INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
15–156/2016/18–1–9–15
DOI: 10.17957/IJAB/15.0045
http://www.fspublishers.org

Full Length Article

Improving the Rice Performance by Fermented Chitin Waste

Nungruthai Kananont[1], Rath Pichyangkura[2], Boonthida Kositsup[1], Wanpen Wiriyakitnateekul[3] and Supachitra Chadchawan[1*]

[1]*Center of Excellence in Environment and Plant Physiology, Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*

[2]*Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*

[3]*Land Development Department Phahon Yothin Road, Chatuchak, Bangkok 10900, Thailand*

[*]For correspondence: s_chadchawan@hotmail.com; Supachitra.C@chula.ac.th

Abstract

Fermented chitin waste (FCW), a by-product obtained from chitinase production using chitin fermentation, was evaluated for its properties for use as soil supplement based plant growth stimulator. Rice growth, yield and photosynthesis parameters were investigated following the growth of rice plants in organic fertilizer supplemented soil with the addition of 0.25, 0.50 or 1.00% (w/w) FCW in comparison with chemical fertilizer application. The application of FCW resulted in an increased photosynthetic pigment concentration and enhanced photosynthesis rate, leading to a significantly higher tiller number, shoot biomass and grain yield. At 30 d after transplantation (DAT), the rice plants grown with 0.5% (w/w) FCW-supplemented soil showed the highest level of new leaf photosynthetic pigments and photosynthesis rate, but they were not significantly different from the plants grown in soil supplemented with 1.0% (w/w) FCW. However, at 60 DAT, the plants grown under 1.0% (w/w) FCW had a significantly higher photosynthesis rate than plants grown in 0.5% (w/w) FCW supplemented soil. The addition of 0.5 or 1.0% (w/w) FCW increased the grain yield 2.7 and 4.3 folds, respectively, compared to that with chemical fertilizer application. The addition of FCW significantly increased the soil pH and organic matter, nitrogen, phosphorus and potassium contents. Therefore, the FCW from a chitin fermenter can be used as a plant growth stimulator for sustainable rice production.

Keywords: Chitin; Growth; Photosynthesis; Rice; Pigment

Introduction

Fermented chitin waste (FCW) is a solid waste by-product obtained from chitinase production by the fermentation of chitin. Chitinase is one of the enzymes that is in demand by, and has been applied in, many biotechnology industries, shellfish waste management (Wang *et al*., 2006), production of single cell proteins and especially, the production of chitooligosaccharides that are used as a plant growth stimulator (Limpanavech *et al*., 2008; Kananont *et al*., 2010; Pornpienpakdee *et al*., 2010) and bio-control product (Chang *et al*., 2007). Chitin is used as the carbon source and inducer for chitinase production. After the chitinase is obtained, the solid FCW from the fermenter, which is mainly composed of non-digested chitin flakes and the microorganisms used for chitin production, is normally discarded.

Several methods of chitin/chitosan utilization for plant growth stimulation have been studied, such as foliage spraying, seed coating and soil supplementation. A positive growth enhancement effect following chitosan application has been reported in many plant species, including coffee (Dzung *et al*., 2011), *Dendrobium* orchid (Limpanavech *et al*., 2008; Pornpienpakdee *et al*., 2010), soybean, mini-tomato, lettuce and rice (Chibu and Shibayama, 1999). Boonlertnirun *et al*. (2008) reported that the application of 80 ppm polymeric chitosan by seed soaking before planting followed by soil application for four times throughout the planting period tended to stimulate the growth and significantly increased the yield of 'Supanburi1' rice, whereas seed soaking before planting followed by foliar application tended to generate an enhanced ability to control plant diseases.

Thailand is a major rice producer and exporter in the world. 'Pathumthani1' rice is the second most popular fragrant rice after Thai Jasmine Rice (Khao Dawk Mali 105 or KDML 105). However, in contrast to 'KDML105' rice, 'Pathumthani1' rice can be grown throughout the year, as its reproduction phase is light-insensitive and it is normally grown in the irrigated areas in Thailand. Unfortunately, seed soaking followed by foliar spraying of chitosan solution on 'Pathumthani1' rice seedlings inhibited their subsequent growth due to the negative growth effects of the acetic acid solvent (Kananont *et al*., 2014).

To cite this paper: Kananont, N., R. Pichyangkura, B. Kositsup, W. Wiriyakitnateekul and S. Chadchawan, 2016. Improving the rice performance by fermented chitin waste. *Int. J. Agric. Biol*., 18: 9–15

Here we aimed to search for a bio-stimulator that can be used to enhance rice production. As chitin and chitosan molecules are similar in their glucose backbone and nitrogen composition, it was speculated that the FCW from the chitinase production industry could be used as a bio-stimulator for rice production. Although FCW has recently been shown to enhance lettuce growth (Muymas *et al*., 2014), there have been no reports on the use of FCW for the enhancement of cereal crop production. Therefore, the effects of FCW on the growth, photosynthesis and yield of 'Pathumthani1' rice were investigated as a plant growth stimulant and to find a useful environmental and economically viable use for the FCW.

