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Nano-fertilizer affects the growth, development, and chemical properties of rice

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Abstract

Polished rice usually lacks essential elements. Cultivation for increased yield with high nutritional value is thus a challenge. A greenhouse experiment was conducted to determine the effects of nanofertilizer application on the yield, total phenolic content (TPC) and antioxidant activity of rice cv. Ilpum. Results showed that agronomic parameters were significantly enhanced by all combination treatments except for that applied with nanofertilizer only. The full recommended rate of conventional and nanofertilizer (FRR-CF+FRR-NF) enhanced the plant height, chlorophyll content, number of reproductive tillers, panicles, and spikelets. The magnitudes of increase over the FRR-CF were 3.6%, 2.72%, 9.10%, 9.10%, and 15.42%, respectively. Similar results were seen in panicle weight (17.4%), total grain weight (unpolished-17.5%, polished-20.7%), total shoot dry weight (15.3%), and harvest index (2.9%). Half the recommended rate of nanofertilizer enhanced TPC, reducing power and 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) scavenging activity by 51.67%, 36.28%, and 20.93% respectively over the FRR-CF+FRR-NF treatment. TPC (557.55 mg GAE/100g) was higher compared to some pigmented rice cultivars. ABTS assay revealed that the scavenging activity could reach 97.23%. However, the highest hydroxyl scavenging activity registered on the FRR-CF+FRR-NF treatment (55.11%). The chelating capacity and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of the rice extracts did not noticeably differ among treatments. TPC appeared indirectly related to total grain yield and total shoot biomass. Alternatively, a strong positive correlation appeared between TPC and reducing power (94%), as well as ABTS radical scavenging activity (89%). Nanofertilizer application promoted the growth, development, TPC, and antioxidant activity in rice, demonstrating the potential to improve crop production and plant nutrition.

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Introduction

Fertilizers have an important role in enhancing food production and quality especially after the introduction of high-yielding and fertilizer responsive varieties. Most of the major crops grown such as rice require large quantities of inorganic inputs. Researches have been conducted to improve rice production but only a few can be seen in the literatures involving nanomaterials (NMs). (He, 2005; Liu *et al.*, 2007; Zhang *et al.*, 2007; Wang *et al.*, 2011; Gong and Dong, 2012; Sirisena *et al.*, 2013; Huang *et al.*, 2014). NMs are defined as materials with a single unit between 1 and 100 nm in size in at least one dimension (Liu and Lal, 2015). Hence, nanofertilizers are either NMs which can supply one or more nutrients to the plants resulting in enhanced growth and yield, or those which facilitate for better performance of conventional fertilizers, without directly providing crops with nutrients (Liu and Lal, 2015). Some studies already proved the significance of nanofertilizers. Some beneficial effects include increase in nutrient use efficiency, better yield and reduced soil pollution (Naderi and Danesh-Sharaki, 2013). The potential contribution of nanofertilizers in improving growth and development of crops lies on its ability for greater absorbance and high reactivity. Nanofertilizers can possibly enter the plant cells directly through the sieve-like cell wall structures if the particle sizes are smaller than the sizes of cell wall pores (5-20 nm). However, no research has refuted either that further absorption of nanofertilizer does occur through dissolution in water/soil solution. In other words, nanofertilizers simply dissolve in solution and release the nutrient(s) as soluble ions. Plants absorb the soluble nutrient ions as indiscriminately as they take in those from dissolved conventional fertilizers. But, the dissolution rate and extent of nanofertilizers in water/soil solution should be higher than those of the related bulk solids because of the much smaller particle sizes and higher specific surface areas of the former (Liu and Lal, 2015).

