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## SHORT COMMUNICATION

# The effect of FRAW towards the growth of chilli seedlings and its associated microorganisms

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## ABSTRACT

This study aims to evaluate the effect of Fermented Rice After-wash Water (FRAW) on chilli growth and to isolate microorganism present in three brands of white rice FRAW. The study showed that FRAW treatment was comparable with NPK fertiliser. In addition, a number of plant growth-promoting microbes associated with FRAW were also isolated. Isolated bacteria and fungi were then characterised according to their morphology and biochemical analysis. Thus the positive effect of FRAW on the chilli was likely due to the plant growth promoting microorganism present in FRAW.

*Keywords:* Fermented Rice After-wash water, biofertiliser, *Capsicum annum*, plant growth-promoting bacteria

## INTRODUCTION

The current world population had reached more than 7.5 billion and estimated to reach almost 10 billion people in 2050 (United Nations, 2017). The increasing number of populations has led to an increase in food demand. To address these challenges, farmers rely on agrochemical products to improve the crop productivity and pest control. Overuse of these agrochemical products, however, introduces new problems to the environment and human health. In accordance with the United Nation 2030 Agenda for Sustainable Development, a goal to manage and reduce the release of chemicals and wastes into the environment was implemented (United Nations General Assembly, 2015). Thus, sustainable agriculture using biofertiliser was proposed to mitigate the use of agrochemical products.

Biofertiliser consists of living microorganisms that can enhance soil fertility and improved plants growth by increasing nutrients availability and uptake (de Souza *et al*., 2015). It is made by fermenting organic matters using available stock culture or from surrounding environment (Shin *et al*., 2017). In Indonesia, domestic waste such as water from rice after-wash is frequently used as biofertiliser (Rosalina, 2008; Istiqomah, 2012; Nasution *et al*., 2013; Wardiah and Rahmatan, 2014; Bayuana, 2015; Bahar, 2016).

Studies have demonstrated the positive effect of rice after-wash water on vegetables such as *Phaseolus radiatus* L. (Istiqomah, 2012), *Lactuca sativa* L. (Wulandari *et al*., 2013), *Brassica rapa* (Wardiah and Rahmatan, 2014) and *Ipomoea reptans* Poir (Bahar, 2016). The growth promoting effect was due to the presence of phosphorus (~14%), magnesium (~13%), calcium (~3%) and other micronutrients such as iron, sulphur, calcium and vitamin B1 (Wulandari *et al*., 2013). Due to the presence of carbohydrates and proteins, rice after-wash water can be fermented to promote beneficial microorganism that can facilitate plant growth (Nasution *et al*., 2013).To date, several studies have demonstrated the positive effect of fermented or non-fermented rice after-wash water. However, microorganism associated with the FRAW has yet to be described.

In this study, chilli was chosen to evaluate the growth promoting effect of FRAW. A single Malaysian in average consumed at least 2-2.5 kg of chillies per year with more than 50% of the supply depends on import (Department of Statistics Malaysia, 2015). Due to suitable climate condition, chilli has gained more interest in local agriculture and urban farming. Therefore, this study would provide the local chilli farmer or urban farmer with an environmentally friendly alternative to agrochemical fertiliser.

## MATERIALS AND METHODS

### Preparation of Fermented Rice After-wash Water (FRAW)

FRAW was prepared from three different rice brands (Faiza Emas; BN, Super (Siam) - Udang Merah; BC, Super - Rama-Rama; BA) according to Carandang and

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Gentry (2013). Briefly, 10 g of rice was mixed with 50 mL sterilised water and left for a week in a bottle at room temperature and covered with sterilised cloth. After a week, the fermentation produced three layers of substances. The middle layer that consists of microbes was separated into sterilised bottle followed by addition of 500 mL of milk to promote lactic acid bacteria (LAB) growth. The mixture was fermented again for another week at room temperature and resulting curd was removed. The solution obtained after removing the curd was known as FRAW and used to treat the chilli seedlings.

**The effect of FRAW towards chilli growth**

The experimental framework to understand the effect of FRAW towards chilli growth involved seed preparation, randomised treatment and analysis. Seeds from F1 chilli were soaked in water that was filtered using 0.2 µm membrane for 24 h until germination. For this study, 50 seeds were chosen randomly and planted into five groups where each group consists of 10 germinated seeds. The seeds were planted into 1:1 ratio of sandy and loam soil and left in the shade. The seedlings were watered 2-3 times a day to maintain the soil humidity. After the seedling start to grow leaves (~10 days), each group was treated with 10 mL of FRAW, liquid NPK fertiliser (NPK) and tap water (control) accordingly. FRAW, NPK and tap water were administered once a week and the seedlings were watered with tap water 2-3 times a day. The plant height, root length and number of leaves were measured at the 5$^{th}$ week. Statistical analysis was performed to evaluate the significant effect of FRAW of different rice brands on the plant height, root length and number of leaves. One-way analysis of variance (ANOVA) with Tukey's multiple comparison tests was used for all parameters.