Materials and Methods

Plant and Fermented Chitin Preparation

'Pathumthani 1' rice seeds were obtained from the Pathumthani Rice Research Center, Bureau of Rice Research and Development, Thailand, while FCW was obtained from OliZac Technologies Co. Ltd., Bangkok, Thailand. Generally, to obtain the chitinase enzyme, a *Bacillus lichenifuels* SK-1 stock culture was grown in minimal medium (0.25% (w/v) yeast extract, 0.03% (w/v) $MgSO_4$, 0.5% (w/v) $(NH_4)_2S_2O$, 0.6% (w/v) KH_2PO_4 and 1% (w/v) K_2HPO_4) with the addition of chitin flakes at a 1:5 (w/v) ratio and incubated at 50°C for 4 d. The supernatant was removed for isolation of the chitinase enzyme and the waste was dried at 60°C for 72 h.

Experimental Design and Rice Cultivation

The experiment was performed in a randomized complete block design (RCBD) with four replications in a greenhouse at the Department of Botany, Faculty of Science, Chulalongkorn University, Thailand. Three levels of FCW (0.25%, 0.50% or 1.0% (w/w)) were added to clay soil (16% sand, 26% silt and 58% clay as (w/w) composition), and thoroughly mixed 7 d before rice seedling transplantation. In addition, soil supplemented with chicken manure fertilizer alone (CMF) or chemical fertilizer (CF), according to the recommendation of the company, were used as controls. For CMF application, 3.31 g of chicken manure was added to the pot 20 and 60 d after transplanting (DAT) of the seedlings. For CF, 1 g of 16-20-0 (N-P-K) CF was added to each pot 20 DAT and then 0.66 g of 46-0-0 (N-P-K) CF was added at 60 DAT. The FCW was mixed into the soil at the designated level at the beginning of the experiment only.

The procedure of transplanting rice cultivations is summarized in Fig. 1. First, seeds were soaked in distilled water for 2 d and then germinated in soil for seedling growth on March 17th, 2012. On the 14th April 2012, the 28-d-old seedlings were transplanted at three seedlings per 28 cm diameter pot containing 5 kg of clay soil supplemented with the indicated amount of FCW, or with either CF or CMF as reference controls. Plants were then grown under natural conditions in a greenhouse during the rainy season in Thailand, maintaining the water level at about 5 cm above the soil surface throughout the experimental period. During this period, the average day time and night time temperature was 34.7°C and 26.8°C, respectively. The average precipitation and relative humidity was 139 mm and 75.2%, respectively.

Analysis of Soil Samples

Soil samples were collected from individual pots before planting and after harvesting in order to analyze the physicochemical properties of the soil, including the pH, electrical conductivity (EC) and the organic matter (OM), organic carbon (OC), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) contents. For soil sample preparation, soil samples were air-dried and then contaminants, such as roots and gravel, were removed. Each dried soil sample was then ground by mortar and pestle and sieved into 2 mm diameter particles for the soil pH and EC analysis and 0.5 mm particles for the chemical composition analysis. The soil pH and EC measurements were performed according to Peech (1965) and Lee *et al*. (2004), respectively, while the Walkley and Black method was used for determining the total soil OM as reported (Nelson and Sommers, 1996). The Kjeldahl method was used for analyzing the total N content as reported (Bremner, 1965). For the mineral content in the soil, the available P was extracted with NH_4F and HCL (Bray and Kurtz, 1945), while the available K, Ca and Mg were extracted by NH_4OAc (Jackson, 1958).

Measurement of Plant Growth, Yield and Leaf Photosynthesis Gas Exchange

The number of tillers/rice plant were counted at 0, 15, 30, 45 and 60 DAT in order to determine the effects of FCW on rice growth. In addition, the day of flowering was recorded when rice plants initially bloomed (flowering stage), and the yield in terms of the number of panicles/plant and filled grains/panicle were determined at the end of experiment (28th August 2012).

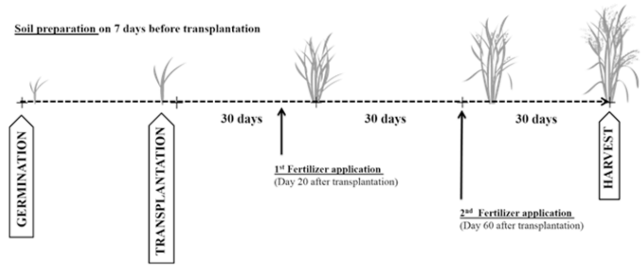

Fig. 1: Rice cultivation process used in this experiment

For determination of the rice leaf photosynthesis gas exchange, the net photosynthetic rate (A_{max}), stomatal conductance (g_s), internal concentration of CO_2 (C_i) and transpiration rate (E) were measured by a portable photosynthesis system (LI-COR 6400, Lincoln, NE, USA) on the three uppermost fully expanded leaves at a photosynthetic photon flux density (PPFD) of 1,200 μmol m^{-2} s^{-1} and CO_2 concentration of 380 μmol/mol. The measurement was performed at 30 and 60 DAT.