Rice is second to the largest commodity produced worldwide. The world dedicated 162.3 million hectares in 2012 for rice cultivation and the total production was about 738.1 million tonnes. The average world farm yield for rice was 4.5 tonnes per hectare, in 2012 (FAOUN, 2014). Rice is one of the most important cereal crops consumed in Asian countries. It is a staple food which contains many nutrients including carbohydrates, proteins, dietary fiber, vitamins and minerals. Moreover, recent studies showed that rice contains biologically active phytochemicals and phenolic compounds (Tian *et al.*, 2005; Aguilar-Garcia *et al.*, 2007). Some special phenolic compounds are present in cereal grains but are not found in significant quantities in fruits and vegetables (Bunzel *et al.*, 2001). These phenolic compounds have been known to have an important role in maintaining health, and have been identified to reduce the risk of chronic diseases like cardiovascular disease, type 2 diabetes, and some cancers (Liu, 2007). The cardioprotective, antimutagenic, and anticarcinogenic effects of phenolic compounds are reported to be generally associated with their antioxidant properties that eliminate free radicals and alleviate lipid peroxidation (Potter, 2005). Scientific studies suggest that pigmented rice have higher antioxidant properties than white rice (Jang and Xu, 2009). However, polished white rice is commonly preferred by the consumers. This is the common form rice is prepared for consumption, thereby reducing the concentration of these compounds in the grain. There were reports stating that phenolic content and antioxidant activity can be increased through soaking and germination process (Sawaddiwong *et al.*, 2008; Islam and Becerra, 2012). Apparently, breeding strategies cannot be taken out of the picture (Yafang *et al.*, 2011) as well as the capability of the growing environment to influence plant growth and composition. Modifying nutrient availability by controlling the quantity and quality could lead to a desirable outcome of improving the nutritive value of crops and that the potential of nanofertlizers could play a vital role in attaining these goals. However, published literatures regarding the potential of nanofertilizer in enhancing these properties are rare. For that reason, this study aimed to elucidate the possible effect of the application of nanofertilizer on the total phenolic content and antioxidant activity of white rice cv. Ilpum. In pursuing such goal, the study also evaluated the influence of nanofertilizer on the growth and development of rice. With the increase in population attended by rising cases of malnutrition and decreasing agricultural lands for crop production, an innovative strategy is a must. It would be very helpful if we use nanofertilizer for specific crops such as rice to minimize the potential negative effects brought about by the extensive use of chemical inputs without compromising production and nutritional benefits.

Materials and methods

The experiment was conducted under greenhouse conditions at the Agricultural Experiment Station and Research Facility, Kyungpook National University, Daegu, South Korea. The experimental set up was arranged in a Completely Randomized Design with five (5) replications.

The treatments include:

1. Control (no conventional fertilizer and nanofertilizer)
2. Full Recommended Rate of conventional fertilizer (FRR-CF)
3. Half Recommended Rate of conventional fertilizer (HRR-CF)
4. Full Recommended Rate of nanofertilizer (FRR-NF)
5. Half Recommended Rate of nanofertilizer (HRR-NF)
6. FRR-CF + FRR-NF
7. FRR-CF + HRR-NF
8. HRR-CF + FRR-NF
9. HRR-CF + HRR-NF

Experimental set up

Soil samples were potted into 15-kg quantities using 31 × 27 cm pots. Split application of the conventional fertilizer was done in the designated pots. Previously grown rice seedlings cv. Ilpum (10-15 days old) were transplanted onto pots and allowed to grow until maturity. The nanofertilizer (N>1.2%; P_2O_5>0.001%; K_2O>0.0001%) used in the experiment was purchased commercially and foliar application was done at seedling, tillering and panicle initiation stages. The dilution dosage was 1 part nanofertilizer to 1,000 parts of water. The nanofertilizer used is a colloidal liquid formula that facilitates nutrient absorption by plant cell for optimal growth. It also contains fungistatic characteristics that retard pathogen replication while allowing plants' passive immune system to withstand biotic and abiotic stresses in complement with enhanced nutrient absorption, allowing plant growth to optimal health in a holistic manner. For these reasons, supplemental application can be of beneficial help to the plant. On the other hand, the conventional fertilizer was applied based on the recommended rate for rice (11kg N - 7kg P_2O_5 - 8kg K_2O/10 are). The experiment was terminated at 100 DAT.

Data Gathering

The data on plant height was gathered from 15 to 75 days after transplanting (DAT) involving five measurement periods. Chlorophyll content was obtained during the maximum tillering stage and heading stage at 45 DAT and 60 DAT, respectively. The SPAD meter (SPAD-502-2900) was used to measure the chlorophyll content of rice. On the other hand, the yield components such as number of reproductive tillers, panicle number, total number of spikelets/grains, grain weight (polished and unpolished), 100-grain weight, and percent filled grains were recorded at harvest. The weight of grains was obtained based on 14% moisture content. The harvest index was also determined as an indicator in crop production.

Antioxidant Properties

Preparation of rice extracts

Rice samples were cleaned, milled and polished using Yamamoto Ricepal 32. Polished rice was pulverized into powder and 0.5 grams of the sample was extracted with 10 ml, 80% methanol (v/v) for 20 hours at ambient temperature. Extractions were filtered using 0.45 *um* PVDF Smartpor and were kept in -20°C until further analysis. All analyses were performed in triplicate.

Determination of total phenolic content

The total phenolic content (TPC) of rice grains was determined using the Folin-Ciocalteu method (Ham *et al.*, 2013) with some modifications. A 50 *ul* extract was mixed with 1 ml of 2% sodium carbonate and 50 *ul* of 50% Folin-Ciocalteu's reagent. The solution was incubated under room temperature for 1 hour. Absorbance readings were measured at 750 nm (Beckman Coulter UV-Vis spectrophotometer) and results were expressed in mg of gallic acid equivalent/100g of residue.

