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Organic fertilizers supplemented with rhizobacteria for promoting the growth of rice (*Oryza sativa* L.)

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Abstract

The effects of organic fertilizer supplemented with halotolerant rhizobacteria on rice growth were investigated. Immobilization of halotolerant rhizobacteria on various carriers was performed. The results showed that filter cake was the best carrier supporting microbial cell survival of 10^9 colony-forming unit per gram (CFU/g). The amounts of nutrients were not different during composting except for phosphorus levels which increased from 0.8-1.13% to 2.17-3.23% at the maturation phase. The germination index of rice seeds in the presence of extracts from the three compost formulas was greater than 80%, indicating the maturity of this compost. The results from pot experiments showed that organic fertilizers supplemented with the mixture of three isolates of halotolerant rhizobacteria formulation 1 (FC), a combination of filter cake and spent wash, and formulation 3 (FC+B), filter cake, spent wash and bagasse supplemented with chemical fertilizer, best promoted the highest plant growth parameters. Three halotolerant rhizobacteria, namely *Pseudomonas azotoformans* I2.1 *Enterobacter aerogenes* P8 and *Bacillus tequilensis* N15 were successfully used in combination with the inoculants to supplement an organic fertilizer. They could potentially promote the germination of rice seeds and the growth of rice plants in pot experiments.

Keywords: Organic-fertilizer, Filter cake, Bio-Fertilizer, Saline soil

1. Introduction

Saline soil is a major problem, causing low crop productivity in Thailand. Soil salinity was investigated and expressed as an electrical conductivity value (EC value), indicated the various levels of salinity as follows: 0-2 deciSiemens (dS/m) (non-saline): salinity effects negligible, 2-4 dS/m (slightly saline): the productivity or yields of sensitive crops might be restricted, 4-8 dS/m (moderately saline): the productivity and yields of many crops were restricted and 8-16 dS/m (strongly saline): only tolerant crops showed satisfying yields and productivity [1]. Under water deficit conditions, soil with salinity appeared to have ion toxicity, low nutrient contents and poor physical properties. Improvement of physical and chemical properties of saline soils can be performed by using organic fertilizers, soil amendment and appropriate cultural practices (by modifying human interactions with the environment). Saline soil is unfertile and normally deficient in nitrogen (N), phosphorus (P), and potassium (K). Amendment of saline soils with compost can increase macro-and micro-nutrients which then improve soil properties so as to be more suitable for growing plants [2].

Organic fertilizer such as compost is produced by the degradation of organic matter by aerobic cellulolytic microorganisms. The degradation rate depends on the potential of microorganisms and types of raw materials for producing compost. Agricultural waste such as the residues from sugar factories are commonly used as substrates for composting. Composting has increasingly become a popular alternative process to reduce agricultural waste. Sugar cane is an important crop in Thailand and its components, especially sugarcane bagasse, are very useful for producing valuable products. The bagasse consists of 45.5% cellulose, 27% hemicelluloses, 21.1% lignin and 6.9% of other organic substances [3]. However, during harvest in Thailand, sugarcane leaves are often burnt for the ease of harvesting stalks, causing atmospheric and environmental problems. Sugarcane leaves were reported to be high in organic matter. Moreover, filter cake is another kind of waste from the sugar industry. Filter cake is a grey-colored sludge, composed of small particles and is rich in organic and inorganic substances. Filter cake has been used before as phosphorus fertilizer for growing rice in acidic soils [4]. Spent wash is a waste from the ethanol industry. It is also rich in nutrients but has high electrical conductivity. Reducing and turning these waste materials into valuable products is one of the Thai government's policies. Reuse of industrial wastes promotes an eco-friendly system in the community; it contributes to the development of agro-industry and raises income for local people. Compost helps to improve the soil structure, while the microbes in compost are able to solubilize nutrients in natural, arid, and saline soils. Moreover, these agricultural wastes are recycled in various ways, including their use as a raw material for production of bio- fertilizer and bio-inoculum carriers. Inoculum carriers serve as media in bio-inoculant production. They control the quality and shelf life of bacterial inoculants by serving as a microenvironment for microorganisms. Besides this, type of carrier and storage temperatures are important factors for determining the shelf life of bio-inoculants [5] and acceptance as agricultural products [6].