**The diversity of microorganism in FRAW**

The diversity of microorganism in FRAW was investigated by isolation of bacteria on nutrient agar (NA) and fungi on potato dextrose agar (PDA). FRAW was serially diluted and spread on both agar. Inoculated NA and PDA were then incubated at 37 °C for 24 h at 30 °C for a week, respectively. Isolated and purified bacteria were characterised microscopically and a series of biochemical assays that includes catalase, methyl red-Voges-Proskauer, arabinose sugar fermentation, triple sugar iron (TSI), Simmon citrate, nitrate reduction and starch hydrolysis. Fungi were characterised based on colony morphology, colour, mycelium structure and pigmentation. Wet mount and lactophenol cotton blue staining were used to observe fungi microscopically.

**RESULTS AND DISCUSSION**

In this study, FRAW were shown to improve the number of leaves, stem height and root length of chilli seedlings (Table 1). However, the effect differs when FRAW was prepared from different brands of white rice. Seedlings treated with Faiza Emas FRAW (BN) demonstrated an increase in shoot height, root length and number of leaves compared to the rest of treatments and control (Table 2). Treatment with BN also resulted in increased shoot height and number of leaves that were comparable to NPK treated seedlings. In contrast, all seedlings treated with FRAW showed a significant root length improvement compared to control and NPK. As shown in Table 2, the root of seedling treated with NPK was not significantly better than tap water treatment (control). In the FRAW treated group, however, seedlings treated with BN showed a superior growth ($p<0.0001$), followed by BC ($p<0.01$) and BA ($p<0.05$). Similar observation was also reported by Budiartoa *et al*. (2006), where the root length and capacity of chrysanthemum was superior when treated with rice husk compared to NPK fertiliser.

**Table 1:** Mean measurement of growth parameter for chilli seedling treated with different brand of Fermented Rice After-wash Water (FRAW) for 5 weeks.

| Treatment | Shoot height (cm) | Root length (cm) | No. of leaves |
|---|---|---|---|
| Control | 5.35±0.19 | 3.00±0.30 | 3±1 |
| NPK | 6.80±0.20 | 3.50±0.20 | 6±1 |
| Super (Rama-rama) (BA) | 6.32±0.67 | 3.80±0.36 | 4±1 |
| Super-siam (Udang Merah (BC) | 6.06±0.24 | 4.00±0.20 | 4±1 |
| Faiza Emas (BN) | 6.46±0.53 | 4.50±0.17 | 6.00 |

The table summarised the mean measurement with standard error. N=3

**Table 2:** A summary of one-way ANOVA statistical analysis of FRAW effect on chilli seedling parameters.

| Parameter | Treatment | | | |
|---|---|---|---|---|
| | NPK | BA | BC | BN |
| Shoot height (cm) | ** | * | ns | ** |
| Root length (cm) | ns | * | ** | **** |
| No. of leaves | ** | Ns | ns | ** |

NPK; NPK fertiliser, BA; Super (Rama-rama), BC; Super-siam (Udang Merah), BN; Faiza Emas, N=3, ns= not significant, * = $p<0.1$, ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$

The growth improvement of FRAW was probably due to several factors such as the composition of FRAW, production of secondary products such as auxin as well as microbial diversity. The differences in rice after-wash water composition and their growth promoting effect were already demonstrated by Wulandari *et al*. (2013). In this study, insignificant effects on *Lactuca sativa* L. shoot height and root length were observed. Thus, they conclude that the effect of different rice after-wash water composition is negligible. In contrast, the positive impact of FRAW on plant growth was demonstrated on *Zea mays* shoot height, root length and leaves number (Nasution *et*

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*al*., 2013). Fermentation of the rice after-wash water could enrich beneficial microbes that promote plant growth.

To the best of our knowledge, this study is the first to describe the occurrence of bacteria and fungi in the FRAW. A total of 24 bacteria and 12 fungi were isolated from the FRAW. As summarised in Table 3, we have isolated and identify several plant growths promoting bacteria and fungi such as *Bacillus* spp., *Lactobacillus* spp., *Pseudomonas* spp. and *Trichoderma* spp. from the FRAW.