Hydroxyl radical scavenging capacity assay

The method of Halliwell *et al.* (1987) was followed in determining the scavenging activity of the extracts using the hydroxyl radical assay. The reaction mixture contained 1750 μl phosphate buffer solution (pH 7.4), 50 μl each of 2-deoxy-2-ribose (80 mM), EDTA (4 mM), $FeCl_3$ (4 mM), H_2O_2 (20 mM), ascorbic acid (4 mM) and 500 μl of rice extracts of various concentration. The mixture was vortexed and incubated for 1 hour at 37°C. Trichloroacetic acid (TCA, 2%) and thiobarbituric acid (TBA, 1%) solutions were prepared and 1 ml of each was added to the mixture and kept in a boiling bath for 15 minutes. After cooling, the pink chromogen revealing the formation of thiobarbituric reactive substances (TBARS) was read at 532 nm using a spectrophotometer (UV-Visible, Tecan Sunrise).

The hydroxyl radical scavenging activity was calculated based on the equation,

Hydroxyl scavenging activity (%) = [(Ac-At)/Ac] × 100

Where,

Ac = Absorbance of the control (without extract)

At = Absorbance of the extract/standard.

Reducing power determination

The reducing power of rice extracts was analyzed according to the method described by Choi and co-workers (2007). Rice extracts (250 *ul*) were mixed with 250 *ul* each of 200 mM sodium phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The mixture was subjected in a water bath at 50°C for 20 minutes. After cooling, 250 *ul* of 10% (w/v) trichloroacetic acid was added and then centrifuged at 5000 rpm for 10 minutes. The supernatant (250 *ul*) was then added to an equal volume of distilled water and 0.1% ferric chloride solution (50 *ul*). The development of blue-green color was read at 700 nm using a spectrophotometer (UV-Visible, Tecan Sunrise) with ascorbic acid as the positive control.

ABTS radical scavenging assay [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)]

The ABTS assay was performed following the method cited by Zheleva-Dimitrova *et al.* (2010) with some modifications. Stock solutions were composed of 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. Preparation of the working solution was done by mixing equal quantities of the two stock solutions and allowing them to react for 14 hours at room temperature under dark condition. The solution was then diluted by mixing 1 ml ABTS solution with 60 ml methanol to obtain an absorbance of 1.4-1.5 units at 734 nm using a spectrophotometer (UV-Visible, Tecan Sunrise). Freshly made ABTS solution (1 ml) was allowed to react with 0.5 ml of plant extracts for 30 minutes and the absorbance readings were taken at 734 nm. The ABTS scavenging potential of the extracts was compared with that of ascorbic acid, and percentage inhibition was calculated following the equation, ABTS radical scavenging activity (%) = [(Ac-As)/Ac] × 100

Where,

Ac = Absorbance of ABTS radical in methanol

As = Absorbance of the ABTS radical solution mixed with extract/standard.

Fe (II) chelating capacity

Determination of the chelating activity of the extracts was done based on the procedure explained by Dinis *et al.* (1994) with some modifications. The sample (150 *ul*) was mixed with 2 mM ferrous chloride (50 *ul*), 5 mM ferrozine (100 *ul*), and 95 % ethanol (1.7 ml) for 10 minutes. The absorbance of the mixture was measured using a spectrophotometer (UV-Visible, Tecan Sunrise) at 562 nm.

Ethylenediaminetetraacetic acid (EDTA) served as the positive control.

DPPH radical scavenging activity assay

The modified method of Brand-Williams *et al.* (1995) was used in determining the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the extracts. The reaction mixture contained 3 ml DPPH working solution (4.73 mg of DPPH in 100 ml ethanol) to which was added 100 µl rice extract. The mixture was shaken and incubated for 30 minutes in the dark at room temperature (30±1°C). The absorbance readings were observed at 515 nm using a UV-Visible spectrophotometer (Tecan Sunrise). The inhibition percentage of the absorbance of the DPPH solution was calculated using the following equation:

Inhibition % = $[(A_{blank} - A_{sample}) \times 100] / A_{blank}$

Where,

A_{blank} = Absorbance of control blank

A_{sample} = Absorbance of the sample extract.

Statistical Analysis

The data gathered was analyzed using the analysis of variance (ANOVA) procedure using SPSS version 21. Comparison of treatment means was determined using Tukey's HSD test.

Results

Agronomic parameters

The plant height was enhanced by the application of full recommended rate of nanofertilizer at 15 and 30 DAT (Fig. 1). Moreover, FRR-NF treatment significantly increased plant height compared to the control as the plant matures. General observations showed that all treatments except for HRR-NF were able to significantly increase plant height. Overall rankings revealed that FRR-CF + FRR-NF treatment performed best. However, the results were comparable to the following: FRR-CF + HRR-NF, HRR-CF + FRR-NF and HRR-CF + HRR-NF.