In this study, filter cake, earthworm manure and saline soil were selected to use as carriers. These residues from the agro-industry are low cost and high in available nutrients and can be converted into valuable products. Halotolerant rhizobacteria were isolated from the rice rhizosphere of saline soils. They possessed potential plant growth properties such as nitrogen fixer, phosphate solubilizer and auxin (IAA) producer [7]. The aim of this study was to test the ability of halotolerant of rhizobacteria (*P. azotoformans* I2.1, *E. aerogenes* P8 and *B. tequilensis* N15) to enhance plant growth on soil at salinity level 6 dS/m. Also, this work investigated the carriers suitable as a supporting material for immobilizing rhizobacterial cells, by producing organic fertilizers supplemented with rhizobacterial cells using residues from the sugar-alcohol industry. These fertilizers were applied to rice grown on normal and saline soil.

2. Materials and methods

2.1 Preparation of microbial inoculums

Three halotolerant rhizobacteria (*P. azotoformans* I2.1 *E. aerogenes* P8 and *B. tequilensis* N15) were isolated from the rice rhizosphere of saline soils. These microbes had the potential to promote plant growth [7]. They were cultured in nutrient broth supplemented with NaCl solution to reach the salinity level of 10 dS/m. The high salinity activated growth in the microbial cells prior to their utilization to stimulate the growth of plant in saline soil. The inoculum was incubated on the rotary shaker at room temperature for 24 h. The culture broth was centrifuged at 5,000 rpm for 20 min and bacterial cells were suspended in sterile distilled water. Each rhizobacterial suspension was counted and the concentration adjusted to 10^8 cell/mL. The three rhizobacteria were used individually in the experiment, and a mixture of the three, combined at a ratio of 1:1:1 (v/v/v), was also tested.

2.2 Efficacy of various carriers as a supporting material for immobilizing rhizobacteria

Filter cake, saline soil and earthworm manure were selected as three different carriers for this study. All carriers were sterilized by autoclaving (121 °C, 30 min) three times. Sterility of the carriers was checked by using agar plate technique. Five milliliters of 10^8 cell/mL of halotolerant rhizobacteria (*P. azotoformans* I2.1*, E. aerogenes* P8 and *B. tequilensis* N15 which was in the form of single inoculum and a mixed culture of three isolates (ratio 1:1:1)) were cultured on 50 g of carrier and transferred to sterilized plastic bags (moisture content adjusted to 40-50%) for incubation at 30 °C for 30 days. The treatment details are presented below. Survival cells of halotolerant rhizobacteria were determined at day 0, 15 and 30 by spread plate technique on nutrient agar.

2.3 Organic fertilizer production

Three halotolerant rhizobacteria isolates (*P. azotoformans* I2.1*, E. aerogenes* P8 and *B. tequilensis* N15) or their mixture (equal volume for each isolate) at the cell densities of 10^8 cell/mL were inoculated at a concentration of 10% (w/v) into 10 kg of filter cake. Then, the inoculated filter cake was turned upside down and irrigated with spent wash every 3 days until day 15. The substrates used for the composting process to produce bio-organic fertilizer were residues from the sugar-alcohol industry such as filter cake, spent wash, bagasse, and sugarcane leaves [8]. Each compost heap was set up using 300 kg of substrate to which 3 kg of starter culture (rhizobacteria immobilized on carrier) was added. Three formulations of substrate mixture were prepared for the production of bio-organic fertilizer as formulation; F1 (FC): a mixture of two substrates (Filter cake: spent wash = 3:0.5 kg, w/v) + 3% of rhizobacteria immobilized on carrier, formulation F2 (FC+L): a mixture of three substrates (Filter cake: spent wash: sugarcane leaves = 2:0.5:1 kg, w/v/w) + 3% of rhizobacteria immobilized on carrier, formulation F3 (FC+B): a mixture of three substrates (Filter cake: spent wash: bagasse = 2:0.5:1 kg, w/v/w) + 3% of rhizobacteria immobilized on carrier. During 60 days of composting, the heap was sprayed with 5 L of spent wash and the pile was turned every 5 days. Compost samples were sampled on day 0, 30 and 60. Temperature, pH, EC, organic matter, total N, P and K and C/N ratio of the composts were measured and analyzed. Additionally, the number of nitrogens fixing bacteria [9], phosphate solubilizing bacteria [10] and cellulolytic bacteria [11] were counted on nitrogen free medium (Ashby's agar), Pikovskaya's agar and carboxymethyl cellulose agar, respectively.