**Table 3:** A summary of bacteria and fungi isolated from FRAW from different brand of white rice.

| Rice Brands | Bacteria | Fungi |
|---|---|---|
| Super (Rama-rama) BA | *Bacillus* sp.<br>*Corynebacterium* sp.<br>*Lactobacillus* sp.<br>*Pseudomonas* sp.<br>*Staphylococcus* sp. | *Aspergillus* sp.<br>*Penicillium* sp.<br>*Rhizopus* sp.<br>*Saccharomyces* sp. |
| Super-siam (Udang Merah) BC | *Bacillus* sp.<br>*Corynebacterium* sp.<br>*Clostridium* sp.<br>*Lactobacillus* sp. | *Paecilomyces* sp.<br>*Pestalotiopsis* sp.<br>*Rhizopus* sp.<br>*Trichoderma* sp. |
| Faiza Emas BN | *Bacillus* sp.<br>*Corynebacterium* sp.<br>*Lactobacillus* sp.<br>*Streptococcus* sp. | *Aspergillus* sp.<br>*Pestalotiopsis* sp.<br>*Rhizopus* sp.<br>*Saccharomyces* sp. |

Details for bacterial and fungal characterisation was stored in Mendeley Dataset and retrievable via http://dx.doi.org/10.17632/87k58mysnd.1

The most common genus in all FRAW was *Bacillus* spp. and *Lactobacillus* spp. Both genera were known to promote plant growth either as biofertiliser or biocontrol agent against plant diseases (Ahemad and Kibret, 2014). *Bacillus amyloliquefaciens*, for example, could produce auxin to promote plant growth (Talboys *et al*., 2014; Yuan *et al*., 2014) and antifungal peptide as a biocontrol agent (Yuan *et al*., 2014; Zhang *et al*., 2017). *Bacillus subtillis* also exhibited an antagonistic effect on pathogenic fungi by producing an antifungal compound that caused structural deformities (Chaurasia *et al*., 2005). *Bacillus thuringiensis* instead were known to produce toxin for insecticides (Jouzani *et al*., 2017). A high frequency of *Lactobacillus* spp. was probably caused by the addition of milk during the FRAW preparation. Lactic Acid Bacteria (LAB) were known endophytes (Minervini *et al*., 2015) in sour dough wheat and effective biocontrol agent for bacterial spot disease in pepper (Shrestha *et al*., 2014). LAB were also known to facilitate plant nutrient absorption by hastening organic compound decomposition (Partanen *et al*., 2010), solubilising phosphate (Shrestha *et al*., 2014; Giassi *et al*., 2016) and fixing nitrogen (Giassi *et al*., 2016).

Another common plant growth bacteria and biocontrol agent that was identified in FRAW from BA is *Pseudomonas* spp. Dinesh *et al.* (2015) reported that *Pseudomonas* spp. did not have direct growth promoting traits such as the production of indoleacetic acid or ammonia towards ginger. *Pseudomonas* spp., however, could synthesise hydrolytic enzymes and facilitate mineral solubilisation in the soil. *Pseudomonas* spp. also inhibit the growth of another plant pathogen such as *Pythium myriotylum* (Dinesh *et al*., 2015).

*Corynebacterium* spp. was also identified from each FRAW. This species can be recovered from various sources such as food (cheese, dairy product), marine sediment, sludge and coral (Bernard and Funke, 2015). However, most *Corynebacterium* spp. are important pathogen such as *C. diphtheria* while non-pathogenic species such as *C. glutamicum* is important for glutamic acid production in the food industry. Therefore, further identification and characterisation of *Corynebacterium* spp. is needed before suggesting the bacteria as a growth promoter or biocontrol agent.

In addition to bacteria, a number of fungi also present in the FRAW that may contribute to the growth promoting effect (Table 3). Fungi such as *Trichoderma* spp. (Zhang *et al*., 2016), *Penicillium* spp.(Shivanna *et al*., 1994) and *Saccharomyces* spp.(Malusá *et al*., 2012) were known to improve plant resistance towards disease. In addition, besides improving plant resistance, *Trichoderma longibrachiatum* T6 was shown to enhance wheat tolerance against salt stress (Zhang *et al*., 2016). *Rhizopus* sp. is a saprophytic fungus that may facilitate the breakdown of organic matter in soil and further improve the soil fertility (Ghosh and Ray, 2011). In contrast, the presence of *Paecilomyces* sp. and *Pestalotiopsis* sp. in BC FRAW have no clear advantages towards the plant growth except for their pathogenic and saprophytic nature (Luangsa-Ard *et al*., 2011; Maharachchikumbura *et al*., 2011). Both *Paecilomyces* sp. and *Pestalotiopsis* sp. may have contributed to a lesser growth efficiency of chillie seedling.

## CONCLUSION

In accordance with our preliminary results, we suggested that FRAW is a potential biofertiliser that is comparable with agrochemical fertiliser such as NPK. The seedlings growth improvement was possibly due to the occurrence of beneficial microorganism and the macro and micronutrients found in FRAW. However, further understanding of the FRAW's effect as a plant growth promoter is imperative.

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