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Assessment of multifunctional biofertilizer on rice seedlings (MR 219) growth in a greenhouse trial

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ABSTRACT

Aims: Food security and safety are current issues around the world. Rice is a staple food for a large portion of the world's population, and in Malaysia around 2.2 million tonnes of rice were consumed annually. Fertilizer input is the key to increase rice production. Malaysian Nuclear Agency (Nuclear Malaysia) has developed multifunctional biofertilizer products in an effort to reduce dependency on chemical fertilizers for crop production.
Methodology and results: Multifunctional biofertilizer products contain indigenous microorganisms that have desired characteristics, which include plant growth promoting, phosphate solubilising, potassium solubilising and enhancing N_2-fixing activity were used in this study. These products were formulated as liquid inoculants, which is suitable for rice production. A greenhouse trial was conducted to evaluate the effectiveness of multifunctional biofertilizer on rice. Multifunctional biofertilizer products were applied singly and in combination with rock phosphate, feldspar and irradiated oligochitosan from a project by Forum for Nuclear Cooperation in Asia (FNCA), Japan. Plants that were given chemical fertilizers and plants not receiving fertilizer or biofertilizer products were used as controls. Fresh and dry weights of rice seedlings were determined. Application of multifunctional biofertilizer combined with irradiated oligochitosan (T3) resulted in significantly higher fresh weights (97.12 g) and dry weights (84.16 g) as compared to non-treated plants (77.39 g fresh weights and 69.56 g dry weights). The results suggested that application of multifunctional biofertilizer in combination with irradiated oligochitosan could increase rice growth.
Conclusion, significance and impact of study: Combination of biofertilizer with oligochitosan increased rice growth in greenhouse trial. Further investigation of this interaction phenomenon should be carried out.

Keywords: multifunctional biofertilizer, irradiated oligochitosan, rock phosphate, feldspar, *Oryza sativa*

INTRODUCTION

Rice is the staple food for most people in Malaysia. Land utilisation for paddy production is 674,928 hectares, which is 76 percent in Peninsular Malaysia (515,657 ha) while Sarawak and Sabah accounted for 18 percent (118,919 ha) and 6 percent (40,352 ha) of the total hectares, respectively (Ramli *et al.*, 2012). Fertilizer is the major input for rice production. According to Food and Agriculture Organization of the United Nations (FAO), 2012, world fertilizer nutrient ($N+P_2O_5+K_2O$) consumption is estimated to reach 180.1 million tonnes in 2012, up by 1.9 percent over 2011. World demand for total fertilizer nutrients is estimated to grow at 1.9 percent per annum from 2012 to 2016 (FAO, 2012). Fertilizer price is increasing due to the demand. In Malaysia, government subsidy on chemical fertilizers helps to maintain the productivity of the paddy farms. However, such subsidy could be a burden for the government. Low cost and effective agriculture inputs are thus needed for sustainable paddy production. Biofertilizer and oligochitosan produced from natural resources could be an alternative to reduce demand for chemical fertilizers. One of the benefits from biofertilizer is the contribution from population of functional microorganisms available in the environment. These microorganisms may enhance plant growth and create healthy rhizosphere. Biofertilizer enhanced the growth of rice (Tan *et al*., 2011), Chinese cabbage (Phua *et al.*, 2012a) and tomato (Phua *et al*., 2012b). Chitosan is produced by deacetylation process of chitin, produced from shellfish wastes or fungi. Irradiation of this polymer led to reduction of molecular weight by scission of glycosidic linkage, thus produced oligochitosan (Gryczka *et al*., 2008). Oligochitosan, an irradiated chitosan was used as plant growth promoter. The effect of chitosan on enhancing leaf area, length of roots and newly developed shoots of *Salix viminalis* L. var. *gigantea* cutting has been reported. Greater fresh and dry weights of these organs were shown in chitosan treated plants. Irradiated chitosan showed greater effect of growth in comparison with the non-irradiated form (Gryczka *et al*., 2008). Radiated oligochitosan showed enhancement of

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rice seedling growth by 15-20% for transplanting (Khairul Zaman *et al.*, 2010). Hien (2004) showed fresh biomass of chrysanthemum plants increased up to 68% with the treatment of 100 ppm of oligochitosan. These previous works showed oligochitosan enhanced growth of plants. Both oligochitosan and biofertilizer could enhance growth of plants. However, different biofertilizer and oligochitosan may have different effects. The present study utilised multifunctional biofertilizer products from Nuclear Malaysia and gamma irradiated oligochitosan produced from a project of Forum for Nuclear Cooperation in Asia (FNCA). There has been no study on joint application of biofertilizer and oligochitosan on plants in Malaysia. Therefore, the objective of the present study is to evaluate effectiveness of biofertilizer and oligochitosan on the growth of rice in a greenhouse trial.