2.4 Determination of organic fertilizer maturity using seed germination method

Each organic fertilizer sample was diluted with distilled water at the ratio of 1:10 and 1:100. Compost suspension was filtered through Whatman No. 1 filter paper. Rice seed surface was sterilized by soaking in 95% ethanol for 5 min and then washed 3 times with sterile distilled water. Ten seeds were placed on filter paper No. 42 in a Petri dish (for triplicate). On day 0 of seed cultivation, 3 mL of the filtrate fertilizer was sprayed onto the filter paper in a Petri dish. The plates were then incubated at room temperature in the dark for 2 weeks. The plates were irrigated with 3 mL of distilled water every 2 days. For the control treatment, rice seeds were irrigated with distilled water. Seed germination (root length and the number of germinated seeds) were measured, and seed germination index (GI) was calculated according to the modified method of Barral and Paradelo [12]. The result was interpreted from the equation Germination Index (GI = (%G × %L)/ 100;(%G) referred to the relative germination and (%L) referred to relative root elongation which was compared to the treatment control (distilled water).

2.5 Effects of organic fertilizer on rice growth in the pot experiment

Seeds of Jasmine rice (*Oryza sativa* L. (KDML 105)) were used in this study. Saline soil (salinity at around 6 dS/m) and non-saline soil (salinity at around 0.7 dS/m) were used, the experiment was completely randomized design with 8 treatments and 5 replications. Each soil sample (12 kg) was packed into a 30 × 26 cm of plastic pot. Five 30-day-old Jasmine rice seedlings were transferred into the pot and cultivated. During cultivation, the water level in the pot was maintained at 5-7 cm above the soil surface. Five g of organic fertilizer was applied to the pot on day 14 and day 30 after the initial cultivation. To test the effects of the organic-fertilizer, eight different treatments were applied to rice seedlings grown in both saline and non-saline soil. All treatments are summarized below. The rice plants were sampled on day 120 of cultivation. Proline in rice plants was determined according to Bates et al [13]. The growth parameters of plants such as shoot and root dry weight, shoot height, and width of leaves and tiller were analyzed in both types of soils. Treatments for studying the effects of organic fertilizer on rice growth in the pot experiment included: T1 control without adding either organic fertilizer or chemical fertilizer formula 15-15-15 of commercial brand , T2 chemical fertilizer treated control, T3 supplemented with organic fertilizer Formulation 1 (FC), T4 supplemented with organic fertilizer Formulation 2 (FC+L), T5 supplemented with organic fertilizer Formulation 3 (FC+B), T6 supplemented with organic fertilizer Formulation 1 (FC) with chemical fertilizer (12.5 kg/ha), T7 supplemented with organic fertilizer Formulation 2 (FC+L) with chemical fertilizer (12.5 kg/ha), T8 supplemented with organic fertilizer Formulation 3 (FC+B) with chemical fertilizer (12.5 kg/ha).

2.6 Statistical analysis

Data were analyzed by the Statistix 8 program and multiple comparisons were performed through the least significant difference (LSD) test at 1% and 5% probability levels. The results and discussion are based on the average of the treatments of each experiment. The standard error of means in bar charts was computed by using Excel plot.

3. Results and discussion

3.1 The selection of carrier for producing bio-fertilizer

Three kinds of carriers [filter cake (FC), saline soil (SS) and earthworm manure (EM)] were used in this study. After sterility checking, a 10^8 colony-forming unit per per millilitre (CFU/mL) of single inoculums (P8, N15 and I2.1) or mixed inoculums (ratio 1:1:1, v/v/v) were fixed on the carriers. The surviving halotolerant rhizobacteria cells were counted at 0, 15 and 30 days of incubation. The numbers of halotolerant rhizobacteria were highest (10^9-10^{10} CFU/mL) at 15 days regardless of the carrier types, then slightly declining at day 30 (data not shown). The most successful carrier for halotolerant rhizobacteria survival (single and mix-culture inoculums) was the filter cake. It is abundant with organic and inorganic substances and therefore most suited for immobilizing rhizobacteria. Being rich in nutrients, filter cake provided a source of food for microorganisms whilst proving harmless to soil and plants. Moreover, filter cake was able to provide the available phosphorus, improve the absorption of phosphorus and accumulation on plants [14]. Therefore, filter cake was selected for use in supporting bacterial cells in the following study.