Table 1. Number of reproductive tillers, panicles and total number of grains as affected by chemical and nanofertilizer application under greenhouse conditions[1].

Treatment	Number of reproductive tillers[2]	Number of panicles[2]	Total number of grains[2]
Control	4 c	4 c	235 d
FRR-CF	30 a	30 a	3213 ab
HRR-CF	22 b	22 b	2424 bc
FRR-NF	4 c	4 c	332 d
HRR-NF	4 c	4 c	297 d
FRR-CF + FRR-NF	33 a	33 a	3799 a
FRR-CF + HRR-NF	29 a	29 a	3266 ab
HRR-CF + FRR-NF	22 b	22 b	2455 bc
HRR-CF + HRR-NF	18 b	19 b	2069 c

[1]Means in a column followed by the same letter(s) are not significantly different at P ≤ 0.05 based on Tukey's HSD test. [2]Numbers per plant.

Fig. 2 shows the chlorophyll content of rice as affected by combined application of conventional and nanofertilizers under greenhouse conditions. Similar to plant height, the chlorophyll content of rice was enhanced by all treatments except for HRR-NF. In general, a decrease in the chlorophyll content of all plants was seen at 60 DAT.

The number of reproductive tillers, number of panicles, and total number of spikelets revealed the same trend (Table 1). These parameters were significantly affected by the application of conventional fertilizer and its combination with nanofertilizer. In addition, the parameters were enhanced best with the application of FRR-CF+FRR-NF.

The panicle weight, total grain weight (unpolished and polished), total shoot dry weight and harvest index were observed to have the same trends in the following order: FRR-CF + FRR-NF > FRR-CF + HRR-NF > FRR-CF > HRR-CF > HRR-CF + FRR-NF > HRR-CF + HRR-NF > FRR-NF > HRR-NF > Control as reflected in Table 2. All treatments were significantly higher over the control except for the treatments applied with nanofertilizer alone. On the other hand, no noticeable differences were seen in terms of 100-grain weight and percent filled grains.

Table 2. Influence of chemical and nanofertilizer application on the panicle weight, total grain weight (unpolished and polished rice), total shoot dry weight and harvest index of rice under greenhouse conditions[1].

Treatment	Panicle weight[2]	Total grain weight[2]		Total shoot dry weight[2]	Harvest index
		Unpolished	Polished		
Control	6.54 d	6.27 d	4.62 d	13.94 e	0.437 c
FRR-CF	78.95 ab	75.92 ab	51.36 b	125.93 abc	0.601 ab
HRR-CF	63.12 bc	60.94 bc	42.44 bc	101.66 bcd	0.596 ab
FRR-NF	8.95 d	8.56 d	6.22 d	17.03 e	0.522 abc
HRR-NF	7.98 d	7.69 d	5.85 d	15.09 e	0.502 bc
FRR-CF + FRR-NF	95.57 a	92.07 a	64.78 a	148.69 a	0.619 a
FRR-CF + HRR-NF	79.63 ab	76.67 ab	52.62 ab	126.09 ab	0.608 a
HRR-CF + FRR-NF	61.61 bc	59.26 bc	40.84 bc	99.26 cd	0.587 ab
HRR-CF + HRR-NF	53.57 c	51.65 c	35.84 c	88.72 d	0.582 ab

[1]Means in a column followed by the same letter(s) are not significantly different at $P \leq 0.05$ based on Tukey's HSD test. [2]Grams per plant.

Antioxidant Properties

Almost similar trend was observed on the TPC, reducing power and ABTS scavenging activity of rice extracts. Although the results were comparable to the control, the application of half the recommended rate of nanofertilizer considerably increased these parameters compared to the other treatments. Compared to the FRR-CF + FRR-NF treatment, the total phenolic content, reducing power and ABTS scavenging activity were enhanced significantly by HRR-NF by 51.67%, 36.28%, and 20.93% respectively (Table 3).

Table 3. Total phenolic content, reducing power and ABTS scavenging activity of rice extracts as affected by chemical and nanofertilizer application under greenhouse conditions[1].

Treatment	Total phenolic content (mg GAE/100g residue)	Reducing power	ABTS radical scavenging activity (%)
Control	557.35 a	0.1359 a	98.27 a
FRR-CF	246.67 c	0.0849 b	78.74 b
HRR-CF	232.84 c	0.0811 b	73.69 b
FRR-NF	400.49 b	0.1227 a	82.60 b
HRR-NF	557.55 a	0.1381 a	97.23 a
FRR-CF + FRR-NF	269.51 c	0.0880 b	76.88 b
FRR-CF + HRR-NF	292.65 c	0.0868 b	76.07 b
HRR-CF + FRR-NF	305.00 bc	0.0911 b	77.31 b
HRR-CF + HRR-NF	248.73 c	0.0780 b	72.77 b

[1]Means in a column followed by the same letter(s) are not significantly different at $P \leq 0.05$ based on Tukey's HSD test.