Measurement of Photosynthetic Pigment Content

After 35 DAT, chlorophyll was extracted from three fully expanded rice leaves in each pot using 80% (v/v) acetone solution as described in Porra (2002). In brief, 10 mL of 80% (v/v) acetone solution was added to 25 mg of rice leaves and kept in the dark for 24 h. Afterwards, chlorophyll *a*, chlorophyll *b* and carotenoid contents were measured at 663.2, 646.8 and 470 nm by a spectrometer, respectively, and calculated using Arnon's method (Arnon, 1949).

Statistical Analysis

The experiment was performed with a RCBD with four replications. Data for each was analyzed using analysis of variance (ANOVA), followed by the Duncan's Multiple Range Test (DMRT) using the IBM SPSS Statistic software and accepting significance at the $p < 0.05$ level. Data are reported as the mean ± 1 standard error or standard variation as indicated. The correlation between yield components and soil properties were performed by using linear regression analysis and linear regression R^2 coefficients were reported.

Results

FCW Increases Soil pH and Nutrition Components

Before rice seedling transplantation, the physicochemical properties of the soil, including the pH, EC and the OM, N, P, K, Ca and Mg contents, were analyzed. The addition of FCW increased the soil pH and OM, N, P and K contents without any significant effect on the EC and the Ca and Mg contents (Table 1). However, the addition of the CF or organic CMF at 20 and 60 DAT should increase the soil nutrition component to a comparable level to that supplemented with 0.25% (w/w) FCW.

FCW Increased the Photosynthesis Rate and Photosynthetic Pigment Contents

To investigate the effects of FCW on rice growth and development, the photosynthetic pigment contents and photosynthesis rate were determined in fresh leaves at the vegetative (30 DAT) and reproductive (booting, 60 DAT or 88 days after germination) stages. The application of FCW significantly increased the photosynthetic pigment contents, where the addition of 0.5% (w/w) FCW resulted in the highest level of chlorophyll *a*, chlorophyll *b* and carotenoid contents, some 1.5 to 1.7 folds higher than the level found in plants grown in soil supplemented with CF (Table 2).

Plants grown in the soil supplemented with FCW not only had a higher level of photosynthetic pigment contents, but they also had a ~1.4 and 1.8 folds higher net photosynthetic rate (A_{max}) at the vegetative stage (30 DAT) than those grown in soil with CF and CFM, respectively, (Fig. 2a). This increase was correlated with the increased stomata conductance (Fig. 2b) and transpiration rate (Fig. 2d), while the intercellular CO_2 concentration was not significantly different from the control plants (Fig. 2c).

On the other hand, at the reproductive (booting) stage the net photosynthesis rate and transpiration rate of the second leaf of plants grown in 0.25–0.5% (w/w) FCW-supplemented soils were significantly decreased compared to the rates in the vegetative stage. This is opposite to the photosynthesis rate of the plants grown in the soil supplemented with CF, which was higher in the reproductive stage (Fig. 2a). A similar phenomenon was also found in the stomatal conductance values (Fig. 2b). The transpiration rate of plants in all treatments were decreased at the reproductive stage (Fig. 2d), while no significant difference was found in the internal CO_2 concentration of plants in both stages (Fig. 2c).

FCW Enhanced the Rice Growth and Yield

When plants were grown in soil supplemented with 0.25% or 0.5% (w/w) FCW, the number of tillers/plant rapidly increased from 15 to 45 DAT, being maximal at 45 DAT (12 and 17 tillers/plant, respectively), and were significantly higher than that in the plants grown in the other treatments at 30 DAT (Fig. 3). However, at 60 DAT the number of tillers/plant decreased in both cases. In contrast, with the higher 1% (w/w) FCW level the number of tillers/plant increased from 15 DAT and especially from 30 DAT, to the highest level at 60 DAT. The decrease in the number of tillers/plant at the booting stage (60 DAT) in the rice plants grown in 0.25% and 0.5% (w/w) FCW, compared to that at 45 DAT reflects the increased proportion of dead tillers in some plants. Interestingly, although the application of 1.0% (w/w) FCW caused a delayed start to the tillering stage it enhanced and maintained the number of tillers/plant until harvesting, where at 60 DAT the rice started booting and no more tillers were formed.

'Pathumthani1' rice normally flowers approximately 90–95 d after germination. In the culture conditions used in this experiment the rice plants grown in soil with 0.25 and 0.5% (w/w) FCW started booting after 93 d of germination, compared to slightly later in those grown in the soil with CF and CMF, at 96.5 and 102 d after germination, respectively, (Table 3). However, the application of 1% (w/w) FCW delayed the onset of flowering to 99 d after germination.