The synergistic effects of carriers and microorganisms can enhance soil properties and the growth of plants. Peat has long been a globally popular carrier for immobilizing microbes [15]. However, the cost of using peat for producing bio-fertilizer is very high. As an alternative, supporting materials from agro industry such as organic waste, industry by-products, plant by-products, mineral soils, and agro-industrial waste have been tested as culture media for microbial growth promotion [16,17]. This study used filter cake, saline soil, and earthworm manure as alternative carriers for supporting cells of microorganisms. These carriers were all able to support and increase the number of bacterial cells.

3.2 Change of physicochemical properties of organic fertilizers during composting process

Three formulations of organic fertilizers were prepared and placed under the shelter with the static pile incubation. The temperature in three piles, which was checked at the center of the heap of fertilizer, started at 26 °C. Later the temperatures of Formulas 2 and 3 rose to 38-40 °C on day 15, whereas that of Formula 1 reached 37 °C on day 30. On day 60, at the curing phase, the temperature of all piles decreased to 27-30 °C. Increases in temperature in the pile were due to the degradation process of microorganisms. At the beginning of the experiment, the pH of all three piles was neutral and EC was around 16-20 dS/m. After 60 days of composting, pH of organic fertilizer had increased to 8.6-8.9 whilst EC was in the same range as the original value. In this series of experiments, we searched for simple and cheap materials among sugar-alcohol industrial waste to produce compost. Filter cake is a byproduct of sugar manufacture consisting of organic and inorganic substances up to 80-90% and is rich in phosphorus [18]. Bagasse is also a residue from sugar mills and has been used as a fuel in many industries. In composting production, bagasse has been used as a bulking material. Moreover, the addition of sugarcane bagasse into the soil can enhance the available water content. In this study, various combinations of these materials were evaluated as substrates for organic fertilizer production. In addition, spent wash was supplemented to the compost heap to provide moisture. Whilst the EC value of spent wash was high, the EC values of all compost piles were around 16-20 dS/m. All three composting piles reached a thermophilic phase on day 15 due to the rise in temperature greater than 38 °C, allowing high levels of microbial activity on the degradation of materials. This increased activity influenced the pH values and changed the composition of nutrients (NPK and organic matter).

3.3 Change of nutrients content in the organic fertilizer piles

During 60 days of composting, total N and total K of all three formulas remained within the range of 0.88-1.33 and 0.85-1.16%, respectively. The total amount of P increased from 0.8-1.13to 2.17-3.23% at day 60. In the previous report of Honig [18], there was an abundance of phosphorus (80-90%) in filter cake in the form of calcium phosphate substance. Also, phosphate solubilizing microorganisms residing in the pile might convert inorganic phosphate into the soluble form of phosphorus. Organic matter of all three formulas was around 34-36% at the beginning and decreased to 28-35% by day 60 of composting. In the conclusion of the Honig studied, the amounts of nutrients in all 3 formulas were in the range of the Thai compost standard i.e., total N, total P and total K greater than 1.0, 1.0 and 0.5%, respectively. Likewise, in this study, the amount of total N, P and K fulfilled the Thai compost standard which is Total N greater than 1% (w/w), Total P greater than 0.5% (w/w) and total K greater than 0.5% (w/w) [19]. Our results showed that filter cake could be composted with various other materials such as bagasse and sugarcane leaves. Filter cake has previously been used as a soil conditioner.