In contrast to the above statement, the highest hydroxyl scavenging activity was observed on the FRR-CF + FRR-NF treatment (55.11%) as shown in Fig. 3. Nonetheless, the result was comparable when combination of half the recommended rate of conventional fertilizer and nanofertilizer was used.

The chelating capacity and DPPH scavenging activity of the rice extracts did not indicate any noticeable differences among treatments. However, it was evident that nanofertilizer application enhanced these parameters.

Discussion

Agronomic parameters

The nanofertilizer used in the experiment is a formulated colloidal farming fertilization supplement that facilitates nutrient uptake, transportation and absorption. As shown in Fig. 1, the FRR-NF treatment significantly increased plant height over the control. In addition, plant height was more enhanced when nanofertilizer was combined with conventional ones, even at a lower application rate. These suggest that nanofertilizer can either provide nutrients for the plant or aid in the transport or absorption of available nutrients resulting in better crop growth. Related study by Liu and Lal (2014) revealed similar findings in soybean. They synthesized a new type of hydroxyapatite phosphorus nanoparticles (NPs) of ~16 nm in size and assessed fertilizing effect of the NPs on soybean in inert growing medium in a greenhouse experiment. The data revealed that growth rate was increased by 33% using phosphorus NPs.

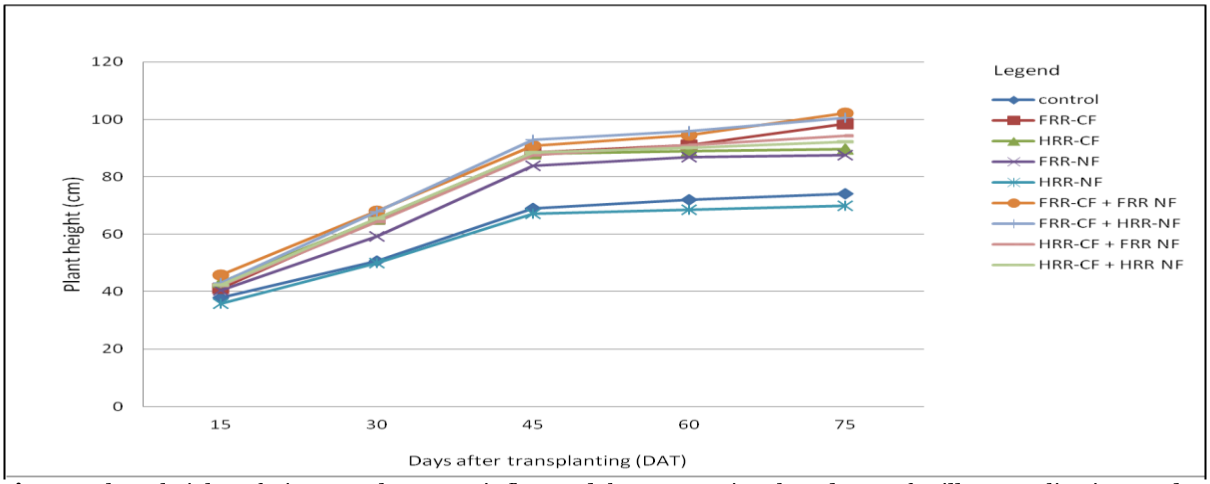

Fig. 1. Plant height of rice cv. Ilpum as influenced by conventional and nanofertillzer application under greenhouse conditions.

Chlorophyll content was recorded at 45 and 60 DAT. Similar to plant height, it was enhanced by all treatments except for HRR-NF (Fig. 2). The FRR-CF+FRR-NF obtained the highest value (2.72% increase over FRR-NF). Chlorophyll is the light harvesting pigment responsible for photosynthesis. Sufficient amount of chlorophyll means greater production of photosynthates responsible for the growth and development of the plant (Hopkins and Huner, 2004). Higher chlorophyll content means higher available N preventing premature senescence. In contrast, a general decrease in the chlorophyll content of plants was seen at 60 DAT. This can be attributed to the actively growing stage of the rice plant. According to Taiz and Zeiger (2006), leaves begin their development as sink organs. A transition from sink to source status occurs later in development. The maturation of leaves is accompanied by a large number of functional and anatomic changes, resulting in a reversal of transport direction from importing to exporting. Nanofertilizer may have affected these processes through its transportation capabilities in terms of penetration and movements within the plant systems.