Table 1: Physicochemical properties of the soil before rice seedling transplantation: the pH, electrical conductivity (EC) and the organic matter (OM), organic carbon (OC), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) contents

Treatment	pH	EC ^{ns} (dS/m)	Total N (%)	P (mg/kg)	K (mg/kg)	Ca ^{ns} (mg/kg)	Mg ^{ns} (mg/kg)	OM (%)	OC (%)
CF	5.01 ± 0.19^{c}	0.40 ± 0.02	0.155 ± 0.019^{c}	20.43 ± 1.81^{d}	93.50 ± 4.12^{d}	905.45 ± 82.31	472.77 ± 35.86	1.82 ± 0.15^{c}	1.06 ± 0.09^{c}
CMF	5.07 ± 0.10^{c}	0.44 ± 0.01	0.166 ± 0.007^{bc}	20.55 ± 2.17^{d}	92.22 ± 0.00^{d}	952.88 ± 28.95	490.88 ± 34.24	1.85 ± 0.17^{c}	1.07 ± 0.10^{c}
0.25% FCW	5.29 ± 0.09^{bc}	0.40 ± 0.03	0.190 ± 0.019^{ab}	32.30 ± 2.79^{c}	122.50 ± 3.00^{c}	873.18 ± 15.42	476.49 ± 21.46	1.95 ± 0.05^{bc}	1.13 ± 0.03^{bc}
0.5% FCW	5.53 ± 0.14^{b}	0.39 ± 0.01	0.169 ± 0.013^{bc}	47.79 ± 5.60^{b}	150.50 ± 3.79^{b}	877.05 ± 79.16	483.17 ± 19.43	2.11 ± 0.04^{b}	1.22 ± 0.02^{b}
1% FCW	5.93 ± 0.30^{a}	0.36 ± 0.07	0.213 ± 0.016^{a}	73.33 ± 8.66^{a}	218.19 ± 19.44^{a}	871.48 ± 93.62	481.44 ± 43.78	2.35 ± 0.14^{a}	1.37 ± 0.08^{a}

Data are shown as the mean ± 1 SD, derived from 4 repeats. Means in a column with a different superscript lowercase letter are significantly different ($p<0.05$; DMRT)

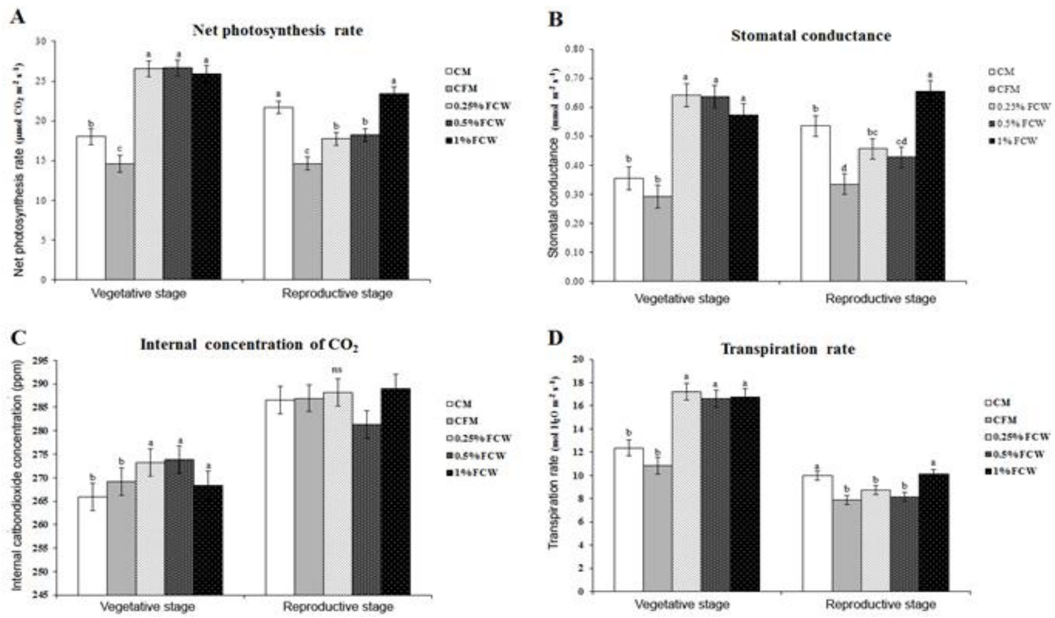

Fig. 2: The effect of FCW supplementation of soil at different concentrations on the (A) net photosynthesis rate, (B) stomatal conductance, (C) internal concentration of CO_2 and (D) transpiration rate on the youngest fully expanded leaves at 30 DAT (vegetative stage) and on the second leaves of the booting stage plants at 60 DAT (reproductive stage)

Data are shown as the mean + 1 SE, derived from 4 independent repeats. Means with a different letter are significantly different ($p < 0.05$; DMRT)

Table 2: The effect of FCW supplementation in the soil on the rice photosynthetic pigment (chlorophyll *a*, *b* and carotenoid) contents at 35 DAT