3.4 Changes in the amount of plant growth promoting microorganisms during composting of organic fertilizer

The numbers of cellulolytic, nitrogen fixing and phosphate solubilizing bacteria in three organic fertilizer formulas were evaluated on days 15, 30 and 60 by plate count on selective culture media (Figure 1). The highest number of viable cells of 2.1×10^8 CFU/g was found in the case of cellulolytic bacteria, while the number of viable cells of nitrogen fixing bacteria was 1.05×10^6 CFU/g and that of phosphate solubilizing bacteria was 5.5×10^4 CFU/g. This indicated that cellulolytic bacteria played a major role in the degradation process of sugar-alcohol industry wastes. In cases of nitrogen fixing and phosphate solubilizing bacteria, they were plant growth promoting bacteria present in the compost which played a role in the conversion of organic and inorganic substances into available forms of nutrients for the plants. Enumeration of microorganisms during composting showed that cellulolytic bacteria were predominant in all compost piles. This is because cellulose is the main component of agro-industry residues.

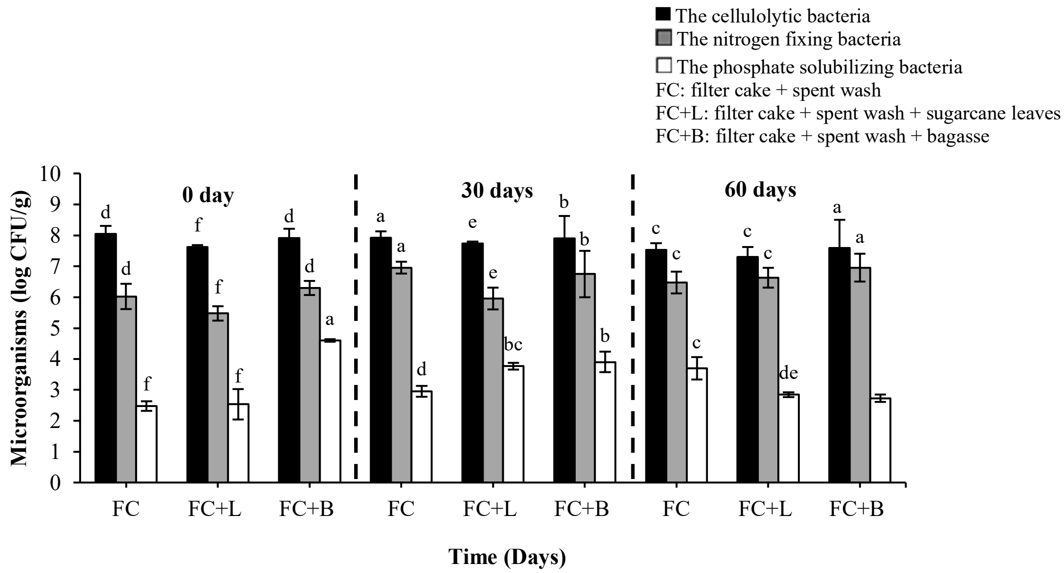

Figure 1 The number of bacteria in three different compost piles, supplemented with different substrates for producing organic fertilizer. Different letters represent significant differences between compared treatments in each of PGPR properties (Cellulolytic, Nitrogen fixing and phosphate solubilizing bacteria) ($p< 0.05$).

3.5 Effects of organic fertilizer on rice seed germination

The GI of rice was determined in order to indicate the maturity of the organic fertilizers and evaluate their qualities on plant growth promotion**.** The results showed that the GI of all three formulas was greater than 80% on day 30 (Table 1), suggesting that the compost prepared by these three formulations supplemented with rhizobacteria was already mature at day 30, whilst demonstrating no phytotoxicity to inhibit plant growth.This constitutes a shorter production time than that of regular compost, where the whole process usually takes at least two months [8]. This might be due to indigenous microbes living in filter cake, spent wash and organic fertilizer with a high potential on substrates degradation. In addition, where the GI index shows a value greater than 100%, the compost product is considered to be a phytonutrient or phytostimulant [12].

Table 1 Effects of various organic fertilizers on the germination of rice seeds.