MATERIALS AND METHODS

Multifunctional biofertilizer

Multifunctional biofertilizer was prepared by culturing two bacteria isolates, namely AP2 and AP3. Both AP2 and AP3 were phosphate and potassium solubilising bacteria isolated from “Natural Farming” compost, produced by Malaysian Nuclear Agency (Nuclear Malaysia). Isolate AP2 was also a plant growth promoter (Phua *et al*., 2012a). Phosphate and potassium solubilising activities were screening by culture isolates on phosphate agar plate (Freitas *et al.*, 1997) and Aleksandrov media agar plates (Hu *et al*., 2006). The plates were incubated at 28 $\pm$ 2 °C for 14 days. Observations were done every two days. Clear zone production indicated positive activities. Phosphate agar plates were prepared by mixing potato dextrose agar (39.0 g); yeast extract (1.0 g) and distilled water (1000 mL). K_2HPO_4 (10% w/v) and $CaCl_2$ (10% w/v) were sterilised separately and added to potato dextrose yeast agar prior to pouring onto plates. Aleksandrov media agar plates were prepared by mixing glucose (5.0 g); $MgSO_4{\cdot}7H_20$ (0.005 g); $FeCl_3$ (0.1 g); $CaCO_3$ (2.0 g); potassium mineral (2.0 g); $Ca(PO_4)_2$ (2.0 g), agar (28.0 g) and distilled water (1000 mL). Plant growth activity was tested for indole-3-acetic acid (IAA) production. The isolates were cultured on TSA amended with 1-tryptophan, followed by overlying them with 82 mm diameter nitrate cellulose membrane and incubation at 28 °C for 3 days. The membranes were overlaid on filter paper saturated with Salkowsky’s reagent (Gordon and Webber, 1950; Alvarez *et al*., 1995). Isolates producing IAA showed pink to red colour after 0.5 to 3 h. Multifunctional biofertilizer were prepared as liquid form. Isolates AP2 and AP3 were grown in nutrient broth (NB). These liquid biofertilizer were cultured for 48 h on an orbital shaker.

Greenhouse experiment

An experiment to evaluate the effects of multifunctional biofertilizer and irradiated oligochitosan on the growth of rice plants was conducted in the greenhouse. Rice variety MR 219 was used in this study. Ten centimeters rice seedlings (approximately 3-4 leaves) were planted in pots containing clay soil mixtures. Irradiated oligochitosan from the project of Forum for Nuclear Cooperation in Asia (FNCA), Japan, was prepared at concentration of 40 ppm. Christmas Island Rock Phosphate (CIRP) and muriate of potash (MOP) were used as sources of phosphate and potassium. Seven treatments were applied to the rice seedlings, viz. Treatment 1 (T1) - 50 mL liquid biofertilizer per pot; Treatment 2 (T2) - 50 mL liquid biofertilizer with CIRP (4.5 g) and MOP (3.5 g) per pot; Treatment 3 (T3) - 50 mL liquid biofertilizer with 40 ppm oligochitosan (spray) per pot; Treatment 4 (T4) - CIRP (4.5 g) and MOP (3.5 g) per pot; Treatment 5 (T5) - oligochitosan (40 ppm) was sprayed ; Treatment 6 (T6) - chemical fertilizers (10 g NPK and 1 g urea) were applied as; Treatment 7 (T7) - non-treated plants as Control. All treatments were given monthly. There were two replications for each treatment with three plants for each replication. The experimental design was a completely randomised design (CRD). Crops were harvested after three months. Fresh and dry weights of the seedlings were measured. Data were analysed by ANOVA with the means separated by Duncan’s test (p < 0.10).

