6	90.41 ± 2.44	77.26 ± 2.03
Average Panicle length (cm)	16.69 ± 0.36	16.83 ± 0.12	16.65 ± 0.05
Rice ears weight (g)	33.69 ± 1.69	39.71 ± 2.2	39.32 ± 1.33
Grain weight (g)	32.29 ± 1.63	38.12 ± 2.12	37.91 ± 1.28
1000-grain weight (g)	20.6 ± 0.11	20.4 ± 0.06	21.6 ± 0.1
Grain numbers (no.)	1860 ± 111.99	2246 ± 79.58	1911 ± 73.99

The results indicated that to meet the maximum plant growth demand, the supply of N from 100% probiotic was insufficient. In addition, the probiotics as a PGPR have the ability to increase the availability of N in soil by the addition of NO_3^-, thereby the roots of rice plant could uptake of N for plant growth. This research has suggested that the existence of probiotics could reduce the use of chemical fertilizer and nutrient leaching. This result was supported by Adusumuli and Laxmi (2011), Barisson and Uphoff (2011), Lavakush*et al*. (2014); Prasanna*et al*. (2015) that implementation of SRI method with an innovation showed significantly increase in vegetative and generative growth; also reduce the use of chemical fertilizer compared to conventional method.

3.2 Detection of Nitrate by Using Raman Spectroscopy

The intensity of Raman Spectroscopy showed different values for each treatment. This research showed that the peak area was found to vary linearly. However, as expected, the highest peak intensity at 455, 544, 1056, 1300, 1581 and 1756 cm^{-1} of the band was found in SRI method with 50% of chemical fertilizer and followed by conventional method and SRI 25% of chemical fertilizer as shown in figure 1. In addition, the peak intensity at 2344 cm^{-1} on 91 days after transplanting showed higher in SRI with 25% of chemical fertilizer than others (Figure 1). It is indicated that the fertilizer could be distributed evenly in the irrigation system.

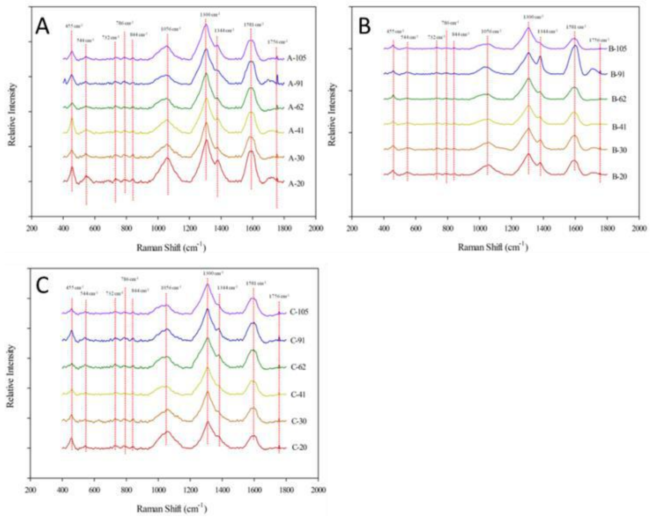

Figure 1. A comparison of Raman intensities of nitrate (NO_3^-) in soil with different method.

The number of 20, 30, 41, 62, 91, and 105 indicate days after transplanting. (A) 50% Fertilizer and Probiotics and SRI; (B) 25% Fertilizer and Probiotics and SRI (C) Customary Agricultural Law 100% Fertilizer. NO_3^- has two main bands in the spectrum, there are NO symmetrically stretched and NO plane deformation. It existed due to the symmetry generated in the state of reducing crystallization (Zapata *et al*., .2018). This research showed that $NO_3^-$was located in 1056 cm^{-1}, SO_4^{2-} was located in 844 cm^{-1}(Figure 1) and NO_2^- was located in 1364 cm-1 (Figure2). In Figure 2, it was seen that the peak of NO_2^- showed higher than NO_3^- and it might be the NO_2^-derived NO_3^-.

Moreover, research studies showed that the location of NO_3^- band was different. The NO_3^- was found in 1056 cm^{-1} using SERS by Gajaraj*et al*. (2013) and 1049 $cm^{-1}$by Zapata *et al*., 2018. It was supported by Zapata and García-Ruiz (2018) that the ranges of spectral were considered by vibrational modes of the anion (due to their covalent bonds) and their shift due to the interaction of cation and anion. Interestingly, the Raman Spectroscopy method could easily detect the concentration of NO_3^- in soil regardless of the change concentration of NO_3^-.