Table 1 shows the number of reproductive tillers, number of panicles, and total number of grains. A significant effect was observed on these parameters when application of conventional fertilizer and its combination with nanofertilizer was done. The data revealed that the FRR-CF+FRR-NF treatment performed best. The percentage increase over the FRR-CF was 9.10%, 9.10% and 15.42%, respectively.

On the other hand, the weight of panicle, total grain weight (unpolished and polished), total shoot dry weight and harvest index were increased significantly by all treatments except for that applied with nanofertilizer alone (Table 2). An increase in harvest index would mean improvement in grain yield. It seems that the function of nanofertilizer at the reproductive stage of rice was only supplemental. Nonetheless, it was evident that nanofertilizer application enhanced the abovementioned parameters. Nanofertilizer may have synergistic effect on the conventional fertilizer for better nutrient absorption by plant cells resulting to optimal growth.

To determine the correlation among TPC, total grain yield, and total shoot biomass, linear regression analysis was carried out. The results revealed that TPC was negatively correlated with the two parameters. The correlation coefficients obtained were 0.6407 (total grain yield) and 0.4940 (total shoot biomass).

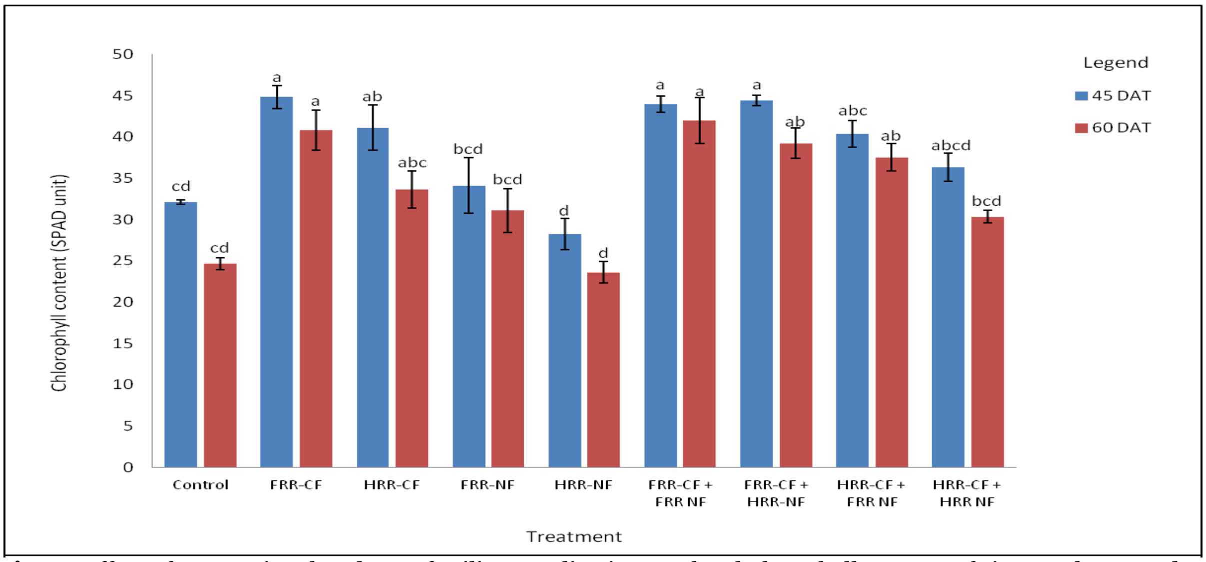

Fig. 2. Effect of conventional and nanofertilizer application on the cholorophyll content of rice cv. Ilpum under greenhouse conditions. Values with the same letter(s) are not significantly different at $p \leq 0.05$ based on Tukey's HSD test. Vertical bars indicate standard error.

Promoted growth and development brought about by the combined treatments can be described by the sink strength. The ability of a sink to mobilize photosynthates toward itself is often known as sink strength, which depends on two factors namely, sink size and sink activity. Sink size is the total biomass of the sink tissue while sink activity is the rate of uptake of photosynthates per unit biomass of sink tissue (Taiz and Zaiger, 2006). As mentioned earlier, nanofertilizer may have affected these processes through its nutrient transportation capability in terms of penetration and movement of a wide range of nutrients, from roots uptake to foliage penetration and movements within the plant.

A number of studies proved the significance of nanofertilizers. For instance, Sirisena *et al.* (2013) obtained higher grain yield in rice with the application of nano-K fertilizer. This is in agreement with the findings of Liu and co-workers (2009) who reported that nanofertilizer application increased crop yield by 20-40%. Another experiment conducted by Delfani *et al.* (2014) obtained the highest yield in black-eyed pea (245 g, a 13.5% increase over the control) when a regular Fe salt fertilizer was combined with Mg-NPs. If nanofertilizer could be made available at the present market price of conventional fertilizers, nanofertilizer application will reduce the cost of conventional ones for crop production, and pollution will be minimized.