Bio-organic fertilizer formulation	(GI) (%) day 0	day 30	day 60
F1 (the extracted solution of filter cake + spent wash + rhizobacteria immobilized on carrier)	31.52^{a}	91.53^{c}	89.65^{c}
F2 (the extracted solution of filter cake + sugar cane leaves + spent wash + rhizobacteria immobilized on carrier)	25.20^{b}	105.66^{a}	112.83^{a}
F3 (the extracted solution of filter cake + bagasse + spent wash + rhizobacteria immobilized on carrier)	18.65^{c}	97.77^{b}	92.03^{b}
F-test	**	**	**
%CV	15.12	1.91	8.21

Different letters represent significant differences among bio-organic fertilizer formulation in the same day.
**mean significant difference at 99%, LSD ($p< 0.01$).

3.6 Effects of organic fertilizer on the growth of rice seedlings in the experimental pot culture

The effects of the three formulations of organic fertilizers with or without the supplementation of chemical fertilizers on the growth of rice seedlings planted in saline and non-saline soils were examined. In saline soils, root dry weight (g), width of leaves (cm) and tiller (plant) of the rice plants showed maximum values in T7 (organic fertilizer FC+L supplemented with chemical fertilizer) and T8 (organic fertilizer FC+B supplemented with chemical fertilizer). These treatments were significantly different from T1 (control) (Table 2).

Table 2 The growth parameters of rice at 120 days of cultivation in saline soils and normal soil after application of organic fertilizers, chemical fertilizer and organic fertilizer + chemical fertilizer.

Treatments	Saline soil					Non-saline soil				
	SDW (g)	RDW (g)	SL (cm)	WL (cm)	T (plant)	SDW (g)	RDW (g)	SL (cm)	WL (cm)	T (plant)
T1: C	22.04^{e}	14.33^{d}	118^{e}	1.13^{cd}	4.50^{d}	29.15^{f}	15.02^{f}	119^{e}	1.75^{d}	4.50^{e}
T2: Chem	44.23^{b}	25.23^{a}	164^{a}	1.28^{abc}	6.25^{abc}	53.05^{c}	20.09^{c}	146^{b}	1.40^{bc}	7.25^{abc}
T3: F1(FC)	34.94^{c}	17.35^{c}	155^{c}	1.23^{bcd}	5.75^{bcd}	33.70^{e}	16.43^{ef}	134^{d}	1.43^{bc}	5.50^{de}
T4: F2 (FC+L)	30.26^{d}	16.33^{c}	146^{d}	1.35^{ab}	5.25^{cd}	35.62^{e}	17.46^{df}	139^{cd}	1.5^{b}	6.0^{cde}
T5: F3 (FC+B)	30.11^{d}	19.31^{b}	143^{d}	1.37^{ab}	6.50^{abc}	39.59^{d}	18.49^{cd}	141^{bc}	1.38^{c}	6.5^{bcd}
T6: F1 + Chem	51.28^{a}	24.36^{a}	163^{ab}	1.10^{d}	7.25^{ab}	60.53^{a}	36.70^{a}	166^{a}	1.63^{a}	7.0^{bcd}
T7: F2 + Chem	48.83^{a}	25.10^{a}	164^{a}	1.40^{a}	7.75^{a}	57.55^{ab}	35.15^{a}	172^{a}	1.7^{a}	8.0^{ab}
T8: F3 + Chem	36.58^{c}	25.59^{a}	158^{bc}	1.43^{a}	7.75^{a}	56.58^{b}	30.23^{b}	168^{a}	1.43^{bc}	8.75^{a}
F-test	**	**	**	**	**	**	**	**	**	**
%CV	4.51	5.54	2.66	8.22	16.48	4.77	5.41	2.86	5.21	17.40

Different letters present significant difference among treatments.
**mean significant difference at 99%, LSD ($p< 0.01$).
Shoot dry weight (SDW), Root dry weight (RDW), Shoot length (SL), Width of leaves (WL), and Tillering (T).

In non-saline soil, the dry weight of root and shoot and width of leaves showed maximum values when fertilizer Treatment 5 (filter cake + spent wash + chemical fertilizer) was added to the soil. The tiller showed positive results after the application of fertilizer Treatment 8 (Table 2).