Treatment	Photosynthetic pigment content (mg/g fresh weight)		
	Chlorophyll *a*	Chlorophyll *b*	Carotenoids
CF	1.43 ± 0.31^{b}	0.50 ± 0.12^{b}	0.56 ± 0.14^{b}
CMF	1.30 ± 0.12^{b}	0.46 ± 0.04^{b}	0.48 ± 0.05^{b}
0.25% FCW	2.27 ± 0.64^{ab}	0.67 ± 0.05^{ab}	0.72 ± 0.06^{ab}
0.5% FCW	2.57 ± 0.50^{a}	0.84 ± 0.11^{a}	0.84 ± 0.11^{a}
1% FCW	2.32 ± 0.20^{a}	0.79 ± 0.25^{a}	0.80 ± 0.25^{a}

CF = soil supplemented with chemical fertilizer
CMF = soil supplemented with chicken manure fertilizer alone
Data are shown as the mean + 1 SD, derived from 4 independent repeats. Means within a column followed by a different letter are significantly different ($p < 0.05$; DMRT)

All three FCW additions (0.25, 0.5 and 1.0% (w/w)) in the soil enhanced the rice production but the significantly highest level of above ground biomass was found in the rice plants grown with 1.0% (w/w) FCW at 3.8 and 10.4 folds higher than the biomass obtained from the soil with CF and CMF treatments, respectively (Table 3). The highest level of grain yield/pot was also found in the 1.0% (w/w) FCW supplemented soil. Although the rice plants grown in soil with 0.5 or 1.0% (w/w) FCW produced a similar number of panicles per plant, supplementation with 1.0% (w/w) FCW increased the spikelets/panicle and filled grains/panicle compared to the other treatments (Table 3).

Discussion

Application of FCW to the soil led to an increased OM level in the soil (Table 1). OM improves the physical, chemical and biological properties of soil, as well as giving a better soil aggregation, available water content and enhanced cation exchange capacity, leading to improved soil fertility (Khaleel *et al*., 1981; Metzger and Yaron, 1987; Matsumoto *et al*., 1999; Farooq and Nawaz, 2014). The addition of FCW also increased the soil N, P and K content, which showed a high correlation with the measured plant yield components. However, no correlation was found between the nutrient level and the day of flowering (Table 4). A very high correlation was found between the measured yield components and the available soil P content, a nutrient that is considered to be important for plant productivity (Hodges, 2010). Therefore, based on this data, P is likely to be an important and limiting nutrient for rice productivity and, since FCW is rich in available P (Table 1), then the addition of FCW to the soil leads to the enhanced rice yield.

Chitin-rich material was recently shown to enhance lettuce growth (Muymas *et al*., 2014), whilst the addition of chitin to the soil was proposed to be a good agricultural practice for plant protection against pathogens and nematodes (Kobayashi *et al*., 2002; Cretoiu *et al*., 2013). Application of chitin to the soil could promote soil microbe diversity (Cretoiu *et al*., 2014). Muymas *et al*. (2011) reported that lettuce cv. 'Red Oak' grown in soil with 20% (w/w) fermented chitin showed the highest growth and also increased populations of soil microbes that can degrade chitin to an efficient nitrogen source. These reports are consistent with our results that the application of FCW enhanced the biomass production and yield of 'Pathumthani1' rice, even though the level of FCW applied to the soil was markedly lower in this rice cultivation reported here. Therefore, it is possible that the appropriate amount of chitin rich material to optimally enhance plant growth and yield may vary from species to species or via interaction with other specific culture conditions.

The FCW supplementation of soil led to an excess available N level, which may account for the prolonged vegetative

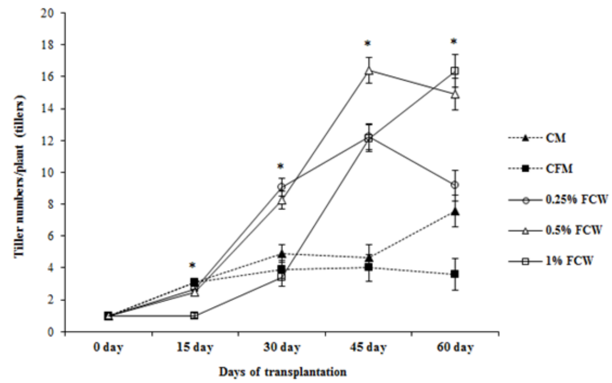

Fig. 3: The effect of 0.25, 0.5 and 1% (w/w) FCW soil supplement on the number of tillers/rice plant at 0, 15, 30, 45 and 60 DAT. Data are shown as the mean + 1 SE, derived from 4 independent repeats. Means with a different letter are significantly different (p < 0.05; DMRT)

Table 3: The effect of 0.25, 0.5 and 1% (w/w) FCW as a soil supplement on the day of flowering, dry weight of shoot biomass and yield in terms of the number of panicles/pot, spikelets/panicle, filled grains/panicle, Grain yield/pot and 100-grain-weight after harvest