Antioxidant Properties

Rice is one staple food in the diet of most of the world's population. Rice has an important role in the concentration of antioxidants ingested daily. In this study, the effect of nanofertilizers on the phenolic content and antioxidant properties were determined from the methanolic rice extracts. Phenolic compounds are secondary metabolites produced by plants throughout their development for several reasons: defense against microorganisms, insects, or herbivores (Crozier *et al*., 2006; Herms and Mattson, 1992); nutrient availability (Herms and Mattson, 1992); exposure to ultraviolet radiation (Rozema *et al*., 1997); and allelopathic interactions (Mann, 1987). Specifically, the availability of key macronutrients during the growth of the plant has considerable potential to affect phenolic accumulation (Parr and Bolwell, 2000).

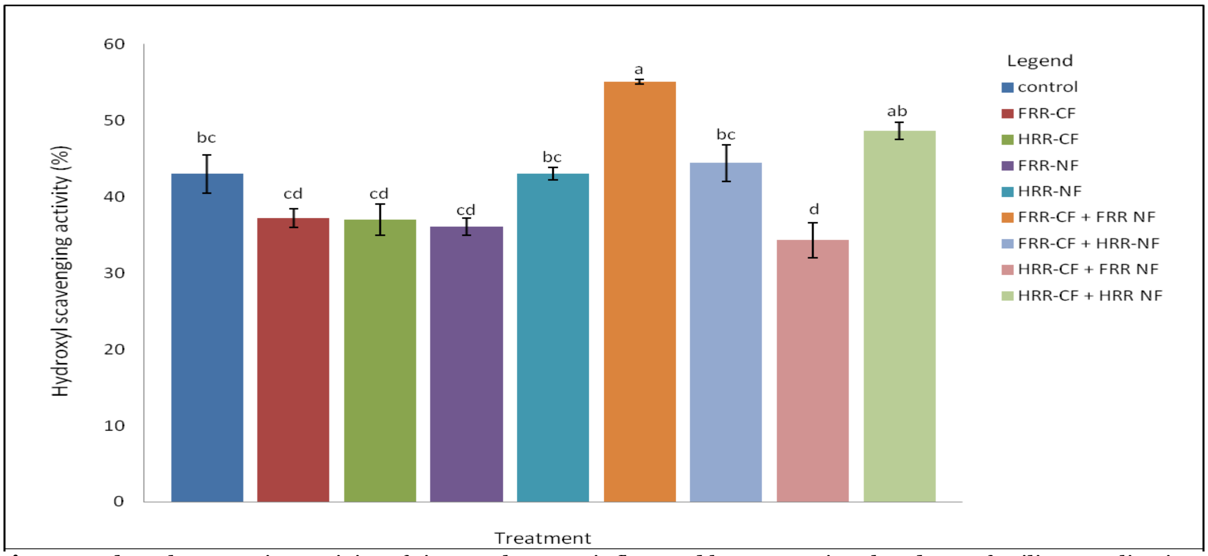

Fig. 3. Hydroxyl scavenging activity of rice cv. Ilpum as influenced by conventional and nanofertilizer application under greenhouse conditions. Values with the same letter(s) are not significantly different at $p \leq 0.05$ based on Tukey's HSD test. Vertical bars indicate standard error.

The HRR-NF treatment showed noticeable increase in the TPC, reducing power and ABTS scavenging activity (Table 3). While the results were comparable to the control, the application of HRR-NF considerably increased these parameters compared to the other treatments. Several studies have indicated that the phenolic compounds in grains have effective antioxidant properties, due to the presence of one or more aromatic rings with one or more hydroxyl groups (Zielinski and Kozlowska, 2000). The TPC of the extracts ranged from 232.84 to 557.55 mg GAE/100g residue. The highest TPC was obtained by applying HRR-NF which was higher compared to the black and red rice cultivars studied by Ham *et al.* (2013). In addition, the experiment conducted by Edwina *et al.* (2014) on different pigmented landraces of rice showed lower values (233.92-251.38 mg GAE/100g). This implies that the TPC can be enhanced in white rice cultivars through nanofertilizer application and can even exceed the pigmented rice cultivars.