The results showed that rice growth was enhanced in both saline and non-saline soil when supplemented with both the organic fertilizer (the mixture of filter cake, bagasse, spent wash and rhizobacteria) and the chemical fertilizer. Plants directly uptake chemical fertilizer for growth while organic fertilizers help to improve the soil structure. Microorganisms in the compost facilitated nutrient cycling in the soil. Bagasse in the compost was utilised as a bulking material; such agents are added in order to provide stability, integrity and porosity to the solid matrix during the process of composting [20]. Moreover, one report showed that one of three bulking agents; gravel, sharps and/or sawdust (1:2 v:v) in a compost mixture of oil gator (350 g), oily sand demonstrated the ability to retain moisture whilst improving drainage, aeration, and the duration of biodegradation of total petroleum hydrocarbons [21]. In this study, two kinds of fertilizer, FC (the mixture of filter cake and spent wash supplemented with chemical fertilizer) and FC+B (the mixture of filter cake, bagasse supplemented with chemical fertilizer) showed a potential to enhance rice growth in saline and non-saline soils, especially, on the tillering capacity of rice.Similarly, the report of Mahfouz & Sharaf-Eldin [22] found that using biological fertilizers allowed a half-portion reduction in the use of chemical fertilizers.

On harvesting day, soil nutrient content was analyzed. The results are shown in Table 3. The results showed that the nutrient content in non-saline and saline soil when treated with T3: organic fertilizer FC (the mixture of filter cake and spent wash) + chemical fertilizer) was greater than that in other treatments. In addition, nutrient content in the fertilizer-applied soils had increased when compared to those in untreated soil. Most of the saline soil contained large amounts of NaCl, which had a major impact on the nitrogen and phosphorus uptake of the rice plants. In this study, the rate of plant growth in saline soil was less than that in non-saline soil, due to the toxicity of salt ions, especially Cl [23]. Halotolerant rhizobacteria played a role in enhancing plant growth whilst being applied in many different formulations. After application into soil, microbe numbers increased and colonization of the rhizosphere soil or at the root followed, promoting plant growth, and activating nutrient uptake by the plant. As a large component of our organic fertilizer, halotolerant rhizobacteria were able to dissolve the nutrients in soils (both normal and saline) rendering them available for plant uptake. These microbes also demonstrated plant growth promoting properties such as nitrogen fixing, solubilization, biological control and phytohormones production.

3.7 Proline content in saline and normal soils

On harvesting day, the shoots of rice grown in saline and non-saline soils supplemented with various fertilizers were collected and their proline contents were determined (Figure 2). In both saline and non-saline soil conditions, a high amount of proline accumulation was seen in the fertilizer-treated, control and chemical fertilizer-treated groups. Accumulation of proline in rice plants was suppressed in the groups supplemented with the organic fertilizer or with the combination of organic fertilizer and chemical fertilizer. To protect plant cells from the high osmotic or stress conditions such as in arid and saline soil, osmo-protectant agents including proline were produced by plant cells. In this study, soils amended with organic fertilizer showed reductions in the amount of proline in rice plants. This indicated that organic fertilizer improved soil chemical properties by acting as a buffer in the soil.

Proline is an amino acid which can be accumulated in many bacteria and plant cells as an osmo-protectant against osmotic stresses [24]. The highest accumulation of proline in rice plants was found when supplemented with chemical fertilizer (T2), which is rich in macronutrients (N, P and K). Normally, plants can directly absorb chemical fertilizer. Soil chemical properties such as salinity can be improved by long term supplementation of chemical fertilizer. Ueda & Takabe [25] reported that proline and hydroxyproline were accumulated at the tip of barley roots under salinity stress condition. In stress environments, microbial cells are damaged. Therefore, in order to survive under stress conditions, microbes secrete proteins as osmo-protectant to prevent cell lysis. Generally, compost can provide essential nutrients (N, P and K) to amend soil physico-chemical properties and re-establish microbial populations and activities at low cost [26].