Treatment	Day of flowering (d)	Panicles/Pot	Spikelets/Panicle	Filled grain/Panicle (seeds)	Grain yield /Pot (g)	100-grain-weight(g)	Dry weight of shoot biomass (g)
CF	96.5 ± 2.4^{bc}	19.0 ± 4.1^{b}	58.9 ± 7.6^{c}	47.0 ± 6.7^{c}	18.38 ± 3.04^{d}	2.20 ± 0.04^{bc}	17.55 ± 1.62^{d}
CMF	102.0 ± 4.3^{d}	9.0 ± 1.4^{c}	36.5 ± 6.9^{d}	28.3 ± 7.3^{d}	5.18 ± 1.15^{e}	2.14 ± 0.03^{c}	6.47 ± 1.01^{e}
0.25% FCW	93.0 ± 1.8^{a}	21.3 ± 1.3^{b}	70.0 ± 3.3^{b}	58.8 ± 2.8^{b}	28.01 ± 2.33^{c}	2.25 ± 0.03^{b}	28.42 ± 1.15^{c}
0.5% FCW	93.5 ± 1.9^{ab}	36.0 ± 0.8^{a}	76.4 ± 4.9^{b}	65.0 ± 5.7^{b}	49.92 ± 6.10^{b}	2.33 ± 0.06^{a}	49.05 ± 2.71^{b}
1% FCW	99.5 ± 1.7^{cd}	36.8 ± 1.5^{a}	110.7 ± 8.9^{a}	93.0 ± 10.3^{a}	79.41 ± 11.15^{a}	2.22 ± 0.05^{b}	67.14 ± 9.48^{a}

CF = soil supplemented with chemical fertilizer (CF)

CMF = soil supplemented with chicken manure fertilizer alone (CMF)

Data are shown as the mean + 1 SD, derived from 12 plants per treatment (four replications with three plants/replicate). Means within a column followed by a different letter are significantly different (p < 0.05; DMRT)

Table 4: Correlation between nutrition levels of N, P, K, Ca, Mg, OM, OC and yield components

Characteristics	Total N (%)	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	OM (%)	OC (%)	100-grain-weight (g)	Grain yield /Pot (g)	Shoot biomass (g; DW)	Panicle/ Pot (Panicle)	Spikelets/ Panicle (Spikelets)	Filled grain /Panicle (seeds)	Day of flowering (d)
Total N (%)	1.000													
P (mg/kg)	0.739^{***}	1.000												
K (mg/kg)	0.432^{ns}	0.716^{***}	1.000											
Ca (mg/kg)	-0.430^{ns}	-0.395^{ns}	-0.523^{*}	1.000										
Mg (mg/kg)	0.206^{ns}	-0.071^{ns}	-0.317^{ns}	0.064^{ns}	1.000									
OM (%)	0.601^{**}	0.855^{***}	0.519^{*}	-0.181^{ns}	0.099^{ns}	1.000								
OC (%)	0.594^{**}	0.853^{***}	0.514^{*}	-0.180^{ns}	0.099^{ns}	1.000	1.000							
100-grain-weight (g)	0.086^{ns}	0.309^{ns}	0.361^{ns}	-0.552^{*}	0.060^{ns}	0.266^{ns}	0.264^{ns}	1.000						
Grain yield /Pot (g)	0.650^{**}	0.912^{***}	0.578^{**}	-0.284^{ns}	0.109^{ns}	0.834^{***}	0.833^{***}	0.429^{ns}	1.000					
Shoot biomass (g; DW)	0.630^{**}	0.913^{***}	0.551^{*}	-0.308^{ns}	0.065^{ns}	0.871^{***}	0.869^{***}	0.471^{ns}	0.973^{***}	1.000				
Panicles/Pot (Panicle)	0.513^{*}	0.825^{***}	0.559^{*}	-0.476^{*}	0.013^{ns}	0.748^{***}	0.748^{***}	0.659^{*}	0.899^{***}	0.933^{***}	1.000			
Spikelets/Panicle (Spikelets)	0.677^{**}	0.907^{***}	0.583^{**}	-0.365^{ns}	-0.051^{ns}	0.782^{***}	0.782^{***}	0.387^{ns}	0.941^{***}	0.905^{***}	0.852^{***}	1.000		
Filled grain /Panicle (seeds)	0.669^{**}	0.905^{***}	0.589^{**}	-0.384^{ns}	-0.044^{ns}	0.777^{***}	0.776^{***}	0.415^{ns}	0.932^{***}	0.896^{***}	0.851^{***}	0.996^{***}	1.000	
Days of flowering (d)	0.043^{ns}	0.071^{ns}	0.157^{ns}	0.268^{ns}	-0.114^{ns}	-0.118^{ns}	-0.116^{ns}	-0.572^{*}	-0.106^{ns}	-0.196^{ns}	-0.352^{ns}	-0.143^{ns}	-0.145^{ns}	1.000