RESULTS AND DISCUSSION

Effects of biofertilizer and oligochitosan on the fresh and dry weight yields of rice in greenhouse trial are shown in Figures 1 and 2. Application of multifunctional biofertilizer combined with irradiated oligochitosan (T3) resulted in significantly higher fresh weights (97.12 g) as compared to non-treated (77.39 g) plants (Figure 1). Figure 2 shows combination treatment biofertilizer with CIRP and MOP (T2) or oligochitosan (T3) enhanced the rice seedling dry weights compared to other treatments. Dry weight for T2 was 84.7 g and T3 was 84.16 g. Although application of treatment 3 (T3) did not show significant higher dry weights as compared to other treatments, it produced the highest dry weights. These results suggested application of multifunctional biofertilizer in combination with irradiated oligochitosan increased rice yields. Application of biofertilizer with CIRP and MOP also increased rice yield. This could be due to phosphate and potassium solubilising activities by the phosphate and potassium solubilising bacteria. Both bacterial solubilisers transformed unavailable phosphate and potassium to available forms for uptake by the rice plants. Tan *et al.* (2012) also reported liquid biofertilizer containing rhizobia and PGPR increased rice vigour and growth. In a separate study Khairul Zaman *et al.* (2010) reported irradiated oligochitosan enhanced growth of rice seedling by 15 to 20%. Anas and Rakhmadina (2013) showed that application of 50 % inorganic NPK fertilizer in combination with oligochitosan, Vitazyme and Azozo biofertilizer increased the number of tillers and yield of rice significantly which had similar effect of application 100 % inorganic NPK fertilizer. Pham (2013) also reported application of oligochitosan and biofertilizer increased cabbage yield of 28.81 % compared to control applied with 100 % recommended NPK rate. In line with these

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results, enhancement of the yields may due to phosphate, potassium solubilising and plant growth promoting activities from biofertilizer microorganisms or plant growth stimulation from oligochitosan. However, the phenomenon of interaction between biofertilizer and oligochitosan to promote plant growth needs further investigation.

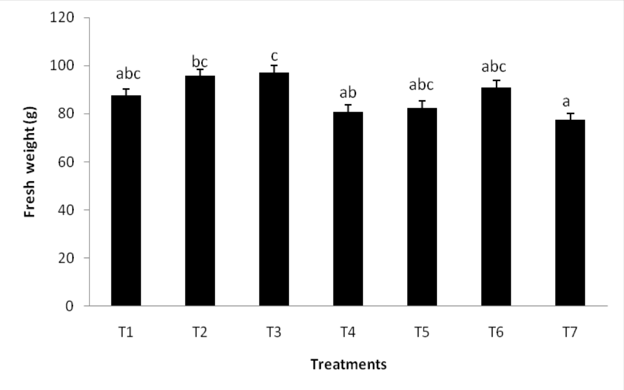

Figure 1: Fresh weights (g) of rice seedlings. Note: Bars with the same letter are not statistically different among the treatments following Duncan's test ($p < 0.10$).

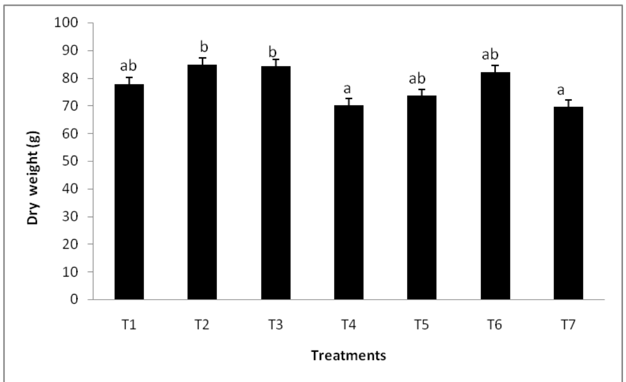

Figure 2: Dry weights (g) of rice seedlings. Note: Bars with the same letter are not statistically different among the treatments following Duncan's test ($p < 0.10$).

CONCLUSIONS

Combination of biofertilizer with CIRP and MOP or oligochitosan enhanced rice seedlings growth in a greenhouse trial. Further investigation of this interaction phenomenon should be carried out. Use of biofertilizers to complement chemical fertilizers would reduce sole dependency on chemical fertilizers.

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