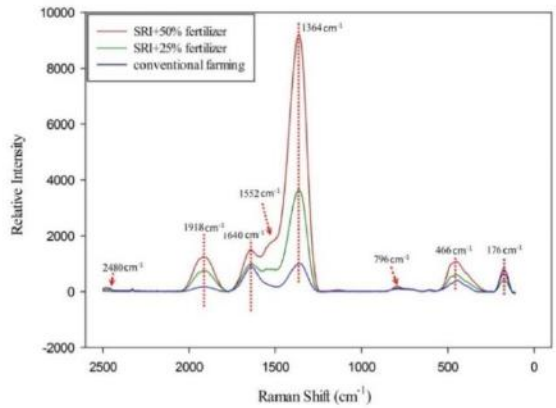

Figure 2. Average Raman Spectroscopy of soil solution extraction on 30 days after transplanting.

The peak intensity at 1364 cm^{-1} was assumed might be the phenolic compound and NO_2^-.

3.3 Detection of Nitrate by using Ion Chromatography

Our interest is to support Raman spectroscopy data by using ion chromatography system. Briefly, the NO_3^- in SRI method with 100% of probiotic and chemical fertilizer is higher than the conventional method on 30 days after transplanting (Table 2). It was because of the existence of probiotic which can release the NO_3^-. However, on 40 days after tansplanting NO_3^-showed decrease as shown in Figure 3. It could be caused by the addition of excess chemical fertilizer in this SRI field. Nearly similar results were also observed by Galloway *et al.* (2008) *in*Vejan*et al.* (2016) which concluded under optimum fertilizer was already able to uptake nutrient for their growth and development, hence the effect of PGPR application seemed to be diminished or fruitless. Futhermore, the excess available nutrient, notably nitrogen, wouldn't be absorbed by plants and instead gets leached into the groundwater.

Table 2. Effect of Cultivation Method on NO_3^-in Different Stages of Rice

NO_3^-						
Days	**conventional farming**		**SRI+25% fertilizer**		**SRI+50% fertilizer**	
20	1.5851	±0.5175	1.5173	±0.4426	1.9609	±0.5757
30	1.8236	±0.5556	3.6548	±0.4684	2.4502	±0.5395
41	0.1423	±0.0095	0.1715	±0.0128	0.3211	±0.1509
62	0.2056	±0.0442	0.2437	±0.0197	0.3411	±0.1110
91	0.5021	±0.0727	0.6215	±0.2647	0.6495	±0.1243
105	0.8292	±0.2130	0.2560	±0.0633	0.3311	±0.0436

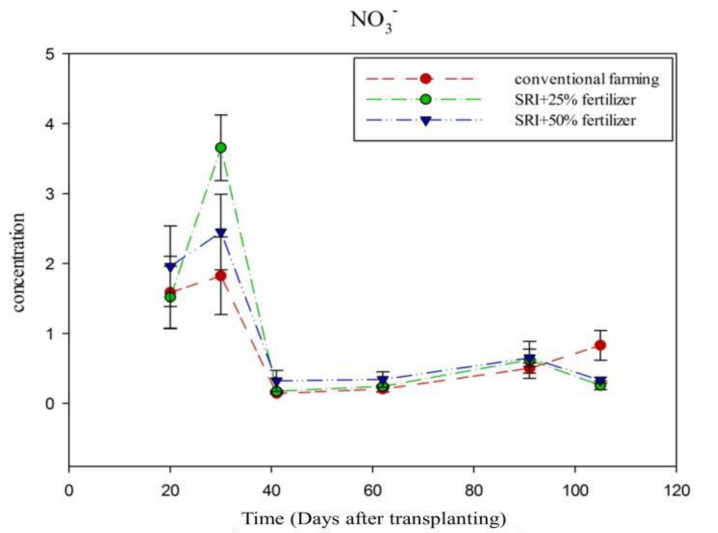

Figure 3. The Trendline ofIon Chromatography on NO_3^- in Rice Field .

4. CONCLUSIONS

The study revealed that the SRI method with 100% of probiotics and 25% or 50% of chemical fertilizer were effective than conventional method. On 30 days after transplanting, the $NO_3^-$and NO_2^{-were} higher than in SRI method with 100% of probiotics and 50% and 25 % of of chemical fertilizer, than conventional method, respectively. However due to the excess of nitrogen, the NO_3^- decreased gradually and the highest NO_2^- derived NO_3^-.

In this study, two soil analysis method were compared by Raman Spectroscopy and Ion Chromatography. Nevertheless, Raman spectroscopy could not completely replace Iron chromathography.

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