OM = Organic matter; OC = Organic carbon. The correlation was performed based on the linear regression and the correlation values in the table are linear regression R^2 coefficients. *, ** and *** represent p< 0.05, 0.01 and 0.001 significance levels stage and delayed flowering time (Hodges, 2010) of the rice compared to those plants in the other treatments. In addition, an increased available N supply can increase the level of photosynthetic pigments, as previously reported in algae (Pancha *et al*., 2014; Zubia *et al*., 2014) and plants (Wang *et al*., 2014), since N is required for chlorophyll synthesis. The increased available N supply, therefore, can lead to increased chlorophyll *a*, chlorophyll *b* and carotenoid contents until it reached the optimal concentration in the medium (Wang *et al*., 2014). In this study, the application of 0.5% (w/w) FCW to the soil of rice plants was optimal for enhancing the leaf photosynthetic pigment contents (Table 3), yet the highest net photosynthesis rate at the reproductive stage was found in the plants grown in 1.0% (w/w) FCW supplemented soil and no significant difference in the net photosynthesis rate was found among the different FCW application levels. Thus, other factors rather than photosynthetic pigments could contribute to the enhanced photosynthetic ability of these rice plants.

It has been shown that the available P supply affects the photosynthesis process in tea leaves, where a P deficiency impaired the electron transport chain from photosystem II (PSII) to photosystem I (PSI). This resulted in ATP production from the light reaction with limited RuBP regeneration and hence a reduced rate of CO_2 assimilation (Lin *et al*., 2009). The increased level of available P in the soils with added FCW could contribute to the increased net photosynthesis rate *via* enhanced electron transport flow. However, K is also used in photosynthesis and is involved in water regulation (Hodges, 2010; Farooq *et al*., 2014). Therefore, the increased available soil K level following the application of FCW may also have played a role in the increase photosynthesis rate and stomatal conductance.

Conclusion

FCW has the potential to be a growth stimulant for rice, where the addition of 1% (w/w) FCW into soil could promote the plant growth and increased rice yield. The application of FCW increased the level of photosynthetic pigments and photosynthesis ability at the vegetative stage, which may be one of the key factors enhancing the rice productivity.

Acknowledgement

This research was financially supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission (WCU-019-FW-57). NK is supported by Chulalongkorn University graduate scholarship to commemorate the 72nd anniversary of His Majesty King Bhumibol Adulyadej. Authors would like to thank Dr. Robert Butcher form valuable comments and revision of the manuscript.

References

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol*., 24: 1–15

Boonlertnirun, S., C. Boonraung and R. Suvanasara, 2008. Application of chitosan in rice production. *J. Met. Mater. Miner*., 18: 47–52

Bray, R.H. and L.T. Kurtz, 1945. Determination of total organic and available forms of phosphorus in soils. *Soil Sci*., 59: 39–45

Bremner, J.M., 1965. Total nitrogen. *In*: *Methods of soil analysis Part 2. Chemical and Microbiological Methods*, pp: 1149–1178. Black, C.A. (ed.). American Society of Agronomy, Inc., Publishers, Madison, Wisconsin, USA

Chang, W.T., Y.C. Chen and C.L. Jao, 2007. Antifungal activity and enhancement of plant growth by *Bacillus cereus* grown on shellfish chitin wastes. *Bioresour. Technol*., 98: 1224–1230

Chibu, H. and H. Shibayama, 1999. Effects of chitosan applications on the early growth of several crops. *Rep. Kyushu. Branch. Crop Sci. Soc. Jpn*., 65: 83–87

Cretoiu, M.S., G.W. Korthals, J.H.M. Visser and J.D. van Elsas, 2013. Chitin amendment increases soil suppressiveness toward plant pathogens and modulates the actinobacterial and oxalobacteraceal communities in an experimental agricultural field. *Appl. Environ. Microbiol*., 79: 5291–5301

Cretoiu, M.S., A.M. Kielak, A. Schluter and J.D. van Elsas, 2014. Bacterial communities in chitin-amended soil as revealed by *16S rRNA* gene based pyrosequencing. *Soil Biol. Biochem*., 76: 5–11

Dzung, N.A., V.T.P. Khanh and T.T. Dzung, 2011. Research on impact of chitosan oligomers on biophysical characteristics, growth, development and drought resistance of coffee. *Carbohyd. Polym*., 84: 751–755

Farooq, M. and A. Nawaz, 2014. Weed dynamics and productivity of wheat in conventional and conservation rice-based cropping systems. *Soil Till. Res*., 141:1–9.

Farooq, M., M. Hussain and K.H.M. Siddique. 2014. Drought stress in wheat during flowering and grain-filling periods. *Crit. Rev. Plant Sci*., 33:331–349

Hodges, S.C. 2010. *Soil Fertility Basics NC Certified Crop Advisor Training*. Soil Science Extension. North Carolina State University. USA

Jackson, M.L., 1958. *Soil Chemical Analysis*. Prentice Hall, Inc., Englewood Cliffs, New Jersey. USA

Kananont, N., B. Kositsup, R. Pichyangkura and S. Chadchawan, 2014. Effects of chitosan and associated solvents on the growth of 'Pathumthani1' rice (*Oryza sativa* L. 'Pathumthani1') seedlings. *J. Chitin Chitosan Sci*., 2: 99–105