The reducing power of bioactive compounds is generally associated with the presence of reductones (Pin-Der-Duh, 1998), which have been shown to exert antioxidant action by breaking the free radical chains by donating a hydrogen atom (Juntachote and Berghofer, 2005). The results showed that the application of HRR-NF alone was significantly higher by 36.28% than the FRR-CF+FRR-NF treatment. Again, nanofertilizer application proved to enhance the antioxidant potential in rice. The outcome of the experiment was comparable to that conducted by Basu *et al.* (2012) in some Indian rice varieties. Antioxidants are secondary metabolites produced when plants are subjected to unfavorable conditions such as limited nutrients. Although nanofertilizer application was supplemental, its better absorption through plant cells somehow provided enough nutrients to enhance antioxidant activities.

Results of the antioxidant activity of the samples as determined by ABTS assay revealed that the scavenging activity can be as high as 97.23% (Table 3). This value was obtained from HRR-NF treated plants. The results were in agreement with those reported by Yodmanee *et al.* (2011) from the pigmented rice cultivars of southern Thailand.

Notably, the observations on the hydroxyl scavenging activity of the extracts were quite the reverse of the previous statements. Among the oxygen radicals, the hydroxyl radicals specifically are the most reactive, and they severely damage almost any adjacent molecule they touch (Aruoma, 1998). The results revealed that the FRR-CF+FRR-NF treatment performed best in scavenging these oxygen radicals (Figure 3). However, there were no noticeable differences seen when HRR-CF + HRR-NF was applied. The results were comparable to those reported by Basu and co-workers (2012) on unpolished rice, which indicate that polished rice can be of equal value when provided with appropriate management practice.

The different treatments did not show significant differences on the chelating effect of the rice extract on ferrous ion. However, it was clear that application of nanofertilizer contributed to the chelating ability. According to Ham *et al.* (2013) the chelating effect of some specialty rice cultivars ranged from 15-80%. It was also reported that Ilpum cultivar obtained around 40%. Based on the results of our experiment, the chelating effect of Ilpum rice extracts was higher (73%) when nanofertilizer application was done. Nanofertilizer facilitates in the absorption of a wide range of nutrients which may form chelation with ferrous iron.

The antioxidant activity of the rice extracts was also evaluated through DPPH scavenging assay. When DPPH radicals encounter a proton-donating substance, such as an antioxidant present in plant extract, the radicals are scavenged. Noticeable differences were not seen among treatments. Nonetheless, it was evident that nanofertilizer application enhanced DPPH scavenging activity. The FRR-NF treatment performed best with 19.53% DPPH radical scavenged. Results were comparable to the data obtained by Edwina *et al.* (2014) and Basu *et al.* (2012) on their study about pigmented rice and unpolished rice. On the other hand, Dutta and co-workers (2012) reported relatively lower DPPH activity (6.01-14.47%) in high yielding rice varieties from Bangladesh.

The relationship among TPC and antioxidant activity was analyzed through linear regression analysis. The results show a strong positive correlation between TPC and reducing power, and TPC and ABTS radical scavenging activity. The analysis shows that TPC contributed to about 94% and 89% on the reducing power and ABTS scavenging activity of the extracts, respectively.

Inverse relationships on yield data and TPC as well as antioxidant capacity can be explained by the “dilution effect”. Fertilization contributed to increases in plant dry matter. Therefore, plants receiving more amounts of fertilizer contained larger amounts of nutrients than the less fertilized plants. However, these amounts were sufficiently diluted by the increased dry matter leading to a decline in nutrient concentrations.

The results may be further explained by the growth-differentiation balance (GDB) framework which is based on the principle that a “physiological trade-off” exists between plant growth and production of secondary metabolite (Herms and Mattson, 1992). Nitrogen, phosphorus, and potassium are the major nutrients needed by the plant for adequate growth and development. When environmental conditions are good and nutrient levels are sufficient, the GDB theory states that plant growth will be favored, with production of photosynthetic proteins receiving resource priority. However, when environmental conditions are poor and the availability of an essential nutrient is limited, the GDB framework proposes that growth allocation for a plant will decrease while the secondary metabolite production that may aid in storage and defense subsequently increase (Herms and Mattson, 1992).

Within the GDB framework, the carbon/nutrient balance (CNB) hypothesis (Bryant *et al.*, 1983) more specifically addresses the effects of fertilization on plant resource distribution. The CNB theory states that when nutrients are limited, plants increase their production of carbon-based compounds, particularly secondary metabolites. Based on the CNB hypothesis, one would therefore expect low fertilization levels to lead to increased concentrations of carbonaceous metabolites such as phenolic compounds.

Conclusion

Application of nanotechnology in agriculture is still in its budding stage. However, it has the potential to revolutionize agricultural systems particularly where the issues on fertilizer applications are concerned. Nanofertilizer application promoted the growth, development, TPC and antioxidant activity in rice and has the potential to improve crop production and plant nutrition. The outcome of this research would be beneficial for other studies involving the application of nanotechnology in the field of agriculture.

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