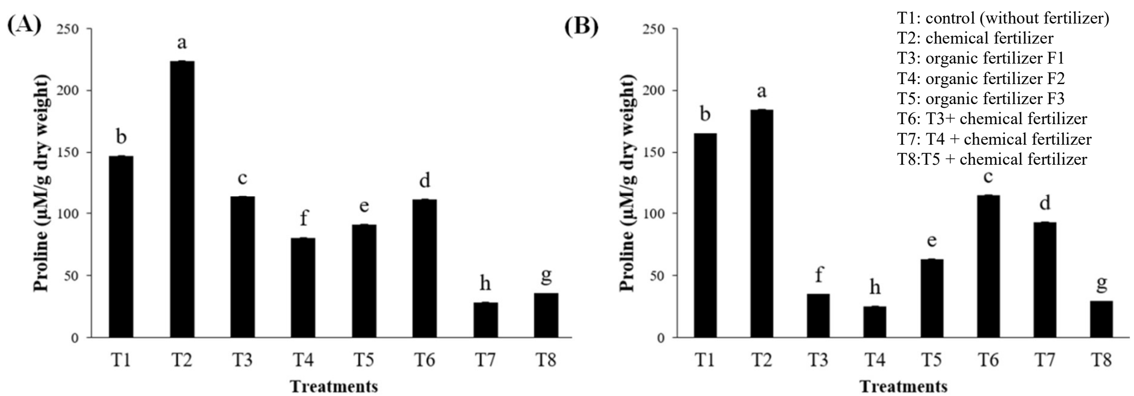

Figure 2 The accumulation of proline in rice plants cultivated in saline soil (A) and normal soil (B) after supplementation with various fertilizers.

Table 3 Analysis of macro nutrient, electrical conductivity, and pH in soil at the harvesting stage.

Treatment	Non-saline soil						Saline soil					
	N (mg/kg)	P (mg/kg)	K (mg/kg)	OM (%)	pH	EC (dS/m)	N (mg/kg)	P (mg/kg)	K (mg/kg)	OM (%)	pH	EC (dS/m)
Original soil	97.6^{bc}	92.3^{d}	114.8^{d}	0.1^{b}	5.5^{bc}	0.8^{b}	111.4^{f}	94.2^{c}	133.0^{d}	0.1^{d}	6.7	6.4^{a}
Chemical fertilizer	122.2^{b}	168.8^{a}	186.7^{c}	0.2^{a}	5.4^{c}	1.2^{a}	152.9^{c}	138.8^{a}	314.3^{a}	0.3^{b}	6.2	6.4^{a}
(FC) + chemical fertilizer	146.8^{a}	118.7^{b}	227.2^{a}	0.3^{a}	7.1^{a}	0.5^{d}	238.8^{a}	145.3^{a}	273.8^{c}	0.4^{a}	5.7	5.5^{bc}
(FC+L) + chemical fertilizer	99.0^{bc}	96.9^{cd}	196.1^{b}	0.2^{ab}	6.8^{a}	0.7^{bcd}	140.4^{d}	113.0^{b}	323.5^{a}	0.3^{bc}	6.0	5.8^{abc}
(FC+B) + chemical fertilizer	147.5^{a}	119.5b	220.9^{ab}	0.2^{ab}	6.^{0b}	0.5^{cb}	169.6^{b}	116.2^{b}	301.9^{b}	0.2^{c}	5.8	5.0^{1c}
F-test	**	**	**	ns	**	**	**	**	**	**	ns	*
%CV	4.8	5.6	4.3	26.9	5.1	22.0	3.5	3.8	1.8	6.2	7.4	8.2

Different letters present significant difference in treatments. *mean significant difference at 95%, LSD (p< 0.05).
** mean significant difference at 99%, LSD (p< 0.01). (FC) + chemical fertilizer: organic fertilizer FC (the mixture of filter cake and spent wash), (FC+L) + chemical fertilizer: organic fertilizer FC+L(the mixture of filter cake + sugar cane leaves + spent wash), (FC+B) + chemical fertilizer: (the mixture of filter cake + bagasse) + spent wash.

4. Conclusion

Organic fertilizer supplemented with bio-fertilizer can provide macro and micronutrients, organic matter and plant growth promoting microorganisms which can improve soil structure and enhance the nutrients cycling in the soil. The bagasse contained in the mixture of organic fertilizer formula 3 might also help to improve soil structure. In addition, this experiment presented methods to reduce the use of chemical fertilizers. The use of half -dose chemical fertilizer supplemented with organic fertilizer increased plant productivity to a level greater than when it was treated with full-dose chemical fertilizer. Moreover, this study demonstrated a low-cost method to culture halotolerant bacteria, which were shown to be phosphate solubilizers that promote plant growth.

5. Acknowledgements

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