Kananont, N., R. Pichyangkura, S. Chanprame, S. Chadchawan and P. Limpanavech, 2010. Chitosan specificity for the *in vitro* seed germination of two *Dendrobium* orchids (Asparagales: Orchidaceae). *Sci. Hort*., 124: 239–247

Khaleel, R., K.R. Reddy and M.R. Overcash, 1981. Changes in soil physical properties due to organic waste applications: a review. *J. Environ. Qual*., 10: 133–141

Kobayashi, D.Y., R.M. Reedy, J. Bick and P.V. Oudemans, 2002. Characterization of a chitinase gene from *Stenotrophomonas maltophilia* strain 34S1 and its involvement in biological control. *Appl. Environ. Microbiol*., 68: 1047–1054

Lee, J.J., R.D. Park, Y.W. Kim, J.H. Shim, D.H. Chae, Y.S. Rim, B.K. Sohn, T.H. Kim and K.Y. Kim, 2004. Effect of food waste compost on microbial population, soil enzyme activity and lettuce growth. *Bioresour. Technol*., 93: 21–28

Limpanavech, P., S. Chaiyasuta, R. Vongpromek, R. Pichyangkura, C. Khunwasi, S. Chadchawan, P. Lotrakul, R. Bunjongrat, A. Chaidee and T. Bangyeekhun, 2008. Chitosan effects on floral production, gene expression and anatomical changes in the *Dendrobium* orchid. *Sci. Hort*., 116: 65–72

Lin, Z.H., L.S. Chen, R.B. Chen, F.Z. Zhang, H.X. Jiang and N. Tang, 2009. CO_2 assimilation, ribulose-1, 5-bisphosphate carboxylase/oxygenase, carbohydrates and photosynthetic electron transport probed by the JIP-test, of tea leaves in response to phosphorus supply. *BMC Plant Biol*., 9: 43

Matsumoto, S., N. Ae and M. Yamagata, 1999. Nitrogen uptake response of vegetable crop to organic material. *Soil Sci. Plant Nutr*., 45: 269–278

Metzger, L. and B. Yaron, 1987. Influence of sludge organic matter on soil physical properties. *In*: *Advances in Soil Science*, pp: 141–163. Stewart, B.A. (ed.). Springer Publishing, New York, USA

Muymas, P., P. Boon-long, S. Chadchawan, R. Pichayangura and K. Seraypheap, 2011. Effect of biomaterial and semi-biomaterial on growth and postharvest quality of 'Red Oak' lettuce. *Agric. Sci. J*., 42: 37–40

Muymas, P., R. Pichyangkur, W. Wiriyakitnateekul, T. Wangsomboondee, S. Chadchawan and K. Seraypheap, 2014. Effects of chitin-rich residues on growth and postharvest quality of lettuce. *Biol. Agric. Hort*., 31: 108–117

Nelson, D.W. and L.E. Sommers, 1996. Total carbon, organic carbon an organic matter. *In*: *Methods of Soil Analysis. Part 3. Chemical Methods*, pp: 961–1010. Bigham, J.M. (ed.). Soil Science Society of American (SSSA), Inc, Publisher, Madison, Wisconsin, USA

Pancha, I., K. Chokshi, B. George, T. Ghosh, C. Paliwal, R. Maurya and S. Mishra, 2014. Nitrogen stress triggered biochemical and morphological changes in the microalgae *Scenedesmus* sp. CCNM 1077. *Bioresour. Technol*., 156: 146–154

Peech, M., 1965. Lime requirement. *In*: *Methods of Soil Analysis Part 2. Chemical and Microbiological Methods,* pp: 927–932. Black, C.A. (ed.). American Society of Agronomy, Madison, Wisconsin, USA

Pornpienpakdee, P., R. Singhasurasak, P. Chaiyasap, R. Pichyangkura, R. Bunjongrat, S. Chadchawan and P. Limpanavech, 2010. Improving the micropropagation efficiency of hybrid *Dendrobium* orchids with chitosan. *Sci. Hort*., 124: 490–499

Porra, R.J., 2002. The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls *a* and *b*. *Photosyn. Res*., 73: 149–156

Wang, S.L., T.Y. Lin, Y.H. Yen, H.F. Liao and Y.J. Chen, 2006. Bioconversion of shellfish chitin wastes for the production of *Bacillus subtilis* W-118 chitinase. *Carbohydr. Res*., 341: 2507–2515

Wang, W., C. Yang, X. Tang, X. Gu, Q. Zhu, K. Pan, Q. Hu and D. Ma, 2014. Effects of high ammonium level on biomass accumulation of common duckweed *Lemna minor* L. *Environ. Sci. Pollut. Res. Int*., 21: 14202–14210

Zubia, M., Y. Freile-Pelegrín and D. Robledo, 2014. Photosynthesis, pigment composition and antioxidant defences in the red alga *Gracilariopsis tenuifrons* (Gracilariales, Rhodophyta) under environmental stress. *J. Appl. Phycol*., 26: 2001–2010

(Received 09 February 2015; Accepted 18 May 2015)